Aspects of Biochemical Kinetics

Libuk7 — December 14, 2003

In this chapter we shall consider several increasingly complex examples of chemical kinetics. Among the goals of our discussion are these:

(a) to enable the reader to write the governing differential equations of biochemical kinetics;

(b) to present several principles that can be used to simplify these equations;

(c) to demonstrate the utility of the simplifications given in (b), especially in view of the fact that computers typically can solve the unsimplified equations with relative ease.

In the course of our discussion we will deal with several fundamental biological situations where kinetic models are essential to understanding — including conformational shifts of channel and receptor molecules, as well as Michaelean and cooperative interactions of substrates with enzymes and ligands with receptors.

1 Interchange between two states of a molecule: molecular level

The biconfigurational molecule We begin the discussion with two simple examples of the types of phenomena to be investigated, shifts in conformation of protein molecules. Ion channels are responsible for the electric conductance of membranes. The channel molecules typically shift spontaneously between open and closed states, with shift rates that are dependent on the local electric field. (Channel ligation also induces shifts, but here we do not

mention this possibility further.) Voltage dependent channel modifications are responsible for such key actions as the passage of electric signals down axons (long "wires" that extend from the cell body) and for bringing about neurotransmitter release. Another example of the shift in conformation between two major states of a large protein molecule occurs in receptors. One of numerous examples is the cyclic-AMP receptor of the cellular slime mold *Dictyostelium discoideum*. The shift between active and densensitized states plays a major role in the adaptation phenomena that are a prominent aspect of the behavior of this "model" organism (Goldbeter [12]). In many other biological contexts, theory and experiment, beginning with Katz and Thesleff [20], support the importance of conformation shifts in adaptation. The conventional description of the shifting of a molecule between two states A and B (e.g. open and closed) is summed up in the **kinetic scheme**

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B \ . \tag{1.1}$$

We shall use the same letters, A, and B in this case, to denote the concentrations (number per unit volume) of the two molecular configurations, as well as to denote their states. The meaning of the **rate coefficients** k_1 and k_{-1} will be explained shortly. Note the assumption that the protein can be described by just two major conformations. This often seems reasonable as a first approximation, but in many circumstances there is evidence for several conformations, for example in channels there are often several closed states (Colquhoun and Hawkes [3]). In yet other circumstances it is possible that the whole idea of identifiable discrete "conformations" may not be appropriate (Liebovitch *et al.* [26]). *Formulation of mathematical model* We define

the rate constant k_1 in (1.1) as follows. Consider a time interval of duration Δt, for which the molecule is in state A at the beginning of the interval. Take Δt to be so small that during an interval of length Δt the only behavior that is likely to be observed is that either (i) the molecule remains in state A or (ii) the molecule shifts to state B and stays in state B. We define $k_1 \Delta t$ to be the probability that (ii) occurs. To be more precise, the smaller is Δt, the better an approximation is $k_1 \Delta t$ to the probability that a molecule that is initially in state A shifts to state B. Let us denote the fractional error in this approximation by $E(\Delta t)$. As Δt approaches zero, we expect that E will

also approach zero. We thus write

$$\begin{aligned}&\text{Probability that during an interval of duration } \Delta t \text{ a}\\&\text{molecule that is initially in state } A \text{ will shift to state } B\\&\quad = k_1 \Delta t\,[1 + E(\Delta t)]\end{aligned} \tag{1.2}$$

where

$$\lim_{\Delta t \to 0} E(\Delta t) = 0\ . \tag{1.3}$$

Similarly, if Δt is sufficiently small then $k_{-1}\Delta t$ is a good approximation to the probability that a molecule that is initially in state B changes to state A and remains in state A. How small should Δt be in order that this approximation be a good one? It must be that the probability $(k_2\Delta t)(k_1\Delta t)$ that $B \to A \to B$ is much less than the probability $k_2\Delta t$ that $B \to A$, for the former event has been assumed to be unlikely: thus we require that $k_1\Delta t \ll 1$, i.e. $\Delta t \ll 1/k_1$. Similarly we require $\Delta t \ll 1/k_{-1}$. Defining k^1 and k_{-1} in (1.1) as we did committed us to certain assumptions concerning the shift of a molecule between two configurations. These assumptions go under the name of **Markov properties**. (Models based on Markov properties are often called **Markov processes**.)

(M1) Transitions between states are *random.*

(M2) The probability that a transition occurs during some time interval *does not depend on the history* of events preceding the time in question. For example, the probability that a receptor that was active at $t = 6$ ms will become desensitized during the time interval (6, 6.01) ms does not depend on how long the receptor was active prior to $t = 6$ ms.

(M3) If environmental conditions are fixed then the overall characteristics of the *transitions* that occur in some time interval *do not depend on the time* at which the observations are made. Note that assumption M2 was used implicitly in arriving at (1.2), since we assumed that there was no influence of previous events on behavior during the time interval Δt. Also, by assumption M3, the rate coefficients k_1 and k_{-1} do not depend explicitly on time. There could be an implicit dependence on time, for example, if the temperature is changing.

2 Interchange between two states of a molecule; population level

We are now ready to derive differential equations for the change in time of concentrations A and B of molecules whose behavior is consonant with kinetic scheme (1.1).
If there are A molecules per unit volume, according to (1.2) the expected decrease in the number of these molecules during a short time Δt is given by

$$\begin{aligned} \text{decrease in } A \text{ molecules} &= \text{total number of } A \text{ molecules} \times \text{fraction} \\ &\qquad \text{that become } B \\ &= A(k_1 \Delta t) \ . \end{aligned} \tag{2.1}$$

Upon changing their conformation, A molecules become B. B molecules, on the other hand, change to A with a probability per unit time of k_{-1}. Thus the following equation describes the expected change in the number of A molecules during the time interval t, $t + \Delta t$:

$$A(t + \Delta t) - A(t) = -A(t) \cdot (k_1 \Delta t) + B(t) \cdot (k_{-1} \Delta t) \ . \tag{2.2}$$

Upon dividing by Δt and taking the limit as $\Delta t \to 0$ we obtain (employing the definition of the derivative dA/dt)

$$dA/dt = -k_1 A + k_{-1} B \ . \tag{2.3}$$

In exactly the same way we obtain the corresponding equation for B

$$dB/dt = k_1 A - k_{-1} B \ . \tag{2.4}$$

The mathematical translation of the kinetic scheme (1.1) is completed by prescribing the initial state of the system, at time $t = 0$:

$$A(0) = A_0 \ , \qquad B(0) = B_0 \ . \tag{2.5a, b}$$

One can think of A_0 and B_0 as measured concentrations at the start of the experiment.
Our equations can be simplified, as is very often the case, by taking advantage of a **conservation law**. For (2.3) and (2.4), the conservation law is

$$A(t) + B(t) = M \ . \tag{2.6}$$

Here M is a constant, the total number of A and B molecules per unit volume (M for total Material). Indeed, given the kinetic scheme (1.1), molecules merely shift between two conformations, so that their total number is conserved (does not change). Of course "real" molecules degrade, but if this process were to be taken into account then the kinetic description (1.1) would have to be modified. (See Exercise 1.) Degradation is often relatively slow, and may therefore be neglected if the process under investigation is being observed for a suitably short time.
The conservation law (2.6) can be derived mathematically. By adding equations (2.3) and (2.4) we obtain

$$dA/dt + dB/dt = 0 \ . \tag{2.7}$$

Since "the derivative of the sum is the sum of the derivatives", (2.7) implies

$$d(A + B)/dt = 0 \ . \tag{2.8}$$

Equation (2.6) then follows, since only a constant has a zero derivative. Note that at time $t = 0$ the total number of molecules per unit volume is $A_0 + B_0$, by (2.5). Thus

$$M = A_0 + B_0 \ . \tag{2.9}$$

It is convenient to use the conservation law (2.6) to express B in terms of A:

$$B(t) = M - A(t) \ . \tag{2.10}$$

Upon introducing (2.10) into (2.3), we "eliminate B" and obtain an equation involving $A(t)$ only:

$$dA/dt = -k_1 A + k_{-1}(M - A) \tag{2.11}$$

or, rearranging,

$$dA/dt = -(k_1 + k_{-1})A + k_{-1}M \ . \tag{2.12a}$$

The initial condition for (2.12a) is that of (2.5a):

$$A(0) = A_0 \ . \tag{2.12b}$$

After problem (2.12) for A is solved, $B(t)$ can be found from (2.10). **Mathematical remark.** The system (2.12a), (2.12b), (2.10) is mathematically equivalent to (2.3), (2.4) and (2.5), but the latter should be employed for numerical integration.

In general, use of conservation laws avoids difficulties of ill-conditioning (excessive sensitivity to inevitable small numerical errors). *Solution and interpretation*

From Appendix 4, Example 1, the general solution to (2.12a) is

$$A = C\exp[-(k_1 + k_{-1})t] + k_{-1}M/(k_1 + k_{-1}) \tag{2.13}$$

for an arbitrary constant C. Imposing initial condition (2.12b) we obtain

$$A_0 = C + k_{-1}M/(k_1 + k_{-1}) \ , \quad \text{or} \quad C = A_0 - k_{-1}M/(k_1 + k_{-1}) \ . \tag{2.14a,b}$$

Together, (2.13) and (2.14b) give the solution for $A(t)$. To determine $B(t)$ we employ (2.10):

$$B(t) = -C\exp[-(k_1 + k_{-1})t] + k_1 M/(k_1 + k_{-1}) \ . \tag{2.15}$$

Let us graph the solutions that we have obtained. To this end we note that at $t = 0$, since $\exp(0) = 1$, (2.13) and (2.14b) indeed yield the correct initial condition $A(0) = A_0$. As time passes the exponential term in (2.13) decays toward zero. This is written

$$A(t) \to k_{-1}M/(k_1 + k_{-1}) \text{ as } t \to \infty \ . \tag{2.16}$$

Similarly, upon employing (2.9), it is easily seen (as the reader should verify) that

$$B(0) = B_0 \ , \qquad B(t) \to k_1 M/(k_1 + k_{-1}) \text{ as } t \to \infty \ . \tag{2.17a,b}$$

Typical graphs of the solutions are provided in Fig. 7.1. **Steady states** of A and B, occur when concentrations are constant. At steady state, $dA/dt = 0$ and $dB/dt = 0$. Here the rate of conversion of A to B should exactly balance that rate of conversion of B to A:

$$k_1 A = k_{-1}B \ . \tag{2.18}$$

Together with the conservation law (2.6), (2.18) determines the ultimate steady states of (2.16) and (2.17b) [as the reader should verify – Exercise 2(a).] Note that the steady state equation (2.18) can be formally obtained by setting the time derivatives equal to zero in (2.3) or (2.4). How fast are the

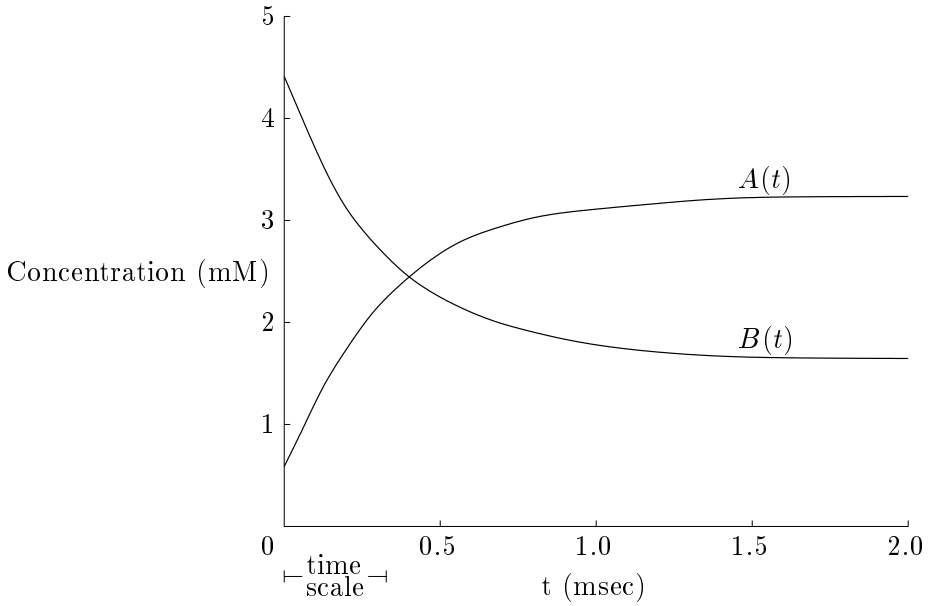


Figure 7.1: Graph of (2.13) and (2.15) when $k_1 = 1$ msec^{-1}, $k_{-1} = 2$ msec^{-1}, $A_0 = 0.6$ mM, $B_0 = 4.4$ mM. Depicted is the predicted time scale for significant variation of $A(t)$ and $B(t)$, namely $(k_1 + k_{-1})^{-1} = 1/3$ msec. [fig71lb7]

steady states of A and B approached? To answer this, consider the following little table.

t	$\exp(-kt)$
0	1
$1/k$	$\exp(-1) = e^{-1} = 0.368$
$2/k$	$\exp(-2) = e^{-2} = 0.135$

(2.19)

The table allows the inference that $1/k$ gives the **time scale** of the function $\exp(-kt)$, in that *a major change in the magnitude of the function takes place in a time interval whose duration is of the magnitude of the time scale*. In the case of $A(t)$ and $B(t)$ it follows from (2.13) and (2.15) that the functions progress toward their ultimate steady states in a time scale $(k_1 + k_{-1})^{-1}$. It could be argued that $2/k$, not $1/k$, is the time required for a "major change" in $\exp(-kt)$. But it is orders of magnitudes that interest us. Is the time scale seconds, minutes, or weeks? A factor of 2 is not important for such considerations. Thus, for simplicity we take $1/k$ as the time scale. *The irreversibility approximation* There are a number of situations wherein the back reaction in the kinetic

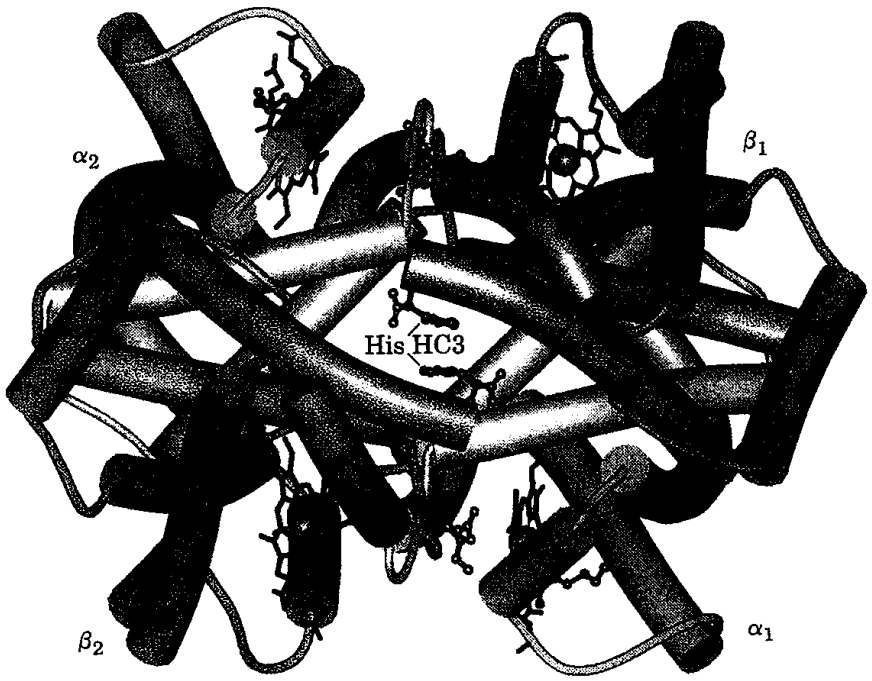


Figure 7.2: Hemoglobin structure (two α subunits and two β subunits). From [31]. Permission to be obtained. [fig72lb7]

scheme (2.1) is "weak", i.e.

$$k_{-1} \ll k_1 \ . \tag{3.1}$$

Under these circumstances, we would expect to find an adequate approximation to the solution by replacing the kinetic scheme (2.1) by the **irreversibility approximation**

$$A \xrightarrow{k_1} B \ . \tag{3.2}$$

The equation for $A(t)$ that corresponds to (3.2) is

$$dA/dt = -k_1 A \ , \tag{3.3a}$$

with initial condition

$$A(0) = A_0 \ . \tag{3.3b}$$

The solution to problem (3.3) is

$$A(t) = A_0 \exp(-k_1 t) \ . \tag{3.4}$$

From (2.10)

$$B(t) = M - A_0 \exp(-k_1 t) \ . \tag{3.5}$$

Comparison of (3.4) and (3.5) with the corresponding exact expressions (2.13) and (2.15) confirms that the irreversibility approximation is indeed a good one when the back reaction rate is relatively small, as assumed in (3.1). **Example.** In the kinetic scheme (1.1), regard the concentration of A as fixed at some value $\overline{A}$. Find B and interpret your answer. (The result will be employed in Section 4.) **Solution.** If we set A equal to a constant value $\overline{A}$ in (2.4) we obtain

$$\frac{dB}{dt} = k_1 \overline{A} - k_{-1} B \ . \tag{3.6}$$

By Example 1 of Appendix 4, this equation has the general solution

$$B = Q e^{-k_{-1} t} + \frac{k_1 \overline{A}}{k_{-1}} \tag{3.7}$$

where Q is an arbitrary constant. The value of Q is set by the initial condition $B(0) = B_0$, yielding

$$B_0 = Q + \frac{k_1 \overline{A}}{k_{-1}} \tag{3.8}$$

and hence

$$Q = B_0 - \frac{k_1 \overline{A}}{k_{-1}} \ . \tag{3.9}$$

Thus the solution to the differential equation (3.6) and the initial condition is

$$B = \left(B_0 - \frac{k_1 \overline{A}}{k_{-1}} \right) e^{-k_{-1} t} + \frac{k_1 \overline{A}}{k_{-1}} \ . \tag{3.10}$$

We infer that if A is a constant $\overline{A}$ in the kinetic scheme (2.4), then B approaches a steady state value of $k_1 \overline{A}/k_{-1}$ in a time of order $1/k_{-1}$. **Exercise**

1. The purpose of this exercise is to experience the stochastic nature of (2.2) when k_1 and k_{-1} are treated as probabilities. Suppose first that these are equal, so that "chance" can be modelled by throwing an ordinary "non loaded" coin. (a) Start with $A = 9$ and $B = 1$, so that $A + B = 10$. Let there be a transition from A to B if your coin gives heads and from B to A if the coin gives tails. Plot the results of a number of tosses.
   (b) In the long run, A and B should be equal, on average. How long does the "long run" take, according to your experimental evidence? How reliable do you think your results are?
   (c) If you are sufficiently knowledgable with a computer, or even with some hand calculators, you can use "random numbers" to do the experiments. Suppose that the probability of A changing to B is 0.6. Given the ability to choose random decimal numbers between 0 and 1, how could this probability be implemented?
   (d) Use a computer to run simulations when A flips to B with probability 0.6 and B flips to A with probability 0.7. What is the long term result? How long do your simulations indicate that it will take for the long term result to be reasonably accurate. [Observations of Coin tossing and observations of the statistics of two-state molecules (such as open or closed channels) amount to the same thing. See Feller [5] for remarkable results concerning coin tossing.]

3 Discussion

Comparison with experiment At this point we can begin to appreciate the usefulness of our mathematical modeling. We first note that comparison with experiment may show that the form of the curves is not correct, in which case the simple model must be revised. Such a failure of our simple model would be very interesting, for it would show that something was definitely amiss with our "common sense" basic assumptions. If the theory is basically sound, one can compare the theoretical predictions of equations (2.13) and (2.15) with experimental results and thereby ascertain the coefficients k_1 and k_{-1} and hence the time scales $1/k_1$ and $1/k_{-1}$ of the conformational shifts (Eigen and Johnson [4]). Thus theory permits us to extract information concerning molecular events (probability of conformation changes) from observations of macroscopic variables (concentrations). *Steady state attractors* The more

one contemplates the basic modeling assumptions that we have made, the more one is impressed. We now know that a giant protein molecule is an enormously complicated dynamic structure (see Fig. 7.2, which only hints at the full structural complexity). Faced with this complexity, it is natural to despair of any simple approach. Indeed the situation can serve as a metaphor for theoretical biology as a whole. Many claim that biology is such a complicated subject that no simple-minded modeling can be of any use. That this is not always a correct view is illustrated by our example. For conformational transformations in particular and for biology in general, a great deal remains to be done, but simple models have enabled important theoretical and conceptual progress. Solutions (2.15) and (3.10) suggest the following important generalization: *after a certain transient period, it is often the case that solutions often approach a steady state.* Remarks. (i) One would not expect a steady state to be attained if conditions were continually changing, and indeed (2.15) and (3.10) were obtained for equations whose coefficients were constants. (ii) Steady states were approached after transients in many models studied in Chapters ? and ?. (iii) Given experience with models in Chapters ? and ?, one would expect that under some circumstances the long term "attractors" of solutions to differential equations might sometimes be oscillatory states, not steady states. This will indeed prove to be the case. A basic assumption of our model is a probabilistic transition between the two states (Markov property M1). Appreciation of the daring of this modeling approach is enhanced if one realizes that the physical processes involved seem deterministic. By suitable application of Newton's laws and the laws of electricity to the various molecules, one should be able to derive a deterministic problem whose solution describes the gross motion of the molecule. (This leaves aside quantum effects.) Indeed models of this kind have been developed and form the basis for extensive numerical simulations that, with the aid of enormous amounts of computer time, are beginning to give information about the dynamics of large molecules.[?] *Probabilistic modeling* Keep in mind that the probabilistic approach is a model. A model need not be "correct": it has to be useful. The classical case of a probabilistic model involves the tossing of a coin and the assumption that there is a certain constant probability that it will land either heads or tails. Here too the problem is deterministic. If one could state exactly how the coin was tossed and could formulate with precision the nature of the material on which it landed, one should be able to calculate all details of the motion including of course whether or not the coin landed heads. One reason that a simple probabilistic

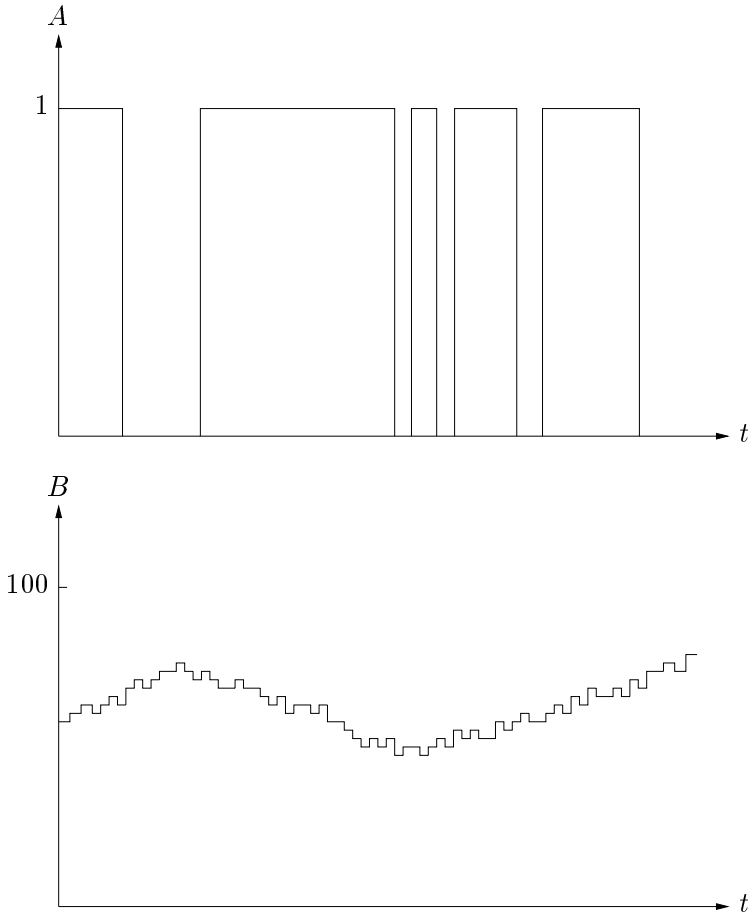


Figure 7.3: Typical graph of the number of molecules in the A configuration when one (top) or 100 (bottom) molecules are being observed. In the bottom graph, and certainly in cases where order of 10^{20} molecules are being monitored (as is frequent), the "jumpy" results can be approximated by a smooth graph, which has derivatives that change smoothly, so that (2.2) can be approximated by (2.3). [fig73lb7]

model is appropriate is that the dynamics are so complicated that solving the relevant equations of an "exact" model is a forbidding task. More importantly, the probabilistic model is appropriate because a slight change in the conditions of the problem is likely to interchange the heads/tails conformations in a coin, or the A/B conformations in a molecule (Keller and Diaconis [21]). Model (2.2) describes probabilistic assumptions for the change in the expected or *average* number of A molecules. "Patch clamp" techniques (for which E. Neher and B. Sakmann received the Nobel prize in 1991) have now made it possible to observe the opening and closing of individual channels. The experimental findings are similar to the top graph of Fig. 7.3. Model (2.2) remains relevant for such situations if A and B are interpreted as the probability that a single channel is respectively open and closed. In contrast with the case where the integrated effect of a large number of channels is ascertained by means of current measurements, single channel recordings yield information not only on mean values of $A(t)$ and $B(t)$ but also on standard deviations and other statistical measures. Direct probabilistic analysis of equations like (2.2), without limiting consideration to approximations like (2.3), can permit exploitation of the more detailed experimental results to obtain a refined picture of channel operations. In particular, from probabilistic models it can be deduced that channel molecules often have more than one type of closed state. See Colquhoun and Hawkes [3]. Here is a way to perform a computer simulation of a stochastic shift between the A and B states. Suppose that at some time t, a molecule is in the A state. Pick a value of k_1 and of δt. By assumption (1.2), during the brief time interval $(t, t+\delta t)$, there is a probability $k_1\delta t$ that there will be a shift to the B state. One can instruct a computer to select a random number between 0 and 1. If that number is between 0 and $k_1\delta t$ then the simulation shifts the configuration to B; otherwise the configuration remains A. By repeating this type of calculation, one can obtain a simulated history of the states, A and B, of a single molecule during some time interval. Repetition of such a simulation yields a history of the stochastic behavior of a number of molecules. One can compare various aspects of this simulated data with experiments. Alternatively, there are analytical methods that will allow conclusions to be drawn if one retains the stochastic character of (1.1). If a simple two state assumption does not fit the data, more states can be postulated. Note from Fig. 7.3 that if only a single channel is under observation then the derivative of $A(t)$ is either zero or infinite, so that passage from (2.2) to (2.3) seems clearly inappropriate. This characterization of the derivative remains true no

matter how many channels are being observed; the number of open channels always changes by integer jumps. Nonetheless, if many channels are being observed, the jumps become less noticeable (Fig. 7.3, bottom graph) and the true jumpy curve can be well approximated by a smooth curve. This smooth curve will have a well-behaved derivative, and it is this curve that we seek when we solve for $A(t)$ in our differential equation formulation of kinetics.

4 A sequence of irreversible transitions: the rate limiting step

Formulation To continue our discussion of kinetics, it is natural to generalize the kinetic scheme of (2.1) to a scheme that contains not two but three different conformations:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \ .$$

Such schemes find numerous application in biology. For example, as we have mentioned, many channel molecules are believed to have several configurations. To give another example the key element in the "calcium-voltage hypothesis" for neurotransmitter release is a voltage sensitive molecule with two inactive conformations (A and B) and one active conformation (C) (Parnas *et al.* [34]). Transitions between conformations are governed by voltage-dependent rate constants. Here we shall consider a special case of the above scheme wherein the transitions are approximated as irreversible:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \ . \tag{4.1}$$

(This situation can, for example, serve as a core model for depolarization-induced release activation in the calcium-voltage hypothesis.) For $A(t)$ and $B(t)$ the appropriate kinetic equations are

$$dA/dt = -k_1 A \ , \qquad A(0) = A_0 \ ; \tag{4.2a,b}$$

$$dB/dt = k_1 A - k_2 B \ , \qquad B(0) = B_0 \ ; \tag{4.3a,b}$$

$$dC/dt = k_2 B \ , \qquad C(0) = C_0 \ . \tag{4.4a,b}$$

Instead of employing (4.4a,b) we can also determine $C(t)$ from the conservation relation

$$C(t) = M - A(t) - B(t) \ , \qquad \text{where} \qquad M \equiv A_0 + B_0 + C_0 \ . \tag{4.4c,d}$$

We shall find exact formulas for the solution to the mathematical problem posed by (4.2)–(4.4). We shall then examine the solution for several parameter ranges in an effort to gain further intuition about chemical kinetics. In so doing we shall be led to the important concept of "rate limiting step" and the corresponding simplification procedure of "lumping". We shall see instances of important concepts, the "quasi-steady state" and the "principle of robustness". *Solution* The solution to (4.2) is

$$A(t) = A_0 \exp(-k_1 t) \ . \tag{4.5}$$

Upon substitution into (4.3a) we obtain

$$dB/dt = k_1 A_0 \exp(-k_1 t) - k_2 B \ . \tag{4.6}$$

Example 2 of Appendix 4 shows that (if $k_2 \neq k_1$) the general solution to (4.6) is

$$B(t) = K \exp(-k_2 t) + \frac{k_1 A_0}{k_2 - k_1} \exp(-k_1 t) \ , \tag{4.7}$$

where K is an arbitrary constant. This constant is determined by the initial condition (4.3b):

$$B_0 = K + \frac{k_1 A_0}{k_2 - k_1} \ , \qquad \text{i.e. } K = B_0 - \frac{k_1 A_0}{k_2 - k_1} \ . \tag{4.8a, b}$$

Equations (4.8b) and (4.7) yield (if $k_1 \neq k_2$)

$$B(t) = B_0 \exp(-k_2 t) - \frac{k_1 A_0}{k_2 - k_1} [\exp(-k_2 t) - \exp(-k_1 t)] \ . \tag{4.9}$$

What behavior does our intuition lead us to expect from (4.1)? The reader is invited to ponder this question before proceeding, and to sketch some graphs that describe the type of solution that he or she expects. If intuition fails, one can proceed to graph the solution [given by (4.5), (4.9) and (4.4c) for $A(t)$, $B(t)$ and $C(t)$ respectively] for several different parameter values. This will provide a background for formulating an appropriate intuitive view of the kinetics described by (4.1). Such a graphing procedure is often required, especially for more complex problems. Indeed, the *molding of intuition via graphs of solutions to selected particular examples*, is an important reason for formulating and solving mathematical models. *Special case*: *fast first*

transition Another aid to intuition is to *consider extreme cases*. For example let us consider the reaction sequence (4.1) under conditions wherein

$$k_1 \gg k_2 \ . \tag{4.10}$$

After some thought many will see what to expect. (Others will need the mathematics to guide them to results that are "obvious" once they have been derived.) A will rapidly transform into B, before B has had a chance to decrease. During this *fast transient* the level of B will approach closely to the value A_0+B_0. Then B will transform into C, relatively slowly, proportionally to $\exp(-k_2 t)$. [More precisely, the "rapid" changes are on the time scale $1/k_1$, which by (4.10) is short compared to the time scale $1/k_2$ for the decay of B.] Our intuition is borne out by inspecting (4.9). Under the conditions (4.10) we may make the approximation

$$B(t) \approx B_0 \exp(-k_2 t) + A_0[\exp(-k_2 t) - \exp(-k_1 t)] \ . \tag{4.11}$$

When $t = 0$ we recover the initial condition $B(0) = B_0$. But since k_1 is relatively large the term $\exp(-k_1 t)$ will rapidly become negligible, yielding the following approximation for the behavior of B *after the initial fast transient*:

$$B(t) \approx (A_0 + B_0) \exp(-k_2 t) \ . \tag{4.12}$$

(We have used the fact that $e^{-k_1 t} \ll 1$ when t is 2 or 3 times $1/k_1$.) See Fig. 7.4. *Fast second transition and the quasi-steady state* Suppose that conditions are opposite to those of (4.10):

$$k_2 \gg k_1 \ . \tag{4.13}$$

What now? Before proceeding, the reader is again invited to try to discern the qualitative behavior without the aid of mathematics. If k_2 is relatively large, then almost as soon as a molecule of B is formed it will be transformed into a molecule of C. We thus expect the kinetics to be dominated by the relatively slow decay of A, which is proportional toexp$(-k_1 t)$. To check our intuition, we again turn to equation (4.9) for $B(t)$. [Remember that according to (4.5), for all parameter ranges the behavior of $A(t)$ is the same, exponential decay.] We see that after a relatively short time, of magnitude $1/k_2$, $\exp(-k_2 t)$ is negligible so that we may approximate (4.9) by

$$B(t) \approx (k_1 A_0/k_2) \exp(-k_1 t) \quad \text{(after the initial fast transient)} \ . \tag{4.14}$$

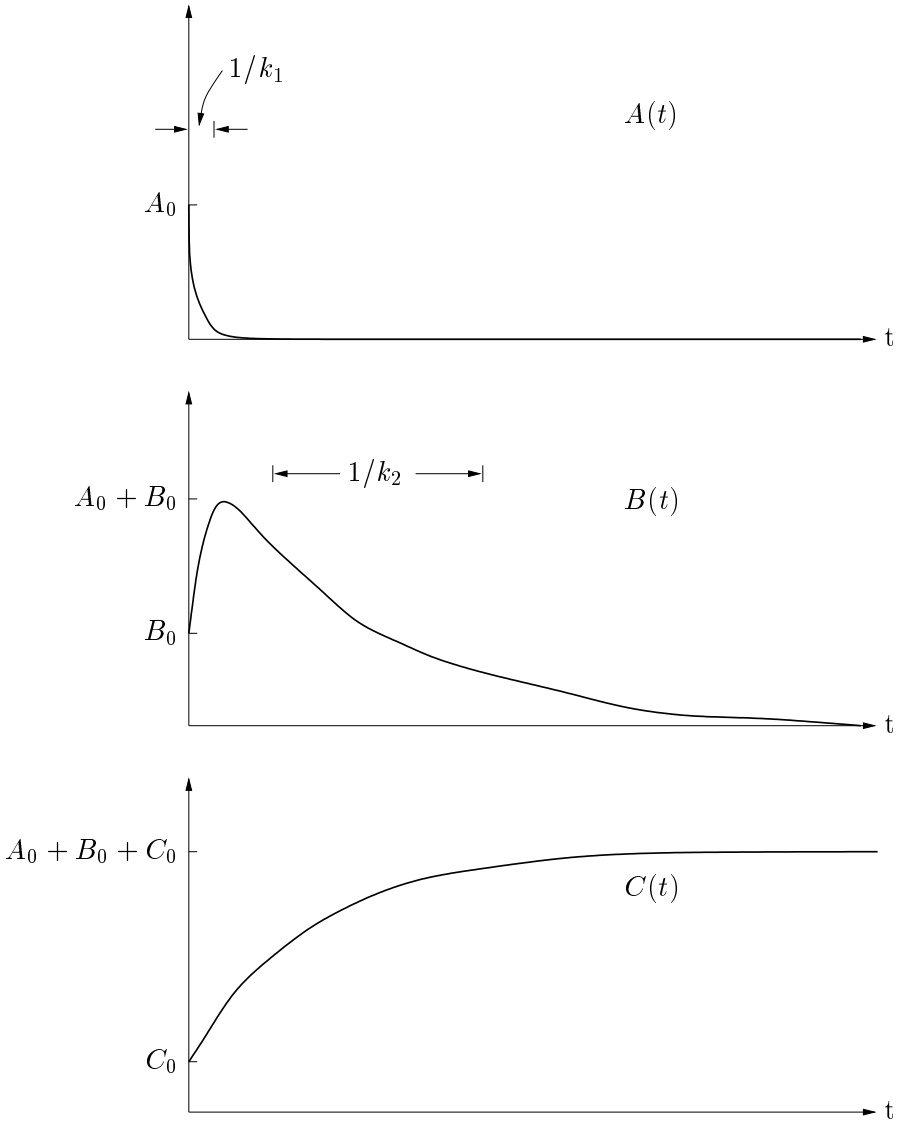


Figure 7.4: Behavior of concentrations A, B, and C according to the kinetic scheme (7.4) when $k_1 \gg k_2$. The widely separated time scales $1/k_1$ and $1/k_2$ are depicted. [fig74lb7]

As we anticipated, for most of the time the decay of B, like the decay of A, is proportional to $\exp(-k_1 t)$. The "initial" concentration of B after the fast transient i.e., after a time of order k_2^{-1}, is given by the value of B in (4.14) when $t \approx 0$:

$$B_{\text{initial}}^{\text{post transient}} \approx k_1 A_0 / k_2 \ . \tag{4.15a}$$

Equation (4.15a) is certainly correct (since it is a deduction from the exact solution) but *why* is it true? To answer this, we observe that B decreases very rapidly during the fast transient. The rapid decrease in B will continue until dB/dt becomes small, but from (4.3a) this will occur when

$$k_2 B \approx k_1 A \ . \tag{4.15b}$$

Since A hardly alters during the initial fast change of B, $A(t) \approx A_0$, and (4.15b) implies (and therefore explains) (4.15a). What turns out to be a very important consequence of our analytic approximation (4.14) for $B(t)$ together with the (exact) expression (4.5) for $A(t)$ is the following relation, which follows directly from (4.14) and (4.5):

$$B(t) \approx (k_1/k_2) A(t) \ , \qquad \text{after the initial transient} \ . \tag{4.16a}$$

We stress that (4.16) *holds for the entire period after the initial transient.* See Fig. 7.5. A key observation is that (4.16) would be obtained from Eq. (4.3a) for dB/dt if we assumed that B was in a steady state in the sense that dB/dt could be set equal to zero. As it is, we say that after a fast transient B is in a **quasi-steady state** with respect to A. A way to rationalize (4.16a) is this. If A were a constant ($A = \overline{A}$) then (as in (3.10), but with k_2 replacing k_{-1}). (4.3) becomes

$$\frac{dB}{dt} = k_1 = A - k_2 B, B(0) = B_0$$

with solution

$$B = (B_0 - \frac{k_1 = A}{k_2}) e^{k_{2t}} + \frac{k_1 = A}{k_2}. \tag{4.16b}$$

Hence, after a transient of time scale $1/k_2$, B would approach a true steady state, which is given exactly by (4.15b) or (4.16a) with $A = \overline{A}$. If A changes only slightly during the time scale of the transient, then (4.16a) should still provide a good approximation to solution behavior after the transient. From (3.4) the time scale for change in A is k_1^{-1}. A will indeed change only slightly during the transient (whose duration is of magnitude k_2^{-1}) when $k_2^{-1} \ll k_1^{-1}$, i.e. when our

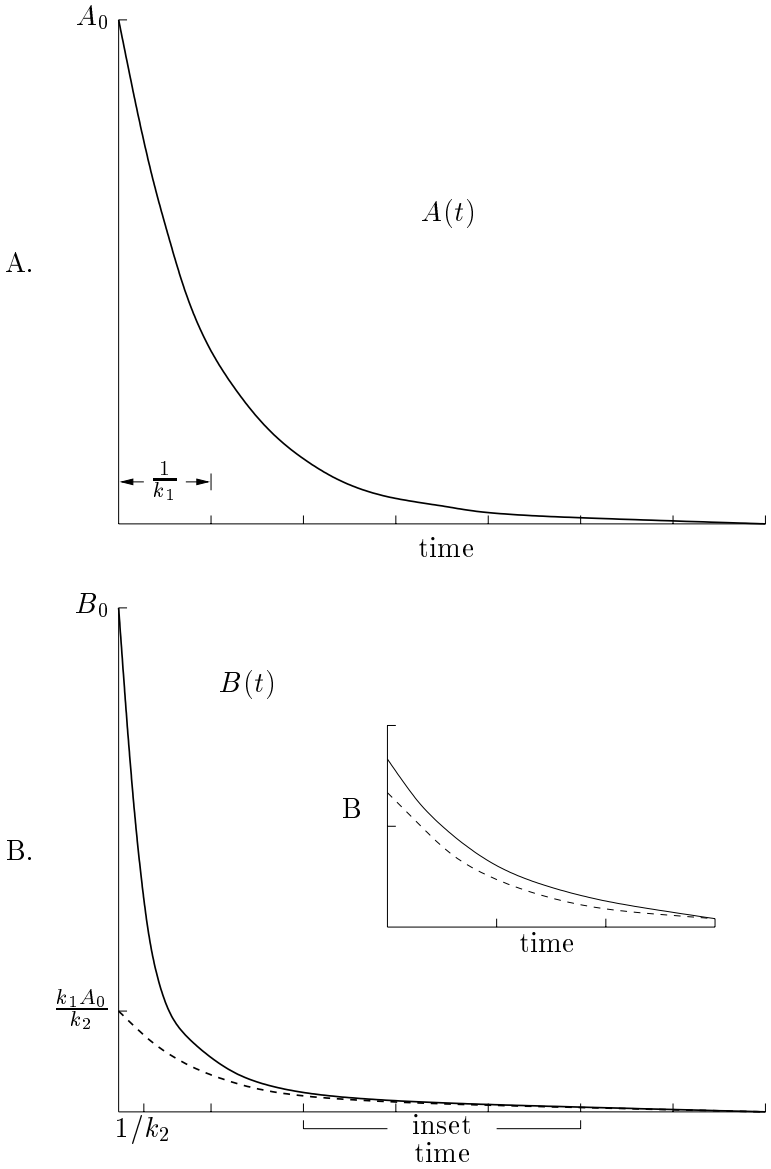


Figure 7.5: Graphs of (2.13) and (2.15) when $k_1 \ll k_2$. In B the dashed line is the quasi-steady state approximation (4.17). This figure is plotted for $k_1 = \frac{1}{3}k_2$. Although the ratio k_2/k_1 is not all that large, still the recommended approximation gives quite good results. The extent of agreement is seen more clearly in the "blown-up" graph displayed as an inset of part B. C. Graphs of $k_2 B/k_1$ when scheme (4.1) holds and $k_2 \gg k_1$. Left: When $A = \overline{A} = constant$. Right: When A varies. [fig75lb7] C. Graphs of $k_2 B/k_1$ when scheme (4.1) holds and $k_2 >> k_1$. Left: When $A = A^b ar = constant$. Right: When A varies.

fundamental assumption (4.13) holds. When $A = \overline{A}$ there is a true steady state wherein B is a constant whose relation to the constant $A = \overline{A}$ is determined by (4.16) with $A = \overline{A}$. When A varies, $B(t)$ is not in a true steady state; rather B is in "quasi-steady-state" whose relation to the relatively slowly varying $A(t)$ is still determined by (4.16). Let us summarize. Suppose that the time scale for variation of A (namely $1/k_1$) is long compared to the time scale $(1/k_2)$ that it would take B to approach a steady state if A were constant. Then, we can regard A as "slowly varying" compared to B. Consequently, after a transient of duration $1/k_2$, B will be in a quasi-steady state with respect to A, as in (4.16). To obtain (4.16), set $dB/dt = 0$ in differential equation (4.3a) for B. See Fig. 7.5.[?] What of C, which we can think of the "product" of the reaction scheme (4.1), starting from the "substrate" A. From Eq. (4.4a) for dC/dt, upon employing (4.16) we find that

$$dC/dt = k_1 A \qquad \text{after the initial transient.} \tag{4.17a}$$

This is an approximate equation for the velocity of the reaction (rate of product formation) in terms of the substrate concentration. Upon substitution of formula (4.5) for A, (4.17a) becomes an approximate differential equation for $C(t)$:

$$dC/dt = k_1 A_0 \exp(-k_1 t) \qquad \text{after the initial transient.} \tag{4.17b}$$

The appropriate "initial condition" for (4.17b) states the concentration of C at the beginning of the period during which (4.17b) is valid. From (4.4 c,d), using our assumption that $k_2 \gg k_1$, we find that to first approximation

$$C(\text{"0"}) = B_0 + C_0 \ . \tag{4.17c}$$

We have used quotation marks around the "initial time" in (4.17c) because this time is not the genuine initial time $t = 0$ but rather a short time (of order k_1^{-1}) therafter. Solution of (4.17b) and (4.17c) gives the anticipated behavior of $C(t)$ [Exercise 2(d)]. The remarkably useful "quasi-steady state approximation" will be considered further in Section 7. *Rate limiting steps* Let us reconsider the two

extreme cases $k_1 \gg k_2$ and $k_2 \gg k_1$ respectively. In the first case we see that the time scale of the $A \rightarrow B \rightarrow C$ transition is the time scale $1/k_2$ of its second step, $B \rightarrow C$, compared to which the time scale $1/k_1$

of the $A \rightarrow B$ transition is negligible. We say that the $B \rightarrow C$ transition is the **rate-limiting step**. The $B \rightarrow C$ transition is the *slowest process; the duration of this process characterizes the overall kinetics*. Similarly, when $k_2 \gg k_1$ we say that the relatively slow $A \rightarrow B$ transition is the rate-limiting step. One cannot blindly apply the generalization "the slowest step is rate limiting". An example is found in the so-called calcium-voltage theory for neurotransmitter release (Parnas *et al.* [35]). According to this theory, the way an electrical signal (depolarization) triggers the release from vesicles of neurotransmitter is by doing two things: elevating intracellular calcium concentration and switching a certain molecule from an inactive state I to an active state A. Reversing the electrical signal (repolarization) is known to terminate release. The action of the electrical signal can be represented schematically as follows where depolarization increases k_1 and k_2 while repolarization increases k_{-1} and k_{-2}.

$$\mathrm{Ca}^{\mathrm{lo}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ca}^{\mathrm{hi}} , \qquad I \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} A .$$

The simultaneous presence of $\mathrm{Ca}^{\mathrm{hi}}$ and A is assumed to be necessary and sufficient for release. Experimental observations show that Ca does not control release kinetics. The theory accounts for these observations by postulating that it is the $I \Leftrightarrow A$ transitions that control release. In the presence of depolarization, k_2 is assumed to be small compared to k_1. Consequently, the Ca concentration rapidly rises, but release does not start until the slower rise in A takes place (the slow step is rate limiting). In the presence of repolarization k_{-2} is assumed to be large compared to k_{-1}. Thus, upon repolarization the concentration of A rapidly drops, terminating release even though the Ca concentration remains high for some time. *The fast step is rate-limiting. The method of exponential peeling* We now briefly consider the question of how to compare with experiment the exact theoretical solution (4.5) and (4.7). In doing so, we shall make use of an important principal: if possible, *transform theoretical predictions so that their graph is a straight line*. (For another application of this principal, see Section 7 below, especially the Lineweaver-Burk plot of Fig. 7.7B.) In the case of (4.5) this is easily done, since

$$\ln A = \ln A_0 - k_1 t . \tag{4.18}$$

Presumably A_0 is known. The unknown parameter k_1 can be estimated from the slope $(-k_1)$ of the anticipated straight-line graph of (4.18). Of course

if the experimental graph is not a straight line, we must construct a better theory. When two exponentials are present, as in (4.7), we proceed similarly but in two stages. Consider the case $k_2 > k_1$. As $\exp(-k_2 t)$ approaches zero faster than $\exp(-k_1 t)$, we expect that for sufficiently large t,

$$\ln B(t) \approx \ln\left(\frac{k_1 A_0}{k_2 - k_1}\right) - k_1 t \ . \tag{4.19}$$

By comparing the predicted straight line of (4.19) with the experimental results, we can estimate $k_1 A_0/(k_2 - k_1)$ and k_1. Since the initial concentrations A_0 and B_0 are presumed known, if the data is sufficiently precise then estimating the slope k_1 and the vertical intercept $\ln\big(k_1 A_0/(k_2 - k_1)\big)$ should permit evaluation of the parameters k_1 and k_2. We can then "peel off" the known portion of $B(t)$, and form the "remainder" $R(t)$:

$$R(t) = B(t) - \frac{k_1 A_0}{k_2 - k_1} \exp(-k_1 t) \ . \tag{4.20a}$$

By (4.7), $R(t) = K \exp(-k_2 t)$. Hence,

$$\ln R(t) = \ln K - k_2 t \ , \text{ where } K = B_0 - \frac{k_1 A_0}{k_2 - k_1} \ , \tag{4.20b}$$

yielding another straight line. In principle, the theoretical results can be tested by seeing whether fitting experimental results for "non-large" t match the slope and vertical intercept of the straight line for $\ln R(t)$ that are predicted with the calculated values of k_1 and k_2. Alternatively, the effects of inevitable experimental error can be diminished by simultaneously taking into account all four observations (two slopes and two vertical intercepts) that relate to the two unknown parameters k_1 and k_2. These parameters can be chosen by the "least squares method" to minimize the sum of the squared deviations between the experiments and the two straight line portions of the logarithmic plot of $B(t)$. If $k_1 > k_2$, the procedure is modified in an obvious way. Furthermore, the procedure is easily extended to an arbitrary number of exponentials. Using logarithms to base 10, which we write "log", is often more convenient than using natural logarithms (ln, "log to the base e). Recall that conversion requires the formula

$$\ln x = (\log x)(\ln e) = 0.434 \log x \ .$$

Suppose we plot the log of some concentration C as a function of time. As the diagram below indicates, exactly the same graph can be interpreted as a graph of C provided that the vertical axis is relabelled. The above approach is called the *method of exponential peeling*. One would anticipate that this method will not work well if k_2 and k_1 are close in value, for then it will be difficult to distinguish between $\exp(k_1 t)$ and $\exp(k_2 t)$. In general, studies show that the method should be used with considerable caution for the results may be very sensitive to small errors in measurement (Lanczos [23]). *The useful fiction of equal transition rates* We have examined with some care the two extreme cases $k_1 \gg k_2$ and $k_1 \ll k_2$. What about the intermediate case $k_1 = k_2$? This is interesting from a mathematical point of view, because our solution formula (4.9) is not defined in this case. [The denominator in the second term of (4.9) "blows up".] To examine the situation more carefully, let us imagine that k_2 and k_1 are close. We write

$$k_2 = k_1 + \varepsilon \tag{4.21}$$

where

$$|\varepsilon| \ll 1 \ . \tag{4.22}$$

If we employ (4.21) to substitute for k_2 in (4.9) we obtain

$$B(t) = B_0 \exp[-(k_1 + \varepsilon)t] - \frac{k_1 A_0}{\varepsilon} \exp(-k_1 t)[\exp(-\varepsilon t) - 1] \ . \tag{4.23}$$

As long as

$$|\varepsilon t| \ll 1 \tag{4.24}$$

we may approximate $\exp(-\varepsilon t)$ by means of a Taylor approximation (Appendix 2):

$$\exp(-\varepsilon t) \approx 1 - \varepsilon t \ . \tag{4.25}$$

With this

$$\frac{1}{\varepsilon}[\exp(-\varepsilon t) - 1] \approx t \ . \tag{4.26}$$

Thus, in the limit as $\varepsilon \to 0$ we may replace (4.23) by

$$B(t) = B_0 \exp(-k_1 t) + k_1 A_0 t \exp(-k_1 t) \ , \qquad \text{when} \quad k_2 = k_1 \ . \tag{4.27}$$

Remarks. (i) The solution (4.9) was definitely not valid when $k_1 = k_2$, yet we obtained formula (4.27) by taking the limit $k_1 \to k_2$ in (4.9). This is

permitted, for the definition of the limit $k_1 \to k_2$ in no way involves the situation when $k_1 = k_2$. (Consult any calculus book.) (ii) Suppose that in spite of what has been said in (i) the reader does not believe that the limiting process is legitimate. No matter. *Any* method, however shaky, may be used to "guess" the solution to a differential equation. Once a guess is obtained, it can be checked by direct substitution. The reader will indeed find that (4.27) is a solution of the differential equation (4.6) and the initial condition (4.3b). (Exercise 3.) (iii) Note the behavior of the solution (4.27) when $t \ll k_1^{-1}$. For this range of t the exponential factors hardly vary, so that $B(t)$ increases linearly. Later B decays slightly slower than exponentially. This is an opportune moment to revisit what may be termed the **principle of robustness** in theoretical work (also see section ?). This principle asserts that if theoretical results are to be trustworthy, they must retain their qualitative and semi-quantitative nature when the underlying model is slightly altered. The principle of robustness would thus lead one to view results with grave suspicion if they depend upon some parameter having *exactly* a certain value. The proportionality to t in (4.27) is just such a result, for this proportionality requires that the kinetic constant k_2 is exactly equal to the kinetic constant k_1. Otherwise the solution is the weighted sum of two exponentials, as in (4.9). No biochemical measurements will ever be able to distinguish between the alternatives that k_1 is exactly equal to k_2, when (4.27) is appropriate, or rather is very nearly equal to k_2, when (4.9) is appropriate. In spite of what has been said in the previous paragraph, the solution (4.27) has more than mere academic value. To see this, turn back to approximation (4.25), which was the key step in the derivation of (4.27). This approximation is valid when $|\varepsilon t| \ll 1$, as has been noted in (4.24). In other words approximation (4.25) is a good one as long as

$$0 \leq t < |\varepsilon|^{-1} \ . \tag{4.28}$$

If the difference between k_1 and k_2 is very small, the interval (4.28) may be very long. Indeed, if ε is small enough then (4.25) may cease to be a good approximation only when $B(t)$ has almost vanished and therefore is (usually) no longer of interest. In such a case we could say that (4.27) is a good approximation "for all time". The time scale for the disappearance of $B(t)$ is $1/k_1$. Thus (4.25) can be regarded as a good approximation "for all time" when

$$|\varepsilon|^{-1} \gg \frac{1}{k_1} \qquad \text{i.e. [by (4.21)]} \quad \frac{k_1}{|k_2 - k_1|} \gg 1 \ . \tag{4.29}$$

Until now we have not considered the other approximation that converts (4.23) into (4.27), namely

$$\exp[-(k_1+\varepsilon)t] \approx \exp[-k_1 t] \ . \tag{4.30}$$

Approximation (4.30) will hold when $|\varepsilon| \ll k_1$ — but this is precisely condition (4.29), so that no new condition is required. **Conclusion.** The two rate constants of (4.1) can be regarded as "close" if

$$|k_2 - k_1| \ll k_1 \quad \text{or equivalently if} \quad |k_2 - k_1| \ll k_2 \ . \tag{4.31a,b}$$

If the rate constants are "close" then a good approximation "for all time" is obtained by assuming that the rate constants are equal, i.e. by employing (4.27). We stress our persistent efforts to approximate and simplify the analytic formulae. These efforts led us to an increased understanding of the nature of the kinetic processes under consideration. "After the fact" many of the conclusions might seem fairly obvious, but it is doubtful whether they could have been arrived at — and/or held with confidence in their validity — without preliminary analytic work.

Exercises

1. Consider the kinetic scheme

$$A \ \xrightarrow{k_1} \ B \ \xrightarrow{k_2} \ C \ \xrightarrow{k_3} \ D \ . \tag{4.32}$$

(a) Write down the differential equations corresponding to (4.32). Instead of the differential equation for $D(t)$, write down the conservation law.
(b) The equations for A and B are the same as (4.2a) and (4.2b), so that the solutions are given by (4.5), (4.7) and (4.8). Find the solution for $C(t)$.
[Hint: For the particular solution try

$$\alpha \exp(-k_1 t) + \beta \exp(-k_2 t) \ ,$$

where α and β are constants that you should determine. Also use the fact that "usually" if for some constants C_i

$$C_1 f_1(t) + C_2 f_2(t) + \cdots + C_N f_N(t) = 0 \quad \text{for all } t \tag{4.33}$$

then $C_1 = C_2 = \cdots = C_N = 0$. But see Exercise 11.]
(c) Write a brief essay discussing how the idea of "rate-limiting step" applies when $k_1 \gg k_3$, $k_2 \gg k_3$.

2. (a) According to (4.7), if $t \ll k_1^{-1}$ and $t \ll k_2^{-1}$, then $B(t) \approx B_0$. But according to (4.27), if $t \ll k_1^{-1}$ then $B(t)$ increases linearly with t. Explain why this difference is "to be expected".
(b) Sketch graphs of $A(t)$, $B(t)$ and $C(t)$ when (4.10) holds, clearly representing the qualitative features of the solution.
(c) When $t = 0$, (4.12) yields $B = A_0 + B_0$. But this is at variance with the initial condition (2.5b). Resolve the contradiction.
(d) Solve (4.17b) and (4.17c). Discuss the behavior of the solution.

3. Verify by direct substitution that (4.27) is a solution of (4.6) and (4.3b) when $k_2 = k_1$.

4. (a) Calculate the next term in (4.25).
(b) It is a reasonable guess [and usually, but not always, a correct guess — see Chapter 6 of Lin and Segel [27] that an approximation is valid if the first term neglected is small compared to the term or terms retained. Use this criterion to justify (4.26).

5. Show that (4.31a) implies (4.31b), and conversely.

6. Show that the relative error of the approximation (4.30),

$$\frac{\exp[-(k_1 + \varepsilon)t] - \exp[-k_1 t]}{\exp[-k_1 t]} \ ,$$

is small as long as $\varepsilon t \ll 1$. Comment on this result in connection with the conclusion of Section 4.

7. (a) Construct the counterpart of Fig. 7.4 for $A(t)$ and $B(t)$ when (4.13) holds for the cases (i) $A_0 > k_2 B_0/k_1$, (ii) $A_0 < k_2 B_0/B_1$. In case (i), but not in case (ii), B initially *increases* even though the $B \rightarrow C$ transition is very rapid. Explain why such behavior is to be expected for sufficiently large values of A_0.
(b) Obtain approximate expressions for $C(t)$ in two ways. (i) Employ the conservation law, using the approximations found for $A(t)$ and $B(t)$. (ii) Solve (4.17b) with the approximate initial condition (4.17c). Use

the (presumably identical) results of (i) and (ii) to sketch a graph of $C(t)$.
(c) Show that if $B_0 \gg k_1 A_0/k_2$ then the time it takes for (4.14) to be valid is approximately $k_2^{-1} \ln(k_2 B_0/k_1 A_0)$. Discuss how this result modifies the sentence that includes (4.14).

8. Graph the qualitative behavior of $A(t)$, $B(t)$ and $C(t)$ when (4.32) and (4.21) hold, for $0 < \epsilon \ll 1$. The graphs should indicate, schematically, behavior on both the time scales $1/k_1$ and $1/\varepsilon$.

9. Show that (4.27) is a correct first approximation to the exact solution (4.9). [Hint: To demonstrate the correctness of (4.27) one must first rewrite (4.9) using (4.21).]

10. (This exercise pursues a mathematical matter that arose in Exercise 1.) Show that there are non-zero values of C_1, C_2, and C_3 such that

$$C_1 f_1(t) + C_2 f_2(t) + C_3 f_3(t) = 0 \quad \text{for all } t \tag{4.34}$$

if

$$f_1(t) = \sin^2 t \ , \qquad f_2(t) = \cos^2 t \ , \qquad f_3(t) \equiv 5 \ .$$

To understand why this example is "special", consult any calculus book for its treatment of the "Wronskian".

11. (Requires knowledge of dimensionless variables.) "Adaptation" is a common phenomenon in biology. At first a biological entity responds to a fixed signal, but later the response becomes negligible. For example, at first people sense a new smell but later they don't notice it. This problem concerns a very simple model of adaptation that appears in a review by Othmer and Schaap [32]).
The model assumes that response is proportional to the concentration of a chemical U, and that U is inactivated by an enzyme V (working in the saturated range, so that the rate of U inactivation is proportional to V and does not depend on U). It is further assumed that the concentrations of U and V are effectively zero in the absence of signal. Suppose that at time $t = 0$ a signal of magnitude S is turned on and kept on, and that the synthesis of U and V occurs at a rate proportional to S, with the constant of proportionality taken as unity

in both cases, for simplicity. The following equations are postulated:

$$\frac{dU}{dt} = S - aU - bV \ , \quad \frac{dV}{dt} = S - bV \ ; \qquad U(0) = 0 \ , \quad V(0) = 0 \ . \tag{4.35a-d}$$

In (4.35), S, a and b are positive constants. (In part (f) below there is discussion of the "coincidence" that the same letter b appears in (4.35a) and (4.35b).)
Let the following dimensionless variables be introduced:

$$u = \frac{U}{S/a} \ , \qquad v = \frac{V}{S/b} \ , \qquad \tau = \frac{t}{b^{-1}} \ . \tag{4.36}$$

(a) Show that the equations become

$$\frac{du}{d\tau} = \alpha(1 - u - v) \ , \quad \frac{dv}{d\tau} = 1 - v \ ; \quad u(0) = 0, \quad v(0) = 0 \ ; \quad \alpha \equiv \frac{a}{b} \ . \tag{4.37a-e}$$

(b) Verify that α is dimensionless.
(c) Find the solution to (4.37b) and the initial condition $v(0) = 0$.
(d) Show that when the solution of (4.37b) is substituted into (4.37a) then the resulting equation can be solved, giving

$$u = \frac{\alpha}{\alpha - 1}(e^{-\tau} - e^{-\alpha\tau}) \ , \qquad \alpha \neq 1 \ . \tag{4.38}$$

(e) Sketch the graph of the solution for $\alpha \gg 1$. This should **not** be done by substituting a few values of τ, but rather by using properties of the exponential functions.
(f) Describe in one or two sentences how the solution for u (and hence U) represents response and adaptation. In particular, give order of magnitude estimates for the *dimensional* response time and adaptation time.
[Note: To give exact adaptation, the simple model we presented requires that the coefficient b of V in (4.35a) be exactly the same as the coefficient of V in (4.35b), i.e. that the number of U molecules destroyed by one V molecule per unit time is exactly equal to the fraction of V molecules per unit time that disappear (because of the finite half life of V). This "fine tuning" is a property of several other adaptation models that have been proposed. A way to avoid fine tuning and thus to obtain "robust" exact adaptation is the subject of a paper by Barkai

and Leibler [1].]
(g) Using a computer, experiment with changing the coefficient b in (4.35b) to c. How "inexact" is the adaptation? Does the answer depend on other parameters? Can you handle the modified problem analytically?

5 Application of nondimensionalization and scaling

Using one of the kinetics problems that we have considered, this section begins with an example of how employing dimensionless variables can advantageously transform a mathematical model. (Appendix 7 provides a different example, in a partially overlapping treatment of nondimensionalization.) It is seen that dimensionless variables can be selected in a variety of ways all of which posess this same advantage. The principle goal of this section is to introduce to the more mathematically inclined the concept of scaled dimensionless variables, a particular way of selecting such variables. Application of this somewhat subtle concept is very useful in obtaining approximate solutions to a large variety of problems that arise in mathematical models in biology and other areas. *Example of the various ways of choosing dimensionless variables* We begin by illustrating the fact that *introduction of dimensionless variables reduces the number of parameters that characterize a problem, and that the same parameter reduction is attained regardless of how the nondimensional variables are defined.* Consider for example (2.12a):

$$dA/dt = -(k_1 + k_{-1})A + k_{-1}M \ .$$

Both rate constants k_1 and k_{-1} have dimensions of 1/time, for they are defined as the average number of $A \rightarrow B$ and $B \rightarrow A$ transitions per unit time. (We can confirm this statement concerning k_1 and k_{-1} by noting that as they must, both dA/dt and $(k_1 + k_{-1})A$ have the same dimensions, concentration/time.) We can choose the reciprocal of either k_1 or k_{-1} as our time scale, or we might choose $(k_1 k_{-1})^{-1/2}$. For definiteness let us define a dimensionless time τ by

$$\tau = \frac{t}{1/k_{-1}} \ , \qquad \text{i.e. } \tau = k_{-1}t \ . \tag{5.1a}$$

The natural way to nondimensionalize A is via its initial concentration. Thus we define a dimensionless concentration

$$a \equiv \frac{A}{A_0} \ . \tag{5.1b}$$

Employing chain rule, we note that upon adopting (5.1a) and (5.1b) we obtain

$$\frac{dA}{dt} = \frac{d(A_0 a)}{dt} = A_0 \frac{da}{dt} = A_0 \frac{da}{d\tau}\frac{d\tau}{dt} = k_{-1} A_0 \frac{da}{d\tau} \ . \tag{5.2a}$$

The same result is obtained more easily by a formal substitution process:

$$\frac{dA}{dt} = \frac{d(A_0 a)}{d(\tau/k_{-1})} = k_{-1} A_0 \frac{da}{d\tau} \ . \tag{5.2b}$$

It is approach (5.2b) that we recommend for future use. Employing (5.2), we find that problem (2.12) for $A(t)$ is transformed into

$$\frac{da}{d\tau} = -\frac{1}{\varepsilon} a + \theta - a \ , \qquad a(0) = 1 \ . \tag{5.3}$$

In (5.3) we have employed the dimensionless parameters

$$\varepsilon \equiv \frac{k_{-1}}{k_1} \ , \qquad \theta \equiv \frac{M}{A_0} \equiv \frac{A_0 + B_0}{A_0} \ . \tag{5.4}$$

Another possibility is to define the dimensionless time by

$$T = \frac{t}{1/k_1} \ , \tag{5.5}$$

in which case (2.12) becomes

$$\frac{da}{dT} = -a + \varepsilon(\theta - a), \qquad a(0) = 1 \ . \tag{5.6}$$

Still another possibility is to employ the concentration T as the concentration unit. If we continue to employ (5.5) to define the dimensionless time, instead of (5.6) we obtain

$$\frac{d\tilde{a}}{dT} = -\tilde{a} + \varepsilon(1 - \tilde{a}) \ , \qquad \tilde{a}(0) = \theta^{-1} \ , \qquad \text{where } \tilde{a} = A/T \ . \tag{5.7}$$

All of the different dimensionless versions of (2.12), namely (5.3), (5.6), and (5.7), contain the two dimensionless parameters ε and θ. This is to be contrasted with the four dimensional parameters of the original problem, A_0, M, k_1 and k_{-1}. We have illustrated the fact that typically there are many ways to choose dimensionless variables, and that all ways lead to the same decrease in the number of parameters that determine the solution of a mathematical model. Moreoever, from the present example and that of Appendix 7 the reader can quickly become proficient at introducing dimensionless variables, which is a straightfoward technical matter. *Scaled dimensionless variables* We now illustrate some of the subtleties that affect advantageous choice of dimensionless variables. When approximate solutions are being sought, it is wise to choice scales for dependent variables (such as concentrations) that estimate their magnitude. Independent variables such as the time should be scaled by "time scales" of the type that we have already discussed. The process of choosing such special dimensionless variables is called **scaling**. In contrast with "standard" nondimensionalization, scaling is a subtle matter. The reader should thus regard the presentation here as a first introduction to an "art" whose mastery can take years of experience. In the *process of scaling* one attempts to *select dimensionless variables so that each term in the dimensional equations transforms into the product of a dimensionless factor, which estimates the approximate size of the term, and the dimensionless term itself, which has an approximate magnitude of unity.* For example, consider the term k_2B in (4.3a). Since B will remain close to its initial magnitude at least for a certain period of time, a suitable scaled dimensionless variable is defined by $b = B/B_0$, with which the term k_2B becomes k_2B_0b. Indeed k_2B_0 gives the magnitude of the term and b is of magnitude unity. After some time, B may change appreciably from its initial value B_0 and our scaling is no longer appropriate. This illustrates an important point concerning scaling, that *different scales may be required in different ranges of the time* [or other independent variables(s)]. In general, *a dependent variable is scaled by a parameter or combination of parameters that provide a typical value of the variable during the time interval of interest.* Let us now consider the question of how to scale the independent variable, choosing as an example the term dB/dt in (4.3). Our task is to select a constant $\widehat{T}$ to define a dimensionless time variable

$$\tau = t/\widehat{T} \ . \tag{5.8}$$

If a typical value $\widehat{B}$ is the scale for B, so that the dimensionless concentration b is defined by

$$b = B/\widehat{B} \tag{5.9}$$

then

$$\frac{dB}{dt} = \frac{\widehat{B}}{\widehat{T}}\frac{db}{d\tau} \ . \tag{5.10}$$

According to the above characterization of the process of scaling, we should choose $\widehat{T}$ so that $\widehat{B}/\widehat{T}$ provides an estimate of the magnitude of dB/dt. If this is successfully done then, as is seen in (5.10), the magnitude of $db/d\tau$ will be unity. We have advocated that $\widehat{T}$ be chosen so that

$$\frac{\widehat{B}}{\widehat{T}} = \left(\frac{dB}{dt}\right)_{\text{typical}} \ . \tag{5.11a}$$

If this is done then

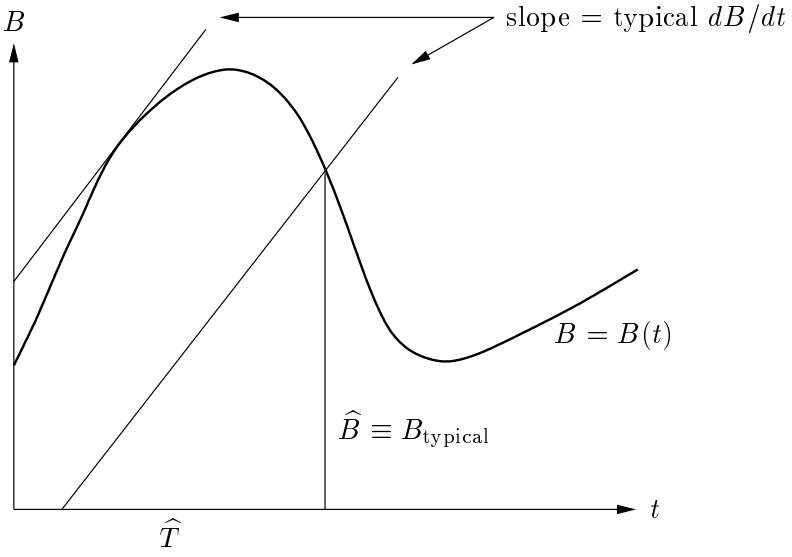
$$\widehat{T} = \frac{B_{\text{typical}}}{(dB/dt)_{\text{typical}}} \ . \tag{5.11b}$$

Figure 7.6A shows that if $\widehat{T}$ is selected according to (5.11) then in a time interval of duration $\widehat{T}$, starting at $t = 0$, the function B undergoes appreciable change. Thus $\widehat{T}$ estimates the time scale for the change in B. Note that according to (5.11b) $\widehat{T}$ is the time it takes for B to increase from zero to B_{typical}, given that B changes at the rate $(dB/dt)_{\text{typical}}$. There are no hard and fast rules for choosing scales. A variant of the procedure suggested above is to choose $\widehat{B}$ as an estimate of the maximum of $|B(t)|$ (in the time interval under consideration) and to choose $\widehat{T}$ so that

$$\frac{\widehat{B}}{\widehat{T}} = \left|\frac{dB}{dt}\right|_{\max} , \quad \text{i.e. } \widehat{T} = \frac{|B|_{\max}}{|dB/dt|_{\max}} \ . \tag{5.12a,b}$$

Depending on whether (5.11) or (5.12) is employed, the dimensionless derivative $db/d\tau$ should be of magnitude unity, or of magnitude less than or equal to unity. As shown in Fig. 7.6B, (5.12) like (5.11) implies that $B(t)$ undergoes significant change in an interval of duration $\widehat{T}$. Observe that $\widehat{T}$ has a simple interpretation according to (5.12b), namely the time it would take to reduce B from its maximum value to zero, when B decreases at a maximal rate. (If $B_{\min}$, the minimum value of B, is comparable to $B_{\max}$ then accurate scaling may require replacing the numerator of (5.12b) by $B_{\max} - B_{\min}$.) Thus,

A.



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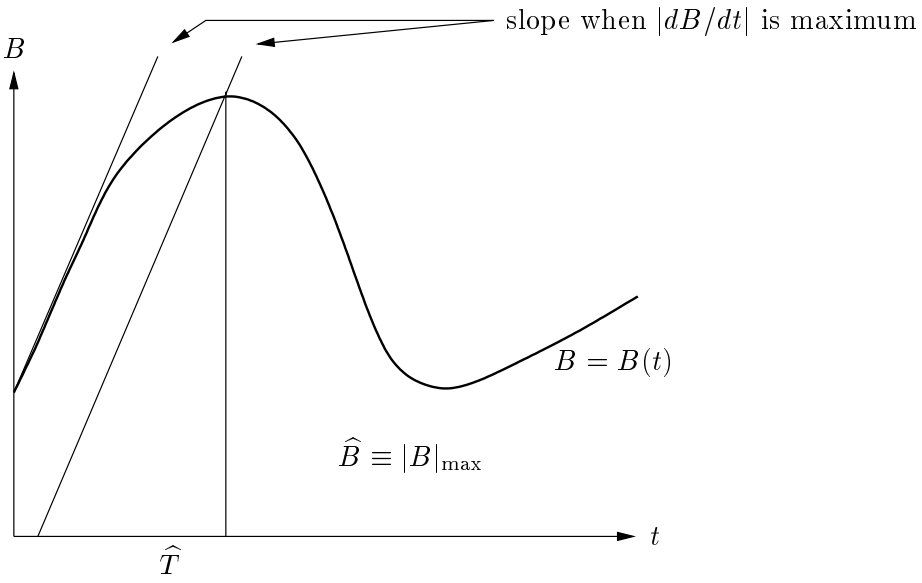


Figure 7.6: Estimating the time scale $\widehat{T}$ for a function $B = B(t)$ according A. to (5.11), and B. to (5.12). [fig76lb7]

given the definition of time scale [under (2.19)] it follows from both (5.11) and (5.12) that *when scaling a function of time, time should be nondimensionalized with an estimate of the time scale.* (In space-dependent problems there are **length scales** that play a role that is exactly analogous to the role of the time scales that we have been considering here.) Let us illustrate the process of scaling on the equations (4.2) when assumption (4.13) is made. We shall choose scales for the period after the fast transient. During this period, A_0 is a good estimate for the magnitude of A, but B has already dropped markedly below its initial value B_0 to the value $B_{\text{initial}}^{\text{post transient}}$ of (4.15). Thus appropriate scaled dimensionless concentrations are

$$a = \frac{A}{A_0} \ , \qquad b = \frac{B}{k_1 A_0 / k_2} \ . \tag{5.13}$$

As we have seen, after the fast transient the solutions decay with a time scale of k_1^{-1}. Thus the appropriate dimensionless time is

$$T = \frac{t}{k_1^{-1}} \qquad \text{i.e. } T = k_1 t \ . \tag{5.14}$$

With (5.13) and (5.14), on being scaled for the post transient region the governing equations (4.2) and (4.3) become

$$\frac{da}{dT} = -a \ , \qquad \varepsilon \frac{db}{dT} = a - b \qquad \text{where } \varepsilon \equiv \frac{k_1}{k_2} \ . \tag{5.15a,b,c}$$

Our basic assumption is that $k_1 \ll k_2$, i.e. that

$$\varepsilon \ll 1 \ . \tag{5.16}$$

Given (5.16), one would immediately think of neglecting the term $\varepsilon db/dT$ in (5.15), yielding

$$b \approx a \ . \tag{5.17}$$

But (5.17) is precisely the quasi-steady state result (4.16), written in terms of our new scaled dimensionless variables. Had we not chosen these variables in a special way, we would justifiably have been worried about neglecting the term $\varepsilon db/dT$. True, ε is very small compared to unity, but db/dT could be large. And a and b could be small compared to unity. However, our choice of scaled variables assures us that a, b and db/dT are in fact neither small nor large but are of magnitude unity. During the fast transient, we must

change our scaling. Now B must be scaled with its initial value B_0 while the time must be scaled with respect to k_2^{-1}, the time scale for the fast transient. Employing Greek letters for our variables in the fast transient layer, we thus introduce

$$\alpha = \frac{A}{A_0} \ , \quad \beta = \frac{B}{B_0}, \qquad \tau = \frac{t}{k_2^{-1}} \ , \tag{5.18}$$

in terms of which the governing equations (4.2) become

$$\frac{d\alpha}{d\tau} = -\varepsilon\alpha \ , \quad \alpha(0) = 1 \ ; \quad \frac{d\beta}{d\tau} = \varepsilon\rho\alpha - \beta \ , \quad \beta(0) = 1 \ . \quad \rho \equiv \frac{A_0}{B_0} \ . \tag{5.19}$$

If we neglect the terms proportional to ε we obtain

$$\alpha \equiv 1 \ , \qquad \beta = e^{-\tau} \ . \tag{5.20a,b}$$

[Note that the neglect of the term $\varepsilon\rho\alpha$ would not be justified, even if $\varepsilon \ll 1$, if $\rho \gg 1$. Indeed, our scaling has automatically indicated an important point. If $\rho \gg 1$, i.e. if $A_0 \gg B_0$, then the term $k_1 A$ in (4.3) is not negligible compared to $k_2 B$, even if $k_2 \gg k_1$.] Approximation (5.20) in the transient layer must smoothly match with the approximation after the transient. This matching is beyond our scope here [see for example Lin and Segel [27], Sections 9.2 and 10.2]. Nonetheless the spirit of matching can be indicated by considering equation (5.15a). What initial condition should be employed for this equation? We need to know the value of a just after the transient, for the equations (5.15) have been derived for the post-transient period. But (5.20a) states that $\alpha \approx 1$ throughout the transient — i.e.

$$A \approx A_0 \ , \qquad \text{so } a \approx 1 \ .$$

Hence the initial condition for (5.15a) is $a = 1$; the appropriate solution is

$$a = e^{-T} \ . \tag{5.21}$$

For this simple example the exact solution is known. One can therefore check that (5.21) and (5.17) give the correct first approximations in the transient region, while (5.20) gives the corresponding approximations after the transient (Exercise 9). By assuming series expansion such as

$$a(T, \varepsilon) = a_0(T) + \varepsilon a_1(T) + \varepsilon^2 a_2(T) + \cdots \tag{5.22}$$

one can improve on the first approximations. Such matters are treated in fuller discussions of scaling, for example that of Lin and Segel [27], Section 6.3.

Exercises

1. (a) Derive (5.6) from (2.12a) both by chain rule and by the quicker method of formally substituting for the dimensional variables in terms of the dimensionless variables defined by (5.2) and (5.5).
   (b) Similarly, derive (5.7).

2. [For the more mathematically experienced reader.]
   (a) Write $\alpha(\tau) = \alpha_0(\tau) + \epsilon\alpha_1(\tau) + \cdots$, $\beta(\tau) = \beta_0(\tau) + \epsilon\beta_1(\tau) + \cdots$ and determine α_0, α_1, β_0 and β_1 from (5.19).
   [Answer: $\alpha_0 = 1$, $\alpha_1 = -\epsilon t$, $\beta_0 = e^{-\tau}$, $\beta_1 = \epsilon p(1 - e^{-\tau})$.]
   (b) Write $a(T) = a_0(T) + \epsilon a_1(T) + \cdots$. Find a_0 and a_1, determining the unknown constants of integration by requiring that for small T, a matches α as closely as possible [$a_0 = e^{-T}$, $a_1 = 0$].
   (c) Using (b) and (5.15b) find a two-term approximation to b, [$b_0 = e^{-T}$, $b_1 = \epsilon e^{-T}$].
   (d) Extend your results to calculate the complete power series in ϵ for all variables. Once again check by expanding (4.5) and (4.9).
   (e) Check your answers to (a), (b) and (c) by comparing with the exact solution of (4.5) and (4.9).

6 Enzyme-substrate-complex and the quasi-steady state approximation

Formulation The reaction scheme symbolized by the **block diagram** of Fig. 7.7 is central to the study of biochemistry. The corresponding kinetic scheme is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P \ . \tag{6.1}$$

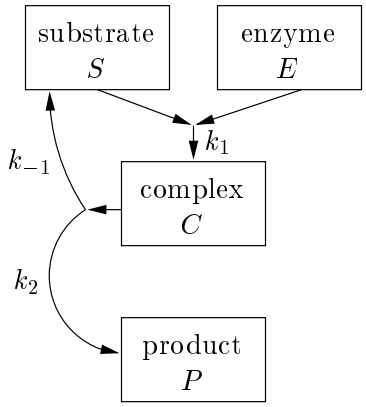


Figure 7.7: A block diagram for an enzyme-substrate-complex reaction. [fig78lb7]

Here E is the concentration of an **enzyme** that catalyzes the transformation of a molecule called the **substrate** of the reaction (denoted by S) into a different molecule called the **product** (concentration P). This is accomplished by means of an intermediate enzyme-substrate **complex** (concentration C) wherein enzyme and complex are bound together. Two remarks on notation.

(i) Frequently the concentration of unbound or *free enzyme* E is denoted by $[E]$ and the complex concentration, of *bound enzime*, by $[ES]$. The precision of this notation is often useful (see ? below), but use of a single letter such as C instead of $[ES]$ makes it easier to write out the somewhat lengthy expressions that are often required in theoretical manipulations. (ii) The notation $k_{\rm cat}$ is often used instead of k_2 ("cat" stands for "catalysis"). For reasons that we will explain at

once, according to the theory of chemical kinetics the differential equations corresponding to the scheme (6.1) are

$$dE/dt = -k_1ES + k_{-1}C + k_2C \ , \quad dS/dt = -k_1ES + k_{-1}C \ , \qquad (6.2a,b)$$

$$dC/dt = k_1ES - k_{-1}C - k_2C \ , \quad dP/dt = k_2C \ . \qquad (6.2c,d)$$

The initial conditions are usually taken to describe a situation where a given amount of enzyme and substrate begin interacting at $t = 0$, at which time neither complex nor product are present:

$$E(0) = E_0 \ , \qquad S(0) = S_0 \ , \qquad C(0) = 0 \ , \qquad P(0) = 0 \ . \quad (6.3a,b,c,d)$$

One can think of preparing a solution of enzyme at concentration E_0, and instantly elevating substrate concentration to S_0 at time $t = 0$. The terms proportional to k_{-1} and k_2 in (6.2) can be regarded as describing changes in the conformation of a molecule (C) and therefore these terms are analogous to expressions derived in Section 7.2. Here the new "conformation" of C is not another connected molecule, as in Section 7.2, but rather two disjoint submolecules. New assumptions are required to obtain the terms k_1ES and $-k_1ES$ in (6.2). Indeed, the **bimolecular reaction** $E + S \xrightarrow{k_1} C$ is postulated by the **law of mass action** to occur at a rate that is proportional to ES. To obtain this "law" we assume that the reacting molecules are far enough apart, i.e. their concentration is low enough, so that each molecule can be regarded as moving independently of all the others. If this is the case, then doubling or tripling (say) either the free enzyme concentration E or the substrate concentration S should double or triple the probability of a collision between E and S. The coefficient k_1 quantitates the probability that a given collision is "successful", i.e. that it results in the union of E and S to form an $E - S$ complex. The meaning of the parameter k_1 can be clarified by considering, for example, the term k_1ES in Eq. (6.2c) for dC/dt. Consider a one micromolar ($\mu M = 10^{-6}M$) solution of enzyme and suppose that the substrate concentration S is fixed at $10\mu M$. Let $k_1 = 10^7$ $\mathrm{M}^{-1}\mathrm{s}^{-1}$ which is the magnitude of k_1 for complex formation between the substrate acetylcholine and the enzyme acetylcholine esterase (Land *et al.* [24]). Then the rate of complex formation is $100\mu M$ per second. Of course, when complex and product begin to form then S does not remain fixed. It is preferable to say that if substrate concentration is initially $10\mu M$ then $1\mu M$ of enzyme will produce complex

at an initial rate of $100\mu M$ per second. Alternatively, at the given initial concentrations of E and S, it takes an individual substrate molecule about 0.1 sec to bind to an enzyme molecule. Formally, our quantitative interpretation of k_1 can be obtained as follows

$$k_1 ES = 10^7\ \mathrm{M^{-1}s^{-1}}(10^{-6}\ \mathrm{M})(10^{-5}\ \mathrm{M}) = 10^{-4}\ \mathrm{Ms^{-1}} = 0.1\ mM s^{-1} = 100\ \mu M s^{-1}\ .$$

From the governing differential equations (6.2) and initial conditions (6.3) it is readily deduced [Exercise (1a)] that the total amount of enzyme, whether in the **free** form E or the **bound** (to substrate) form C, remains constant:

$$E(t) + C(t) = E_0\ . \tag{6.4}$$

In other words, Eq. (6.4) expresses the fact that the total amount of enzyme is conserved, according to the assumed scheme (6.1). With the substitution $E = E_0 - C$, (6.2b) and (6.2c) become two equations in two unknowns:

$$\begin{aligned} dS/dt &= -k_1(E_0 - C)S + k_{-1}C\ , \\ dC/dt &= k_1(E_0 - C)S - (k_{-1} + k_2)C\ . \end{aligned} \tag{6.5a, b}$$

Once (6.5a) and (6.5b) are solved [subject to initial conditions (6.3b) and (6.3c)] then E can be found from (6.4), and P from (6.2d) or from the substrate-product conservation equation [Exercise (1a)]

$$S(t) + C(t) + P(t) = S_0\ . \tag{6.5c}$$

The quasi-steady state approximation (*QSSA*) No formulas that give solutions to the pair of differential equations (6.5a) and (6.5b) are known. But in an extensive class of "steady state" situations, the equations can be simplified and important formulas can thereby be derived. All biochemistry texts discuss the important consequences of assuming a "steady state" for the complex C. Often, no justification is given for this assumption (Fersht [7], Stryer [47]). Other sources may mention that the assumption is justified under conditions, which are readily arranged in the laboratory, wherein the experiment begins with a *large excess of substrate*, i.e. S_0 is large. One can indeed argue that if there is a great deal of substrate then $S \approx S_0$ for a considerable period of time. During this time it seems reasonable to consider the approximate equation obtained by setting $S = S_0$ in (6.5b). This gives

$$\frac{dC}{dt} = k_1 E_0 S_0 - (k_1 S_0 + k_{-1} + k_2)C\ . \tag{6.5d}$$

The initial condition is $C(0) = 0$. By Exercise (1b) the solution is

$$C = \overline{C}[1 - e^{-\lambda t}] \tag{6.5e}$$

where

$$\overline{C} \equiv \frac{E_0 S_0}{K_m + S_0} \ , \qquad \lambda \equiv k_1(S_0 + K_m) \ , \qquad K_m \equiv \frac{k_{-1} + k_2}{k_1} \ . \tag{6.5f,g,h}$$

Thus C approaches a **steady state** $\overline{C}$ for large time. By definition, a dependent variable (such as C) is in a steady state when that variable does not change with time. (Here, indeed, (6.5f) is the solution of (6.5d) when $dC/dt = 0$). *At steady state there is a balance between processes that produce* C [$E + S \rightarrow C$ at rate $k_1 E S_0 = k_1(E_0 - C)S$] *and processes that "destroy"* C [$C \rightarrow E + S$, $C \rightarrow P$], at rates $-k_{-1}C$ and $k_2 C$ respectively. Before C passes from its initial value $C = 0$ to a value close to its steady state $\overline{C}$, there is a **transient period** where C varies. As can be seen from (6.5e) this period has duration of magnitude λ^{-1}. During this transient period, C increases from its initial value of 0 and approaches $\overline{C}$. C increases because the production terms dominate the destruction terms. The reason for this dominance is as follows. C is initially zero, and C will remain small for a while. When C is small, production is relatively large, since almost all the enzyme is free; by contrast destruction is minimal, since there is little C that can break apart into enzyme and either substrate or product. The decrease in the amount of free enzyme E and the concomitant increase of C will lead to a balance between production and destruction. This perfect balance is expressed by $dC/dt = 0$, indeed the condition for a steady state. However large S is, eventually the irreversible conversion of complex to product will cause S to diminish significantly below its initial value S_0. Thus the approximation $C \sim \overline{C}$, which is valid after the transient (which is also called the **induction period**), will cease to be accurate. But since S is changing slowly, the post-transient balance between production and destruction of C should continue to hold, to a good approximation. That is, we expect that as S slowly decreases, a good approximation will be obtained if dC/dt is taken to be zero. If dC/dt is set equal to zero in (6.5b), but without fixing S at its initial value S_0, there results a generalization of (6.5f):

$$C = \frac{E_0 S}{K_m + S} \ . \tag{6.6}$$

As the reader should check, the above approximation (6.6) is obtained by setting $dC/dt = 0$ in (6.2c). Note however that C is not in a true steady state, for dC/dt is only "approximately zero". In fact C slowly decreases in parallel with the decrease in S. Thus theorists prefer to speak of the **quasi-steady state approximation** (**QSSA**) (or sometimes the "pseudo-steady state approximation"). We have already discussed a simple example of the QSSA in Section 4. Let us now examine the consequences concerning S and P of the quasi-steady state result (6.6) for C. Upon substituting (6.6a) into (6.2b) and (6.2d), we obtain

$$\frac{dS}{dt} = -\frac{k_2 E_0 S}{K_m + S} \ , \tag{6.7a}$$

and

$$\frac{dP}{dt} = \frac{k_2 E_0 S}{K_m + S} \ . \tag{6.7b}$$

To solve (6.7a) for S we require an initial condition. Since the initial amount of substrate is very large, it is reasonable to assume that very little substrate disappears during the initial transient induction period. Thus we can assume as an *approximate initial condition*

$$S(0) = S_0 \ . \tag{6.8}$$

The "initial time" in (6.8) is *approximated* by $t = 0$ but in fact the initial conditions are relevant at a time just after the fast transient, for only then does (6.7a) become a suitable approximation. If the transient is brief compared to the time that S changes significantly according to (6.7a) then indeed (6.8) can be said to give conditions at a time that is "almost" at the beginning of the period during which substrate is transformed into product. We repeat for emphasis that operationally the QSSA consists of two approximations. (i) After the fast transient induction period one can set $dC/dt \approx 0$, which yields the differential equation (6.7a) for $S(t)$. (ii) One can employ (6.8) to describe the "initial condition" of S, just after the induction period. *Important inferences from the QSSA* It is customary to define the **velocity**

of a reaction, denoted by V, as the rate of product formation:

$$V \equiv \frac{dP}{dt} \ . \tag{6.9a}$$

With this, and the definition

$$V_{\max} \equiv k_2 E_0 \tag{6.9b}$$

(6.7b) becomes

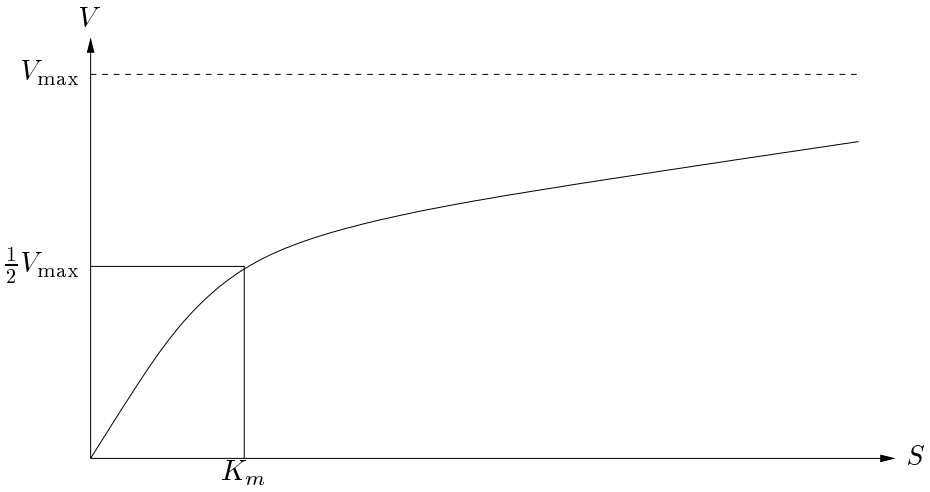
$$V = \frac{V_{\max} S}{K_m + S} \ . \tag{6.10a}$$

As depicted in Fig. 7.8A, $V_{\max}$ is the maximum possible reaction velocity, which is approached when the substrate concentration is very large: more precisely, we see from (6.10) that $V \approx V_{\max}$ when $S \gg K_m$. K_m, the **Michaelis constant** defined in (6.5h), provides the substrate concentration at which the reaction proceeds at half its maximum rate. There are two regimes of behavior that follow from (6.10). In the **linear regime**, when $S \ll K_m$, V is proportional to S : $V \approx (V_{\max}/K_m)S$. In the **saturated regime**, when $S \gg K_m$, the value of V is approximately constant, independent of the amount of substrate ($V \approx V_{\max}$) Simplifying assumptions of linearity or saturation for the conversion to product of a given substrate are often made as part of mathematical models of complex biochemical processes. Comparison of (6.10a) with experiment can provide estimates of $V_{\max}$ and K_m. In (4.18) we gave an example of manipulating a formula so that its graph is a straight line, thereby rendering easier the comparison of theory and experiment. Another example is obtained by writing (6.10a) in the form

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \left(\frac{1}{S} \right) + \frac{1}{V_{\max}} \ . \tag{6.10b}$$

This **Lineweaver-Burk plot** of $1/V$ as a function of $1/S$ is a straight line (Fig. 7.8B) which enables ready estimation of the slope $K_m/V_{\max}$ and intercept $1/V_{\max}$, and hence of $V_{\max}$ and K_m. Especially interesting are situations where measurements do not conform to the expected straight line of the Lineweaver-Burk plot (6.10b). Then a more elaborate theory must be sought. See Section 9. In principle, the Lineweaver-Burk plot (6.10b) can be compared with experiment by measuring the amount of product and the amount of substrate at several different times in a single experiment. From a practical point of view, it is often easier to take data right after the start of several different experiments at different initial substrate levels, in each of which the substrate concentration is assumed to be the various known values of S_0 at the start of the experiment. But "right after the start" of an experiment

A.



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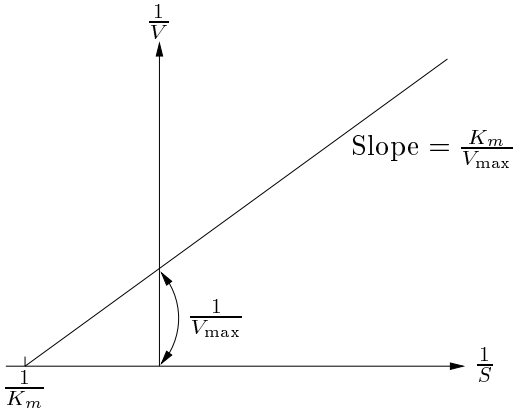

Figure 7.8: A. Graph of Eq. (6.10a), the velocity of the reaction (6.1) as a function of the substrate concentration, according to the QSSA. The graph illustrates the biochemical interpretations of the Michaelis constant K_m and the maximal velocity, $V_{\max}$. B. Plotting the same data in the form (6.10b), so that the graph is a straight line (Lineweaver-Burk plot). [fig79lb7]

must be after the induction period, otherwise the QSSA is not valid. Could it be that during the induction period so much substrate has turned into product that S_0 is not the "initial concentration" as is usually assumed in experiments? This question will be dealt with shortly. In this connection, aside from practicality there is another reason why data to compare with the Lineweaver-Burk plot are taken at the start of several experiments. Once product has accumulated, the neglect in (6.1) of the back reaction between E and P becomes problematical. Of course, it could be that the back reaction rate k_{-2} is so small that this is not a problem. (The term corresponding to the back-reaction, $k_{-2}EP$, is zero initially, when $P = 0$, and is expected to remain small for some time after the start of the reaction.) It is worth noting that, unless the back reaction rate is very large the conditions that we will now derive for the validity of the QSSA remain valid when the back reaction is taken into account (Segel [39]). *Statement of conditions for the validity of the QSSA* With the background provided here, especially with the concept of "time scale", we can reach a clearer understanding of the QSSA. We will shortly demonstrate the following. (i) The time scale $t_{_C}$ for the duration of the transient period before the QSSA is valid can be estimated in terms of the parameters of the governing equations (6.2) and (6.3) as

$$t_{_C} = \frac{1}{k_1(S_0 + K_m)} \ . \tag{6.11a}$$

(ii) The time scale $t_{_S}$ for substrate change in the period during which the QSSA is expected to describe the formation of product is estimated by

$$t_{_S} = \frac{K_m + S_0}{k_2 E_0} \ . \tag{6.11b}$$

(iii) A necessary condition for the validity of the QSSA equations (6.6), (6.7) and (6.10) is that there is a **fast transient**, i.e. that $t_{_C} \ll t_{_S}$. Employing the estimates (6.11a,b) for t_C and t_S, one finds that this condition takes the form

$$\frac{k_2 E_0}{k_1(S_0 + K_m)^2} \ll 1 \ . \tag{6.11c}$$

(iv) The approximate initial condition (6.8) is valid providing

$$\epsilon \ll 1 \qquad \text{where } \epsilon \equiv \frac{E_0}{S_0 + K_m} \ . \tag{6.11d}$$

It turns out that $\varepsilon \ll 1$ implies (6.11c). Consequently the condition $\varepsilon \ll 1$ is expected to be sufficient to assure the validity of both the equations and the initial conditions of the QSSA. A rough quantitative guess for the interpretation of $\epsilon \ll 1$ might be "take ϵ to be at least an order of magnitude smaller than unity", i.e. $\epsilon < 0.1$. *Simulations of the QSSA* Before turning to the tech-

nical problem of demonstrating claims (i)–(iv), we will consider the QSSA further in light of these claims. First of all, let us examine some computer simulations that have been carried out to check these claims. Such simulations (computer-generated approximate solutions of the relevant differential equations) provide an excellent way to test our reasoning. The results of a typical computer "experiment" are shown in Fig. 7.9. Plotted in this figure are the ratio of substrate concentration S to its initial concentration S_0, the ratio of product concentration P to S_0 (which is the final product concentration, since all substrate is transformed into product by the irreversible process (6.1)), and the ratio of complex concentration C to the complex concentration $\overline{C}$ that is defined in (6.5f). (We expect $\overline{C}$ to be an approximation to the maximal complex concentration, which should occur at the end of the fast transient.) It is commonly believed that for the QSSA to be valid that the initial substrate concentration S_0 must be much larger than the initial enzyme concentration E_0. In Fig. 7.9, these two concentrations are taken to be equal. However $\epsilon = 0.1$. Even though S_0 is not large compared to E_0, nonetheless — as predicted here — the QSSA is valid after a fast transient induction period. It is seen that the time scale for the induction period is indeed $t_{_C}$. [See Exercise 3(c).] Moreover, Fig. 7.9 confirms that the purported time scale $t_{_S}$ is indeed appropriate for major post-transient changes in the dependent variables, for the substrate concentration has changed significantly when $T \equiv t/t_{_S} \approx 1$, i.e. when $t \approx t_{_S}$. Note in Fig 7.9 the fast transient during which the substrate concentration S decreases below its initial value. During this same transient, the complex concentration C increases from zero to a value close to the value predicted by the QSSA. It is convenient also to graph the course of S and C during the reaction by plotting the point (C, S) as time varies. Accordingly, the quasi-steady state equation (6.7a) is plotted as a dashed line in Fig. 7.10. It is seen that when ϵ is small then indeed equation (6.7a) is an excellent approximation, after a fast transient. (As the figure shows, the transition from $S/S_0 = 1$ to the heavy QSSA curve is rapid. The arrows on the figure show that the time scales $t_{_C}$ and $t_{_S}$ play their predicted roles.) Case (iii) of Fig. 7.10 verifies that if (6.11c) holds but (6.11d)

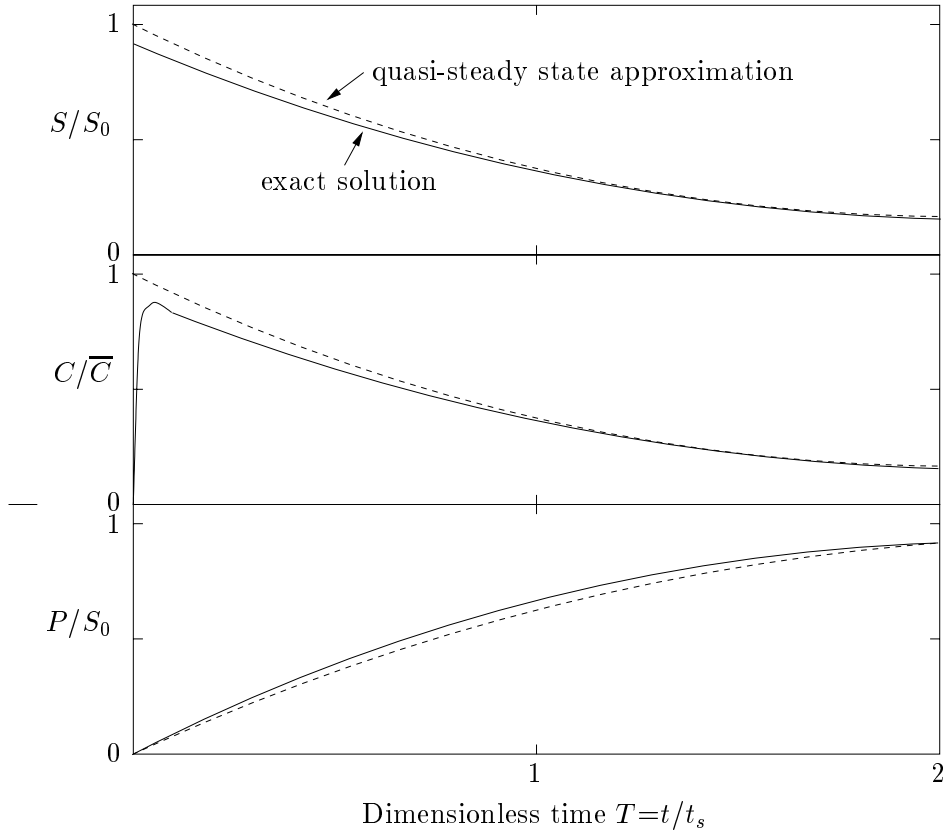

Figure 7.9: Dimensionless numerical solutions of the unapproximated equations (6.5a,b) and (6.2d) [solid lines] and of the quasi-steady state approximations (6.7a), (6.6) and (6.7b) [dashed lines]. $\overline{C}$ and t_s, the scales for complex concentration and time ($T = t/t_s$), are defined in (6.5f) and (6.11b). $E_0 = S_0$ but $\epsilon = 0.1$, where $\epsilon \equiv E_0/(S_0 + K_m)$. [Examination of (6.25) shows that the dimensionless solutions depicted in the figure depend only on the dimensionless parameters $\epsilon, \kappa \equiv k_{-1}/k_2$ and $\sigma \equiv S_0/K_m$. Here $\kappa = 10$, $\sigma = 1/9$.] Redrawn from Segel [39]. [fig710lb7]

does not, then there is still a fast transient, after which (6.7a) is an excellent approximation. However S decreases appreciably during the transient, so that (6.8) is not a suitable initial condition. (The appropriate initial condition can be calculated. See Exercise 5.) *Verification of conditions for validity of QSSA* We now begin our demonstration of conditions (i)–(iv) above. As a prerequisite to our discussion, let us first estimate the duration of the transient induction period after which the QSSA is expected to be valid. We denote this "transient time scale" by $t_{_C}$. To estimate $t_{_C}$, the time for rapid increase of complex concentration, we observe that during the brief transient we do not anticipate a marked decrease in substrate concentration. Since we seek only a rough estimate of $t_{_C}$, during the transient we can approximate S by its initial value S_0 even if S_0 were to decrease rather substantially, say to half of its initial value. Hence, during the transient (6.5b) can be replaced by the approximate equation (6.5d) that we have already considered. We see from (6.5e) that C approaches a steady state $\overline{C}$ with a time scale $t_{_C} = \lambda^{-1}$. Formula (6.11a) of Condition (i) follows from definition (6.5g) of λ:

$$t_{_C} = \frac{1}{k_1(S_0 + K_m)} \ . \tag{6.11}$$

It will prove useful to generalize our argument slightly. Until now we have thought of the transient as due to a sudden increase in substrate concentration from a value $S = 0$ (with the corresponding complex concentration $C = 0$) to the value $S = S_0$. Consider a more general situation in which the substrate concentration has been held for a long time at some fixed value $S = S_f$. Then the complex will be at a steady state value C_f. Suitably altering (6.5f), we find [Exercise 1(b)] that

$$C_f = \frac{E_0 S_f}{K_m + S_f} \ . \tag{6.12}$$

If at time $t = 0$ the substrate concentration is now suddenly switched to a fixed value $S = S_0$, then the complex concentration obeys (6.5d) but with the initial condition

$$C(0) = C_f \ . \tag{6.13}$$

The solution is [Exercise 1(b)]

$$C = \overline{C} + (C_f - \overline{C})e^{-\lambda t} \ . \tag{6.14}$$

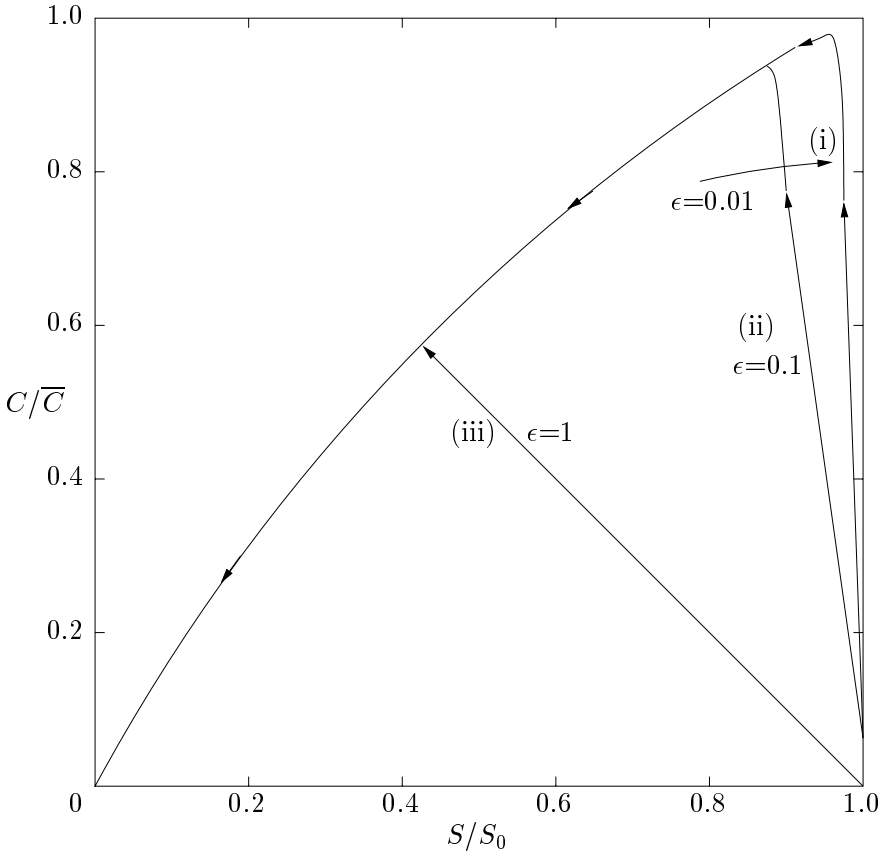

Figure 7.10: Numerical solutions for dimensionless complex and substrate concentrations in the $C-S$ plane (phase plane) for three values of ϵ, of the unapproximated equations (6.5a,b). Starting from the initial state $S = S_0$, $C = 0$ (lower right corner) the point (S, C) moves rapidly upwards and somewhat leftwards until it turns sharply left and thereafter follows the dimensionless version of curve (6.7a) of the QSSA. The times $t = t_{_C}$, $t = 3t_{_C}$, $t = t_{_S}$, and $t = 3t_{_S}$ are successively indicated on each curve by an arrow head. [See Exercise 3(c) for the simple calculations required to translate these times to the dimensionless time $T = t/t_{_S}$ that was used here and in Fig. 7.8.] Parameters: $\sigma = 1$; $\kappa = 10$ in (i) and (ii), $\kappa = 100$ in (iii). [fig711lb7]

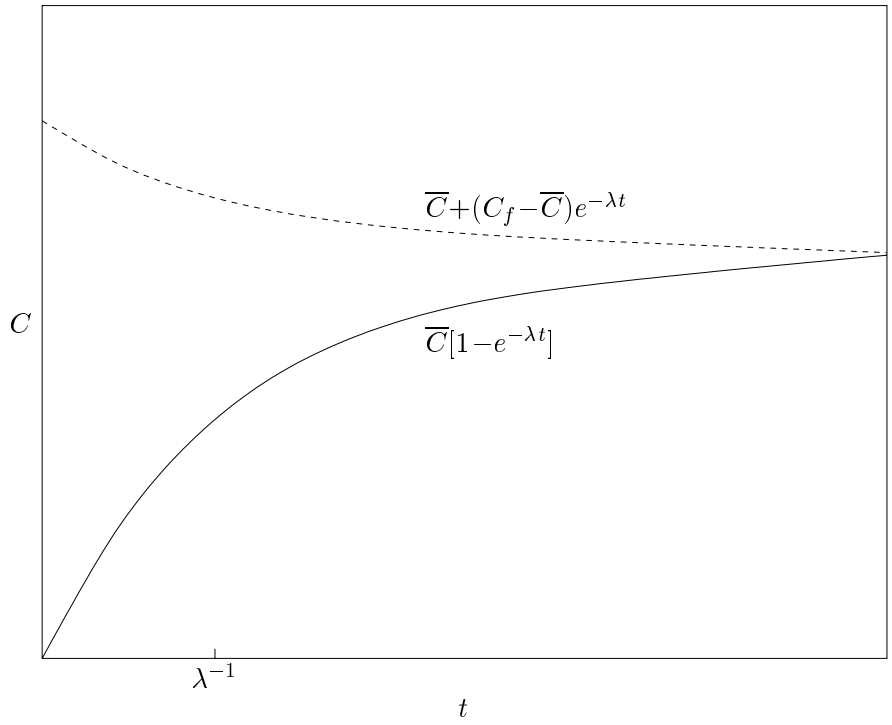

Figure 7.11: Solid line: graph of the formula (6.5e), the time course of the complex when the substrate concentration S is instantaneously switched from $S = 0$ to $S = S_0$. Dashed line: graph of formula (6.14), the counterpart of (6.5e) when S is switched from $S = S_f$ to S_0. The time scale $t_{_C} = \lambda^{-1}$ is seen to be independent of the initial substrate values. Graphs show the particular case $S_0 = K_m$, $S_f = 2S_0$. [fig712lb7]

It follows that $t_{_C} \equiv \lambda^{-1}$ is not only the time scale for the initial transient after which the QSSA is expected to be valid, as in (6.11a) but $t_{_C}$ is also, as in (6.14), the time scale for the complex C to reach a steady state after *any* (instantaneous) change in the substrate concentration to a new fixed value. See Fig. 7.11. Given the more general characterization of $t_{_C}$, one would expect that the complex C will remain close to a steady state with respect to changing values of the substrate S provided that $t_{_C}$ is small compared to the time scale $t_{_S}$ for a significant change in S. For if $t_{_C} \ll t_{_S}$, C can "keep up" with the changes in S. Under these conditions it is legitimate to replace the true steady state equation (6.5f), for fixed S_0, by (6.6) the approximate counterpart of this equation for slowly varying S. This yields the quasi-steady

state of C with respect to the slowly varying concentration of S. Many find the requirement $t_{_C} \ll t_{_S}$ for the validity of the QSSA not easy to accept at first. Let us therefore approach this condition in a slightly different way. Recall our discussion of *why* C approaches a steady state in equation (6.5d) for the complex C when the substrate S is taken to be a constant S_0 – because production and destruction come into balance. Now let the substrate decrease according to the original kinetic scheme (6.1). This decrease provides an additional slowing of the production term k_1ES, so that the time scale $t_{_C}$ for attainment of steady state when S is fixed will tend to be a mis-estimate. There are two reasons for the error. One reason is direct: the true production rate k_1ES is over-estimated by k_1ES_0. The second reason is indirect: since complex production is over-estimated the complex concentration is over-estimated and consequently the complex destruction rate is over-estimated. It is conceivable that both the true growth of the complex destruction and the true decrease in complex formation are so slow that it takes a very long time before any semblance of a steady-state is approached. But if S changes only slightly during the time $t_{_C}$ then indeed the essence of the matter is expected to be the same as when S is fixed; there should be a steady state for C, appropriate not to S_0 but to the present value S of the substrate concentration: (6.5f) can be replaced by (6.6). When will S change only slightly during the time $t_{_C}$? When the time scale $t_{_S}$ for a significant change in S is long compared to $t_{_C}$. Our next task, then, is to estimate $t_{_S}$. For this we employ the following characterization of a time scale (which was also introduced in Libuk7, Section 5):

$$t_{_S} \equiv \text{ time scale for significant change in } S \approx \frac{\text{magnitude of a significant change in } S}{\text{typical magnitude of } dS/dt} \,. \tag{6.15a}$$

(Here is an analogy to (6.15a). The time scale for journey of 132 kilometers with a speed that varies from zero to 70 kilometers is 100 kilometers/(50 kilometers per hour) = 2 hours. With good luck, on a fast road, the journey could take not much more than an hour; with bad luck, maybe three hours. But "a couple of hours" is the right order of magnitude.) It is the long term decay of S whose time scale is given by $t_{_S}$. We are therefore concerned with events after the transient, so that the QSSA equation (6.7a) provides the appropriate expression for dS/dt in (6.15a). For the value of S in (6.7a) we substitute a typical magnitude of S, namely S_0. It might be thought more appropriate to employ $S_0/2$, the average of the initial and final values of S, but such numerical factors are inappropriate for order of magnitude estimates. (1 mM and 0.5 mM are both of magnitude "millimolar".) Similarly

the magnitude of a significant change in S is also taken to be S_0. Hence (6.15a) yields

$$t_{_S} \approx \frac{S_0}{k_2 E_0 S_0/(K_m + S_0)} = \frac{K_m + S_0}{k_2 E_0} \ . \qquad (6.15b)$$

We can now express our necessary condition for the validity of aspect (i) of the quasi-steady state assumption: that the duration of the fast transient, $t_{_C}$, is small compared to $t_{_S}$. From (6.11) and (6.15b), we see that the condition $t_{_C} \ll t_{_S}$ is

$$\frac{k_2 E_0}{k_1 (S_0 + K_m)^2} \ll 1 \ . \qquad (6.15c)$$

Part (ii) of the QSSA requires, as was assumed in (6.8), that the change in the substrate concentration during the fast transient is small compared to the initial substrate concentration S_0. We denote this change by ΔS. We can find an estimate of ΔS by multiplying the maximal rate of decrease of S by the approximate duration $t_{_C}$ of the transient. The maximal value of $|dS/dt|$ will occur at the very beginning of the reaction. From (6.2b) with the initial conditions $C = 0$ and $S = S_0$, we see that this maximum is $k_1 E S_0$. Thus, from (6.11)

$$\left|\frac{\Delta S}{S_0}\right| \approx \frac{1}{S_0}\left|\frac{dS}{dt}\right|_{\max} \cdot t_{_C} = \frac{k_1 E_0 S_0}{S_0} \cdot \frac{1}{k_1 (S_0 + K_m)} = \frac{E_0}{S_0 + K_m} \ . \qquad (6.16)$$

Consequently a second necessary condition for the validity of the quasi-steady state assumption is

$$\epsilon \ll 1 \qquad \text{where } \epsilon \equiv \frac{E_0}{S_0 + K_m} \ . \qquad (6.17a, b)$$

It is readily shown [Exercise 1(c)] that

$$\frac{k_2 E_0}{k_1 (S_0 + K_m)^2} < \frac{E_0}{S-) + K_m}$$

i.e. that $\epsilon \equiv E_0/(S_0 + K_m)$ exceeds the left side of (6.15c). Accordingly, if (6.17a) holds then certainly (6.15c) holds. Thus (6.17) emerges as *the* necessary condition for the validity of the QSSA. To summarize our results, suppose that relatively little substrate disappears during the fast transient, which is assured by (6.17). Then the time scale of complex adjustment to substrate changes is much shorter than the time scale of these changes (complex adjustment is fast compared to substrate change, i.e. $t_{_C} \ll t_{_S}$, which

is assured by (6.15c)) and the QSSA should be fully valid. Our analysis predicts that if parameters are such that (6.17) is false but (6.15c) is true then substrate concentration will diminish noticeably during the fast transient induction period. Nonetheless, although the standard QSSA initial condition (6.8) is not a valid approximation in this case, the assumed validity of (6.15c) implies that the fundamental QSSA differential equations (6.6) and (6.7) should hold after the transient. *Further comments on the QSSA*

When condition (6.17) holds we see from (6.16) that after the transient the complex concentration is just a small fraction of the substrate concentration. This certainly guarantees a relatively small decrease of substrate concentration during the transient. Indeed "undetectable complex" is a rule of thumb sometimes used by kineticists to signal the appropriateness of the QSSA. For references, see Turányi *et al.* [48], p. 172. At this point the reader is recommended to reread the discussion in Section 4 where the first example of a QSSA was presented. This involved the slow irreversible change of a "substrate" A into a "product" C via a slow irreversible transition to an intermediate state B, together with a fast irreversible transition to C:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \ ; \qquad k_1 \ll k_2 \ .$$

There is much in common between the two uses of the QSSA. And in the example of Section 4, all approximations are transparently correct, for they were derived from an exact solution to the problem. Note that in the example of Section 4, when A is fixed then the time scale of B is $1/k_2$, which is fast compared to the time scale $1/k_1$ for the change of A. But after the initial fast transient (during which A can indeed be well approximated by a constant) then the time scale for the change of B is $1/k_1$ — since the quasi-state variable B tracks changes in the slower variable A during the period of validity of the QSSA. The analogy is tight with the enzyme-substrate example. There the quasi-steady state variable C has a time scale $t_{_C}$ when S is fixed, but then varies with a time scale $t_{_S}$ when, during the QSSA, C tracks S. Two more things worth noting are these. The various time scales depend on the parameter values. For different sets of parameters, behaviors can be entirely different (for example when $k_1 \gg k_2$ in the example of Section 4). Note also that a given variable has different scalings for different values of the dependent variables. This latter fact is the heart of singular perturbation approaches to

the solution of mathematical problems [27]. The condition validating the QSSA formula (6.7a) is

$$t_C \ll t_S \quad \text{where} \quad t_S = S_0/(dS/dt)_{\text{qssa: max}} \ , \quad \text{i.e. } t_C \ll S_0/(dS/dt)_{\text{qssa: max}} \ . \tag{6.17a, b}$$

Estimate (6.17b) was provided earlier in (6.15a). We have written the lengthy subscript "qssa:max" to indicate the fact that in the denominator of (6.17b) we need the maximal value of dS/dt during the period after the fast transient. The second part of the qssa is the assumption that little substrate disappears during the transient. As in (6.16) the justification for this step requires

$$t_C \ll S_0/(dS/dt)_{\max} \ . \tag{6.17c}$$

In the denominator of (6.17c) we need the "genuine" maximum of dS/dt. This maximum is expected at $t = 0$ (when $t = 0$ substrate concentration is maximal since none has been bound in complex nor turned into product). Thus

$$\left(\frac{dS}{dt}\right)_{\max} > \left(\frac{dS}{dt}\right)_{\text{qssa: max}} \tag{6.17d}$$

and if (6.17c) holds then certainly (6.17a) holds. That is, if condition (6.17c) for negligible substrate loss during the fast transient holds then the classical QSSA formulas are valid. This provides a general justification for the kineticists "rule of thumb", mentioned above, that undetectable complex justifies a QSSA. (Keep in mind that if (6.17c) does not hold but if (6.17a) does hold then the QSSA formula for the reaction velocity is valid after the fast transient even though considerable substrate disappears during the transient.) **Worked example.** Construct an analogy between the quasi-steady state approximation and the possibility that a helicopter H can retain a fixed position with respect to an airplane S despite its evasive movements. **Solution.** Let the fixed position of H be "on the tail" of S, say one kilometer behind and two kilometers above. If such a position can be maintained, then H is in a quasi-steady state. For in following S, H can be continually exercising all sorts of complex maneuvers, so that H certainly isn't in a true steady state. But it is in a steady state with respect to the changing position of S. Let time t_S be the time scale for significant changes in the position of S. Let t_C be the time scale for H to achieve similar changes in its own position. If $t_C \ll t_S$ then, after a time of magnitude t_C, H should be able to reach and retain its desired position with respect to S. See Fig. 7.12. The condition

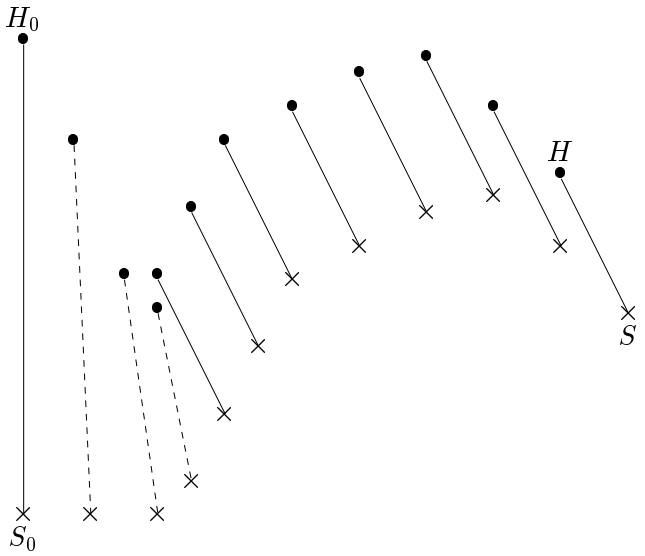

Figure 7.12: A helicopter H that can keep a fixed position with respect to a (small) airplane S. At first the two aircraft are in a steady state (H_0, S_0) with the airplane parked on the ground and the helicopter hovering far above. After a time, S takes off and then dives. The helicopter tries to retain a fixed position close to and above S. As they move to the right, the minute by minute positions of the two aircraft are denoted by dots and crosses respectively. When a QSSA is attained, the positions of the two aircraft are joined by a solid line; a dashed line denotes the relative positions of the aircraft during the transient period before the QSSA is attained. [fig713lb7]

$E_0 \ll S_0 + K_m$ of (6.17) renders precise the intuitive feeling that the QSSA should be appropriate if the initial substrate concentration S_0 is large enough. It is particularly important that large S_0 is sufficient but not necessary. If $K_m \gg E_0$ then S_0 need not be large compared with the initial enzyme concentration E_0, and there are many *in vivo* situations where S_0 and E_0 are comparable (Sols and Marco [46]). Even when E_0 is relatively large, a QSSA may be enabled by a change of variables (Borghans *et al.* [2]). Our whole discussion is also relevant (suitably modified) to the binding of a ligand, such as a hormone, to a receptor. For this situation, E corresponds to receptor concentration and S to ligand concentration. (See Exercise 4.) It is frequently the case *in vivo* that ligand concentration is comparable to or smaller than receptor concentration. Nonetheless the

quasi-steady state approximation is often valid in these situations, because of the presence of K_m in (6.17). An example is the binding of the ligand acetylcholine to its post-synaptic receptor (Parnas *et al.* [33]). Typical biological models involve rather a large number of kinetic equations for the various important interacting chemicals. The quasi-steady-state assumption offers a most important tool for simplifying these models. Examples abound. See Goldbeter [10] **or** [11] for the use of the quasi-steady state assumption in rendering tractable models for oscillations in glycolysis and in cyclic AMP secretion in the cellular slime mold. Or consult work by Segel and Perelson [43] and by Fishman and Perelson [8] that exploits the fact that in immunology chemical reactions occur on time scales of milliseconds to seconds that are far shorter than the time scales of days or weeks that characterize changes in the immune system. Also see Segel and Goldbeter's [41] QSSA treatment of the "relaxation oscillations" that can occur in a model for glycolysis and many other biological contexts. It turns out that justifying a QSSA by estimating time scales as we have done here is not a simple matter and generally requires considerable experience. Nonetheless researchers can make good progress in testing a conjectured quasi-steady state assumption by comparing the results with computer simulations (for example as in Perelson and Brendel [36]). In the case just cited, later analytical investigation conformed the earlier semi-empirical approach (Segel and Perelson [44]). A recent study carefully examines, by a combination of numerical and analytical methods, the question of which variables in a complex kinetic scheme can profitably be approximated by a QSSA (Turányi *et al.* [48]). The alert reader may have noticed that there is an element of circularity in our reasoning. To calculate the decrease in substrate concentration during the fast transient, we have assumed that there is a fast transient preceding the period when the QSSA is valid; we have assumed the existence of the very QSSA whose validity we are trying to establish. Similarly, in estimating the maximum magnitude of dS/dt for use in the estimate (6.15a) we employed the QSSA equation (6.7a) for dS/dt. In fact, what is being attempted is establishment of conditions under which the QSSA is a **consistent approximation**. We advocate using the QSSA and the resulting Lineweaver-Burk plot to estimate K_m, which in turn should be employed to verify condition $E_0 \ll S_0 + K_m$ for the validity of the QSSA. This establishes the consistency of the approximation. Of course, often $E_0 \ll S_0$, in which case the consistency of the QSSA is established a priori. A simple example illustrates the differ-

ence between consistent and inconsistent approximations. Consider the quadratic equation

$$x^2 - 0.01x - 4 = 0 \ .$$

Suppose that for some reason it is decided that the term $0.01x$ is negligible compared to 4. Neglecting the supposedly negligible term, and regarding $x^2 - 4 = 0$ as an approximate equation, one obtains the approximate roots $x = 2$ and $x = -2$. For both these roots, indeed $|0.01x| \ll 4$. The approximation is thus consistent and our faith in the approximation is strengthened. (Note the "circular reasoning" that was used to establish consistency: we assumed that a certain term was neglibible, we simplified the mathematical problem in question by neglecting that term, we found what we hope is an approximate solution by solving the simplified problem, and then we evaluated the size of the neglected term using that approximate solution.) Suppose on the other hand that someone feels that 4 is negligible compared to $0.01x$. The approximate equation $x^2 - .01x = 0$ gives the two roots $x = 0$ and $x = 0.01$, for neither of which is $4 \ll |0.01x|$. Thus this "approximation" is inconsistent, so that one can put no faith in it. See Lin and Segel [27] for further discussion of consistency in approximations. There it is shown that on relatively rare occasions, when a problem is "ill conditioned", consistency is not enough to assure the suitability of an approximation. This is unusual but it is not unheard of. (Turányi *et al.* [48] discuss this matter in some detail.) Usually consistent approximations are accurate, while inconsistent approximations can be accurate only by improbable luck. Two types of motivation for making a quasi-steady state assumption have been illustrated by our discussion, rendering calculations simpler and rendering theoretical results more biologically meaningful. In the immunological example just cited (Segel and Perelson [43]), the largest computers envisioned could not solve moderately inclusive models of the immune system since chemical accuracy requires sub-millisecond resolution but the necessity to model various significant immunological phenomena implies that the numerical integrations must track weeks or months of system kinetics. The quasi-steady state assumption does away with the necessity to compute the fast chemical kinetics; the great disparity in time scales generally means that the assumption will yield accurate results. By contrast, the classical use of the quasi-steady state assumption to simplify the basic enzyme-substrate-complex equations (6.5) is not primarily justified by reasons of computational efficiency. With appropriate numerical methods (for "stiff" equations — see Gear [9], as

well as Turányi *et al.* [48]) the original equations (6.5a) and (6.5b) can be solved almost as rapidly as the simplified equations (6.7a) and (6.7b). In the present case the decisive role of the quasi-steady-state approach lies in the biophysical meaningfulness of (6.7b) and the ease with which $V_{\max}$ and K_m can be obtained from experiment with the aid of the Lineweaver-Burk plot (Fig. 7.8B). *Mathematical development of the QSSA via scaled variables* Our developments can be used as another illustration of the use of scaled variables (see Libuk7, Section 5). Consider the initial transient period. During this period its initial concentration S_0 is a good scale for S. C should be scaled by its concentration $\overline{C}$, an estimate for the maximum value of C. To scale t the appropriate time scale, $t_{_C}$, should be employed. Hence we introduce the variables

$$s \equiv S/S_0 \ , \qquad c \equiv C/\overline{C} \ , \qquad \tau \equiv t/t_{_C} \ , \tag{6.19}$$

with which (6.5a) and (6.5b) become [Exercise 3(a)]

$$\frac{ds}{d\tau} = \epsilon\left[-s+\frac{\sigma}{\sigma+1}cs+\frac{\kappa(\kappa+1)^{-1}}{\sigma+1}c\right] , \qquad \frac{dc}{d\tau} = s-\frac{\sigma}{\sigma+1}cs-\frac{1}{\sigma+1}c \ , \tag{6.20a, b}$$

with initial conditions

$$s(0) = 1 \ , \qquad c(0) = 0 \ , \tag{6.21a, b}$$

where the dimensionless parameter ϵ is given in (6.17b). The other two dimensionless parameters are

$$\sigma \equiv S_0/K_m \ , \qquad \kappa \equiv k_{-1}/k_2 \ . \tag{6.21c, d}$$

Since variables are scaled, when $\epsilon \ll 1$ (6.20a) can be approximated by $ds/d\tau = 0$. From (6.21a), $s \equiv 1$. Substitution of $s \equiv 1$ into (6.21b) gives

$$\frac{dc}{d\tau} = 1 - c \ . \tag{6.22}$$

With (6.21b), (6.22) implies

$$c = 1 - e^{-\tau} \ . \tag{6.23}$$

Thus we "automatically" obtain, in dimensionless form, the initial layer approximation $S \equiv S_0$ and equation (6.5d) for C. Better approximations

can be obtained by series expansions, in powers of ϵ. After the fast transient, t_S is the time scale. The scales for S and C remain appropriate. Upon introducing

$$T \equiv t/t_S \ . \tag{6.24}$$

we obtain the following scaled dimensionless equations [Exercise 3(b)]:

$$\begin{aligned} \frac{ds}{dT} &= (\kappa+1)(\sigma+1)\left[-s + \frac{\sigma}{\sigma+1}cs + \frac{\kappa(\kappa+1)^{-1}}{\sigma+1}c\right] , \\ \epsilon\frac{dc}{dT} &= (\kappa+1)(\sigma+1)\left[s - \frac{\sigma}{\sigma+1}cs - \frac{1}{\sigma+1}c\right] . \end{aligned} \tag{6.25a, b}$$

As a first approximation we set $\epsilon = 0$ in (6.25b), obtaining

$$c = \frac{(\sigma+1)s}{\sigma s + 1} \ . \tag{6.26}$$

This is the dimensionless version of (6.6). On substituting (6.26) into (6.25a) we obtain an equation for s, thereby "automatically" obtaining the dimensionless version of the central quasi-steady state equation (6.7a). A procedure exists for establishing appropriate initial conditions for (6.25), and therewith systematically obtaining more accurate approximations for s by **matching** the transient and post transient solutions in their "overlap region" of common validity. Once several terms in the power series (in ϵ) for s have been determined, the corresponding approximation for b is readily obtained by expanding (6.25b). The spirit of the calculations is similar to those required in Exercise 5.2. For a detailed account, see Lin and Segel [27]. The central idea is that concentrations at the end of the transient region must match those at the beginning of the post transient region. Since $s \equiv 1$ throughout the transient region, to first approximation, it is natural to take $s(0) = 1$ as the first approximation to the initial condition for (6.25a). From (6.23), we see that c approaches unity toward the end of the transient (when τ is large). Thus $c(0) = 1$ is the first approximation to the initial condition for (6.25b). The conditions $c(0) = s(0) = 1$ are indeed consistent with (6.26). Our scaled equations can be used to generate further understanding of Fig. 7.11. Note first that the equations of (6.20), which are appropriate during the induction period, have the form

$$\frac{ds}{d\tau} = \epsilon f(s,c) \ , \qquad \frac{dc}{d\tau} = g(s,c) \ . \tag{6.27}$$

Because ϵ is small, we anticipate that $ds/d\tau$ will be small compared to $dc/d\tau$. Consequently, when examining Fig. 7.11 when $\epsilon = 0.01$ we are not

surprised that s hardly changes, yielding a solution trajectory that moves almost vertically throughout the brief duration of the induction period. Nor is it surprising that the induction period comes to a close when the solution trajectory nears the curve $g(s,c) = 0$ (which is equivalent to the curve (6.26) of the QSSA). The reason is that if g is nearly zero then both $ds/d\tau$ and $dc/d\tau$ are small. Both c and s now change at the same rate, so that the solution trajectory will no longer rise vertically. Moreover, since both c and s now change slowly, a new time scale is appropriate. This brings us to (6.25), which has the form

$$\frac{ds}{dT} = K\, f(s,c)\ , \qquad \epsilon\frac{dc}{dT} = K\, g(s,c)\ , \tag{6.28}$$

where K is a constant. Now the smallness of ϵ suggests, and Fig. 7.11 confirms, that the solution trajectory will remain very close to the QSSA curve $g(s,c) = 0$. As will be illustrated elsewhere in this book, equations of the form (6.27) are found in several areas of theoretical biology, and indeed in many other scientific subjects. Our example illustrates typical features of solution behavior: rapid vertical transit to $g(s,c) = 0$, followed by slow motion along this curve. (The rapid transit is vertical since the slowly changing variable is measured along the horizontal axis.) In the present instance, this motion terminates in the point $S = 0$, $C = 0$, corresponding to complete consumption of substrate. In other examples, more exotic behavior is found, but the solution still consists of rapid vertical motion together with slow motion along $g(s,c) = 0$. See for example Segel and Goldbeter's [41] study of "relaxation oscillations" in cAMP concentration.

Exercises

1. (a) Derive (6.4) from (6.2) and (6.3). Similarly derive (6.5c).
   (b) Verify (6.5e), (6.12) and (6.14).
   (c) Show that if (6.17a) holds then (6.15c) holds.
   [Hint: Show that $t_c/t_s = \epsilon F$ where $F < 1$.]
   (d) Why is formula (6.9b) for $V_{\max}$ intuitively reasonable?
   (e) Show mathematically that (6.10a) implies that $V_{\max}$ is the maximum value of V.

2. (a) By calculating dV/dS from (6.10), show, using (6.7a), that for $S \le S_0$, $V(S)$ is maximal when $S = S_0$.
   (b) From (6.7) we see that a consequence of the QSSA is that dS/dt is

the negative of dP/dt. Show this directly from an appropriate conservation law.

3. (a) As practice in employing dimensionless variables, verify (6.20).
(b) Similarly, verify (6.25).
(c) Show that the time $t = t_{_C}$ mentioned in the caption of Fig. 7.11 corresponds to $T = \epsilon/\big[(1+\kappa)(1+\sigma)\big]$, where T is the dimensionless time $t/t_{_S}$. Thus verify that in Fig. 7.11 $t_{_C}$ indeed gives a good estimate of the duration of the fast transient.

4. Receptor molecules (concentration R) reversibly bind with ligand (concentration L) to give bound ligand (concentration B). Free (unbound) ligand irreversibly decays. The mathematical model includes the following equations:

$$\frac{dL}{dt} = -k_1 RL + k_{-1}B - kL \ , \qquad \frac{dB}{dt} = k_1 RL - k_{-1}B \ ; \qquad (6.29a,b)$$

$$L(0) = L_0 \ , \qquad B(0) = 0 \ , \qquad R(0) = R_0 \ . \qquad (6.30)$$

(a) What is the kinetic scheme for this situation?
(b) Write the equation for dR/dt and *prove* that $R + B = R_0$.
(c) Show that if a quasi-steady state assumption is made on B, then

$$dL/dt = -kL \ . \qquad (6.31)$$

(d) Demonstrate that after the fast initial transient, if $L(0) \approx L_0$ is appropriate just after this transient, then

$$B = \frac{R_0 L_0 e^{-kt}}{(k_{-1}/k_1) + L_0 e^{-kt}} \ . \qquad (6.32)$$

Sketch a graph of this function. Is the behavior of B in accord with intuition? Explain.
[Hint: do not solve any differential equations. Instead use the quasi-steady state equation for B together with the fact that $R = R_0 - B$.]
(e) If $R = R_0 - B$ is substituted into the equation for dB/dt, one obtains

$$dB/dt = k_1(R_0 - B)L - k_{-1}B \ . \qquad (6.33)$$

Why is the time scale during the initial fast increase of B given by $1/(k_1 L_0 + k_{-1})$?

(f) Why is the following a necessary condition for the quasi-steady state assumption?

$$\frac{k}{k_1 L_0 + k_{-1}} \ll 1 \ . \tag{6.34}$$

(g) Show that $L(0) = L_0$ is an appropriate initial condition for (6.31) if

$$(k_1 R_0 + k) \cdot \frac{1}{k_1 L_0 + k_{-1}} \ll 1 \ . \tag{6.35}$$

Does condition (f) imply condition (g) or vice-versa? Why is your answer intuitively reasonable?

5. Show that if (6.15c) holds but (6.17a) does not then the right hand sides of (6.20a) and (6.20b) are approximately proportional, so that $dc/ds \approx \epsilon^{-1}$. By consulting Fig. 7.10, deduce the appropriate replacement for the initial conditions (6.8). (If you get stuck, see Segel and Slemrod [45]).

6. Give intuititive reasons for the following mathematical results.
(a) When the quasi-steady state assumption is valid, then (but not otherwise) the rate of product formation is approximately equal to the rate at which the substrate concentration increases — as shown by (6.7).
(b) The maximum reaction velocity is proportional to the initial enzyme concentration, with the constant of proportionality as given in (6.9b).
(c) If (6.17a) holds then (6.15c) holds.

7. Consider the following kinetic scheme for the reaction of an enzyme E with a substrate S, and an inhibitor I. (P = product; C and D are complexes.)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \ , \qquad C \xrightarrow{k_2} E + P \ , \qquad E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} D \ . \tag{6.36}$$

The following are the equations for S, C, D and I:

$$\begin{aligned} \frac{dS}{dt} &= -k_1 ES + k_{-1} C \ , & \frac{dC}{dt} &= k_1 ES - (k_{-1} + k_2) C \ , \\ \frac{dD}{dt} &= k_3 EI - k_{-3} D \ , & \frac{dI}{dt} &= -k_3 EI + k_{-3} D \ . \end{aligned} \tag{6.37}$$

Initial conditions are $E(0) = E_0$, $S(0) = S_0$, $C(0) = D(0) = 0$, $I(0) = I_0$.
(a) Write the differential equation for dE/dt.
(b) In one or two sentences, explain why we expect that

$$E(t) + C(t) + D(t) = E_0 \ . \tag{6.38}$$

(c) Show that (6.37) implies (6.38). [This provides a check on (a).]
(d) If we make a quasi-steady state assumption $dD/dt \approx 0$ and $dC/dt \approx 0$ we obtain 2 equations for C and D in terms of S and I. Show that solving these equations gives

$$C = \frac{E_0 S}{S + K_m(1 + \frac{I}{K_3})} \tag{6.39}$$

where $K_m = (k_{-1}+k_2)/k_1$, $K_3 = k_{-3}/k_3$. Since $dP/dt = k_2 C$, this gives an expression for the reaction velocity V (= rate of product formation dP/dt). Let $V_{\max} \equiv k_2 E_0$. On a single pair of axes, draw graphs of V as a function of S for two fixed values of I, and use these graphs to show that indeed I inhibits the reaction.

8. This problem concerns another approach to the quasi-steady state assumption. The idea of the new approach (Borghans et al., *Bulletin Math Biology*, 1996 ???) is to consider the total substrate concentration $\overline{S}$, instead of the free substrate concentration S, where

$$\overline{S} \equiv S + C \ . \tag{6.39}$$

(a) Show that in terms of $\overline{S}$, the equations (6.5a,b) become

$$\frac{d\overline{S}}{dt} = -k_2 C \ , \ \frac{dC}{dt} = k_1[(E_0 - C)(\overline{S} - C) - K_m C] \ , \tag{6.40a,b}$$

with initial conditions

$$\overline{S}(0) = S_0 \ , \ C(0) = 0 \ . \tag{6.41a,b}$$

Take for granted (but see part (f) of this problem) that for all parameter values it is quite a good approximation to neglect the C^2 term in (6.40b), which gives

$$\frac{dC}{dt} = k_1[-(E_0 + K_m + \overline{S})C + E_0\overline{S}] \ . \tag{6.42}$$

Thus from now on consider (5a) and (6) as the basic equations for $\overline{S}$ and C. In particular, the QSSA is found by setting $dC/dt = 0$, obtaining

$$C = \frac{E_0 \overline{S}}{E_0 + K_m + \overline{S}} \tag{6.43a}$$

and

$$\frac{d\overline{S}}{dt} = \frac{-k_2 E_0 \overline{S}}{E_0 + K_m + \overline{S}} \ . \tag{6.43b}$$

(b) Explain why the time scale t_C for the fast change in C is given in (6.44), where during a time of order t_C, C approaches $\overline{C}$:

$$t_C = \frac{1}{k_1(E_0 + S_0 + K_m)} \ , \ \overline{C} = \frac{E_0 S_0}{E_0 + K_m + S_0} \ . \tag{6.44a, b}$$

(c) Justify the following estimate for the time scale of substrate change, after the fast transient:

$$t_{\overline{S}} = \frac{E_0 + S_0 + K_m}{k_2 E_0} \ . \tag{6.45}$$

Show that the condition $t_C \ll t_{\overline{S}}$ can be written

$$\frac{k_1(E_0 + S_0 + K_m)^2}{k_2 E_0} \gg 1 \ , \text{ i.e. } \left(1 + \frac{E_0 + S_0}{k_2/k_1} + \frac{k_{-1}}{k_2}\right)\left(1 + \frac{S_0 + K_m}{E_0}\right) \gg 1 \ . \tag{6.46a, b}$$

(d) Find the condition that justifies using $\overline{S}(0) = S_0$ as the initial condition for (6.43b). (Use the estimate $k_2\overline{C}$ for $|d\overline{S}/dt|$ during the transient, but justify this approximation.) You should find that this condition turns out to be the same as (6.46a). (e) Why does condition (6.46b) show that using $\overline{S}$ instead of S gives a QSSA that is valid for a wider parameter range than the standard QSSA? (f) This part of the problem concerns a partial justification of neglecting the C^2 term in (6.40b). We will consider only the quasi-steady state situation, where $dC/dt = 0$. Show that it is consistent to neglect the quadratic term in the resulting equation for C, as follows. The quadratic term is small compared to the linear term if

$$C \ll E_0 + K_m + \overline{S} \ . \tag{6.47}$$

With the quadratic term neglected, C is given in (6.43a). With this value of C, show that (6.47) can be written

$$1 \ll \left(1 + \frac{\overline{S}}{E_0} + \frac{K_m}{E_0}\right)\left(1 + \frac{E_0}{\overline{S}} + \frac{K_m}{\overline{S}}\right) \ . \tag{6.48}$$

Condition (6.48) is valid if $\overline{S}$ is either large or small compared to E_0. What about when $\overline{S} = E_0$?

7 Multiple subunit enzymes and proteins: cooperativity

Often data on reaction velocity does not yield the straight line predicted by the Lineweaver-Burk plot that was discussed in Section 6. Since the simple theory does not work, more complex assumptions must be made in an effort to understand the mechanism of enzyme action. Illustrative steps toward a more comprehensive theory will be taken in this section. *Dimer for which binding induces concerted conformational change*: *Formulation* Enzymes typically consist of multiple subunits. Several of the principles that we wish to illustrate can be illustrated by considering the model for a two subunit **dimeric** enzyme that is diagrammed, in two equivalent ways, in Fig. 7.13. The appropriate kinetic scheme is

$$\begin{aligned} E + S &\underset{k_{-1}}{\overset{2k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_p} E + P, \\ C_1 + S &\underset{2k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2 \xrightarrow{2k_q} C_1 + P \ . \end{aligned} \qquad (7.1a, b)$$

Here C_1 denotes the concentration of complexes of the dimeric enzyme molecule E with a single substrate molecule S. C_2 denotes the concentration of the complex of E with two substrate molecules. P denotes the concentration of product. The model assumes that binding of a substrate molecule S to one subunit of the enzyme E changes the enzyme conformation in a **concerted** fashion, i.e. both the subunits change in the same way. A second binding produces another concerted conformational change. Such changes alter the likelihoods of substrate binding and dissociation. The rate constant k_1 concerns the probability of binding substrate S to a single site, and is thus termed **site specific**. The **statistical factor** 2 that appears in Fig. 7.13 takes into account the fact that there are two sites at which S can bind to the dimer E and thereby change E to C_1. Other statistical factors appear for analogous reasons. Thus, the differential equations corresponding

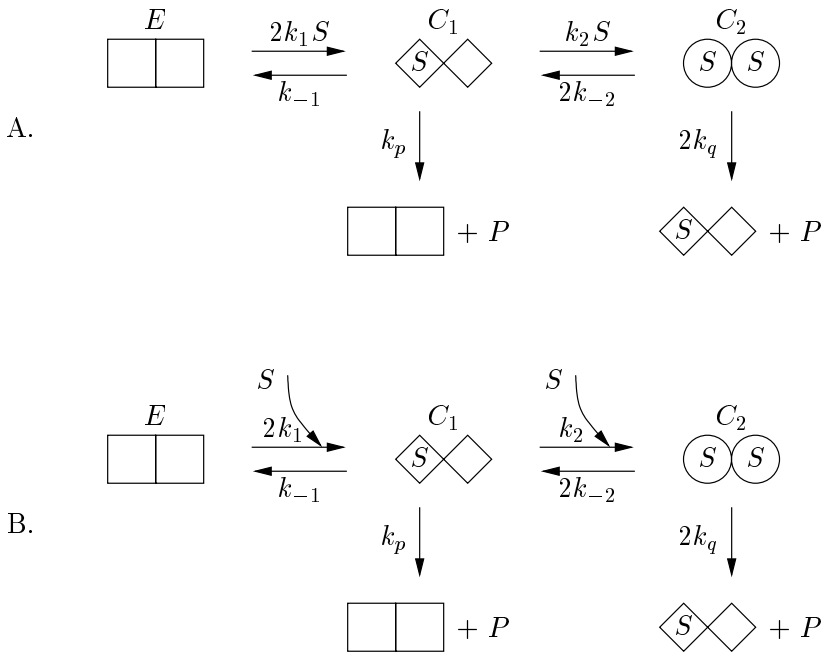

Figure 7.13: A. A simple possible model for product formation of a product P from a substrate S catalyzed by a cooperative dimeric enzyme E. Different shapes denote different conformations of subunits. B. An equivalent alternative to the diagram of A, where the role of substrate S is more explicitly indicated. [fig715lb7]

to Fig. 7.13 and Scheme (7.1) are

$$\begin{aligned}
\frac{dS}{dt} &= -2k_1ES - k_2C_1S + k_{-1}C_1 + 2k_{-2}C_2 \ , \\
\frac{dC_1}{dt} &= 2k_1ES - (k_{-1} + k_p)C_1 - k_2C_1S + (2k_{-2} + 2k_q)C_2 \ , \\
\frac{dC_2}{dt} &= k_2C_1S - (2k_{-2} + 2k_q)C_2 \ , \\
\frac{dP}{dt} &= k_pC_1 + 2k_qC_2 \ .
\end{aligned} \qquad (7.2a-d)$$

E is determined from the conservation law

$$E = E_0 - C_1 - C_2 \ . \qquad (7.3)$$

We consider the following initial conditions:

$$S(0) = S_0 \ , \qquad E(0) = E_0 \ , \qquad C_1(0) = C_2(0) = P(0) = 0 \ . \tag{7.4}$$

Consequences of a quasi-steady state assumption (*QSSA*) In analogy with the case of a monomeric enzyme, let us make quasi-steady state assumptions on both complexes:

$$\frac{dC_1}{dt} = 0 \ , \qquad \frac{dC_2}{dt} = 0 \ . \tag{7.5}$$

Given (7.5), Eqs. (7.2b) and (7.2c), with E expressed by the conservation equation (7.3), can be regarded as a pair of linear equations for C_1 and C_2, in terms of S. Solving these equations, one obtains, after some algebra [Exercise 1(b)],

$$C_2 = \frac{C_1 S}{2K'_m} \ , \qquad C_1 = \frac{SE_0/K_m}{1 + (2S/K_m) + (S^2/K_m K'_m)} \ , \tag{7.6a.b}$$

where we have employed the Michaelis constants

$$K_m = \frac{k_{-1} + k_p}{k_1} \ , \qquad K'_m = \frac{k_{-2} + k_q}{k_2} \ . \tag{7.7a, b}$$

Let us introduce the maximal velocity V_q for product-formation from the doubly-bound enzyme:

$$V_q = 2k_q E_0 \ . \tag{7.8}$$

Let us also introduce the dimensionless variable s and the dimensionless parameters α and β:

$$s = \frac{S}{K_m} \ , \qquad \alpha = \frac{k_q}{k_p} \ , \qquad \beta = \frac{K'_m}{K_m} \ . \tag{7.9a - c}$$

With these, employing (7.6a) and (7.6b), we obtain [Exercise 1(b)] from Eq. (7.2d)

$$V(s) = \frac{\alpha^{-1} s(\beta + \alpha s)}{\beta + 2\beta s + s^2} \ , \tag{7.10}$$

where V is the dimensionless reaction velocity

$$V = (dP/dt)/V_q \ . \tag{7.11}$$

Equation (7.10) (and its generalizations) is sometimes called the "Adair equation". Clearly, a Linewearer-Burk plot of (7.10) does not in general yield a straight line (Exercise 1(e)).

Protein binding We now show that virtually the same formulae that we have derived so far for **product formation by a dimeric enzyme** can also be applied to the case of a molecule S (usually called a **ligand** in this context) **binding to a dimeric molecule** E. To adapt our calculations for binding to a dimeric protein we have only to set to zero the product-formation rate constants k_p and k_q in the scheme (7.1) and the corresponding equations (7.2). The concentrations C_1 and C_2 of the singly and doubly bound dimer are given by (7.6) as before, but with $k_p = k_q = 0$. The modified constants K_m and K'_m of (7.7) (with $k_p = k_q = 0$) are termed **dissociation constants**. Like Michaelis constants, dissociation constants have the dimension of concentration. The analog of the dimensionless reaction velocity V of (7.11) is the **saturation function** Y, defined as the fraction of sites that are bound with ligand. Since C_2 contains two bound sites and since the total number of sites on E_0 dimers is $2E_0$

$$Y = \frac{C_1 + 2C_2}{2E_0} \ . \tag{7.12}$$

From (7.6), with $k_p = k_q = 0$, we find that

$$Y(s) = \frac{s(\beta + s)}{\beta + 2\beta s + s^2} \ . \tag{7.13}$$

Note that formula (7.13) is a special case of (7.10), with $\alpha = 1$. This can be regarded as a useful mathematical coincidence [but see Exercise 1(c)], since our forthcoming mathematical analysis of expression (7.10) for the reaction velocity $V(s)$ will also serve for the analysis of formula (7.13) for the saturation function $Y(s)$. We further note that when $\beta = 1$, i.e. $K_m = K_{m'}$, then (7.13) reduces to

$$Y(s) = \frac{s(1 + s)}{1 + 2s + s^2} = \frac{s(1 + s)}{(1 + s)^2} = \frac{s}{1 + s} \ . \tag{7.14}$$

Analysis of Michaelean binding It will turn out to be helpful in later discussions to digress here to perform an analysis of (7.14). To make our intuitive discussions a little easier to understand, we rewrite (7.14) in dimensional variables, remembering that now $k_p = k_q = 0$. Since $s = S/K$, (7.14) becomes

$$Y = \frac{S}{S+K} , \qquad K = \frac{k_{-1}}{k_1} . \tag{7.15a,b}$$

We derived (7.15) as a limiting case of a dimeric enzyme E with two identical sites and no product formation. In the absence of product formation, E is just a dimer that binds substrate S. The two sites of E are identical and have the same value of $K = k_{-1}/k_1$. According to the law of mass action the binding fraction Y will be the same whether pairs of sites happen to be joined or each monomeric site moves independently of all other sites. In other words, one expects that (7.15) will result from the kinetic scheme

$$F + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C , \tag{7.16}$$

where F is a free (unbound) monomer and Y is the quotient of the steady state value of C and the total monomer concentration. This expectation is easily verified [Exercise 1(d)]. Equation (7.15) for Y is said to represent **Michaelean binding**, in analogy with the almost identical formula (6.10a) for the velocity V of a Michaelean monomeric enzyme. As expected, as S increases, the binding fraction Y asymptotically approaches its maximum possible value $Y = 1$. Often the situation $Y = 1$ when all sites are bound is referred to as a **(fully) saturated state**. The dissociation constant K is the half-saturation concentration, for when $S = K$ then $Y = \frac{1}{2}$. Another interpretation of K can be seen if (7.12) is simplified for small values of S, more precisely for values of S such that $S \ll K$. Then (7.15) reduces to

$$Y \approx \frac{S}{K} \qquad \text{or} \qquad Y = K_{_A} S \quad \text{where } K_{_A} = K^{-1} . \tag{7.17}$$

Thus the reciprocal of K, which is often called the **association constant** $K_{_A}$, is the slope of the straight line that approximates the graph of Y as a function of S when S is small. In mathematical language, the line $Y = S/K$ or alternatively $Y = K_{_A} S$ is tangent to $Y(S)$ at the origin (see Fig. 7.14A). Formally, the tangency of $Y = S/K$ follows from (7.15):

$$\frac{dY}{dS} = \frac{K}{(S+K)^2} , \qquad \left.\frac{dY}{dS}\right|_{S=0} = \frac{1}{K} . \tag{7.18a,b}$$

Another helpful interpretation of K_A, can be obtained by employing the Taylor approximation [a version of Eq. (5) in Appendix 2] of (15), keeping in mind that $Y(0) = 0$:

$$Y(\Delta S) \approx Y(0) + \left.\frac{dY}{dS}\right|_{S=0} \Delta S = \frac{\Delta S}{K} \ . \tag{7.19}$$

That is

$$Y(\Delta S) \approx \left(\frac{1}{K}\right) \Delta S \qquad \text{or} \qquad Y(\Delta S) \approx K_A \Delta S \tag{7.20}$$

for small S. Equation (7.20) implies that if ligand concentration is increased from zero to a small value ΔS then there is a proportional increase in the fraction of sites bound. The constant of proportionality is the association constant K_A. The terms **affinity constant** or simply **affinity** are also used for the proportionality factor K_A. There is one more slightly different interpretation of K_A, as long as the tangent line $Y = K_A S$ is a good approximation to $Y(S)$. In such circumstances

$$\begin{aligned} Y(S + \Delta S) - Y(S) &\approx K_A \cdot (S + \Delta S) - K_A \cdot S \\ &\approx K_A \Delta S \text{ i.e. } \Delta Y \approx K_A \Delta S \ . \end{aligned} \tag{7.21}$$

Thus, *for low concentrations, when* $Y \approx K_A S$, we see that *the affinity is the ratio between the increase in fractional binding* Δy *caused by (small) increases* Δs *in the ligand concentration.* (For example if $K_A = 5$ mM^{-1} then (7.21) implies that if the substrate concentration is increased by 0.01 mM then Y is increased by 5%.) The larger the affinity, the larger the increase in binding for a given increase ΔS in the ligand concentration. Figure 7.14A shows that $Y(S)$ falls below the tangent line $Y = K_A S$ as S increases. Why should this happen? This reason is this. True, each individual potential binding partner F for substrate S remains unchanged in its affinity (measured by K_A) for substrate. But for higher values of S, when ΔS new molecules are added these molecules are confronted with fewer unbound sites F. The reason is that at higher S, more sites are bound and thus fewer free sites F are available for binding; **saturation** of the binding sites is occuring. Therefore, the larger S is, the smaller is the increase in binding fraction ΔY for a given substrate increment ΔS. Since for arbitrary S Eq. (7.21) generalizes to

$$\Delta Y = Y(S + \Delta S) - Y(S) \approx \frac{dY}{dS}(S)\Delta S \tag{7.22}$$

we thus expect that the derivative $dY(S)/dS$ decreases as S increases. Moreover, the derivative should approach zero when $S \gg K$, since for such large values of S binding is almost saturated so that there are virtually no free sites left. Indeed, we see from (7.18a) that

$$\frac{d^2Y}{dS^2} = \frac{d}{dS}\left(\frac{dY}{dS}\right) = \frac{-2K}{(S+K)^3} \ . \tag{7.23}$$

Formula (7.23) confirms that $dY(S)/dS$ decreases toward zero as S increases (the second derivative is negative). The rate of decrease, owing to saturation, is quantitated by the second derivative. This rate continually decreases to zero as S increases, starting from its maximum at $S = 0$, where it has a value of $-2/K^2$. *Nature of graph for velocity dependence on substrate* Let

us return from our digression concerning $Y(S)$ and examine the behavior of the graph of expression (7.10) for the dimensionless reaction velocity V. To this end, as is always useful in examining graphs, we calculate the first and second derivatives of V:

$$\frac{dV}{ds} = \alpha^{-1}\beta\frac{\beta + 2\alpha s + (2\alpha - 1)s^2}{(\beta + 2\beta s + s^2)^2} \ , \tag{7.24a}$$

$$\frac{d^2V}{ds^2} = -2\alpha^{-1}\beta\frac{\beta(2\beta - \alpha) + 3\beta s + 3\alpha s^2 + (2\alpha - 1)s^3}{(\beta + 2\beta s + s^2)^3} \ . \tag{7.24b}$$

We see from (7.24a) that if $2\alpha > 1$ then $dV/ds > 0$, so that V continually increases with s. If $2\alpha < 1$, when s is sufficiently small, $dV/ds \approx \alpha^{-1}$ and thus is positive, but dV/ds is negative when s is sufficiently large — so that the term $(2\alpha - 1)s^2$ dominates in the numerator of (7.24b). That is, when $2\alpha < 1$ then V increases when s is small but V *decreases* for all sufficiently large s. It may seem surprising that adding substrate can decrease the rate of product formation, but this latter result makes sense upon further consideration. Since $2\alpha \equiv 2k_q/k_p$, if $2\alpha < 1$ then the maximum rate of product formation from C_1 (k_pE_0) is larger than the maximum rate of product formation from C_2 ($2k_qE_0$); the latter process is less efficient than the former. When substrate concentration grows sufficiently, the relatively inefficient process becomes more and more dominant. From (7.24b) we observe that d^2V/ds^2 is positive for small s if and only if $\alpha > 2\beta$. This same second derivative is negative for large s if and only if $2\alpha > 1$. When both

of these conditions on α hold then the graph of V shifts from concave up to concave down as s increases. Under such circumstances the rising graph of $V(s)$ is said to be **sigmoidal**. Compare Fig. 7.14B. **Results for binding and their interpretation: cooperativity** To translate our results concerning expression (7.10) for V to the binding saturation curve (7.13) we set $\alpha = 1$. Here dY/ds is always positive, and hence, as expected, the extent of binding Y is always an increasing function of the (dimensionless) substrate concentration s. d^2Y/ds^2 is positive for small s if and only if $\beta < \frac{1}{2}$ and is always negative for large values of s. We now consider the concept of cooperativity. For the moment, let us fix our attention on the case of protein-ligand binding. In this case, K_m and K'_m are dissociation constants. If $K'_m < K_m$ ($\beta < 1$) then the affinity of the second binding of ligand to protein is higher than the affinity of the first such binding. This situation is identified with **positive binding cooperativity**. Similarly $K'_m > K_m$ ($\beta > 1$) is identified with **negative binding cooperativity**. The intuitive idea is that positive binding cooperativity results when binding of a ligand to one site of a dimer somehow causes binding to the second site to become easier; the sites somehow "cooperate" positively, for example via a conformational change, to raise the affinity of the second binding. If the first binding makes binding to the second site less likely (lower affinity) then the "cooperativity" is deemed negative. Let us try to give our intuitive idea more precision, using as an example the dimensional version of Eqn.(7.13):

$$Y(S) = \frac{S(\beta K + S)}{K^2\beta + 2\beta KS + S^2} \ , \quad K = \frac{k_{-1}}{k_1} \ , \quad K^1 = \frac{k_{-2}}{k_1} \ , \quad \beta = \frac{K^1}{K} \ . \tag{7.25}$$

Suppose that we are faced with some theoretically derived expression $Y_{th}(S)$, of which (7.25) is an example, for the fraction of sites bound. What kind of cooperativity does $Y_{th}(S)$ express? To answer this question we assume that Y_{th} is proportional to S for small S:

$$Y_{th}(S) \approx \frac{S}{K_{th}} \text{ for } S \text{ small} \ , \tag{7.26}$$

for some constant K_{th}. This assumption holds for (7.25), since

$$Y(S) \approx \frac{\beta KS}{K^2\beta} \text{ i.e. } Y(S) \approx \frac{S}{K} \text{ for } S \text{ small} \ . \tag{7.27}$$

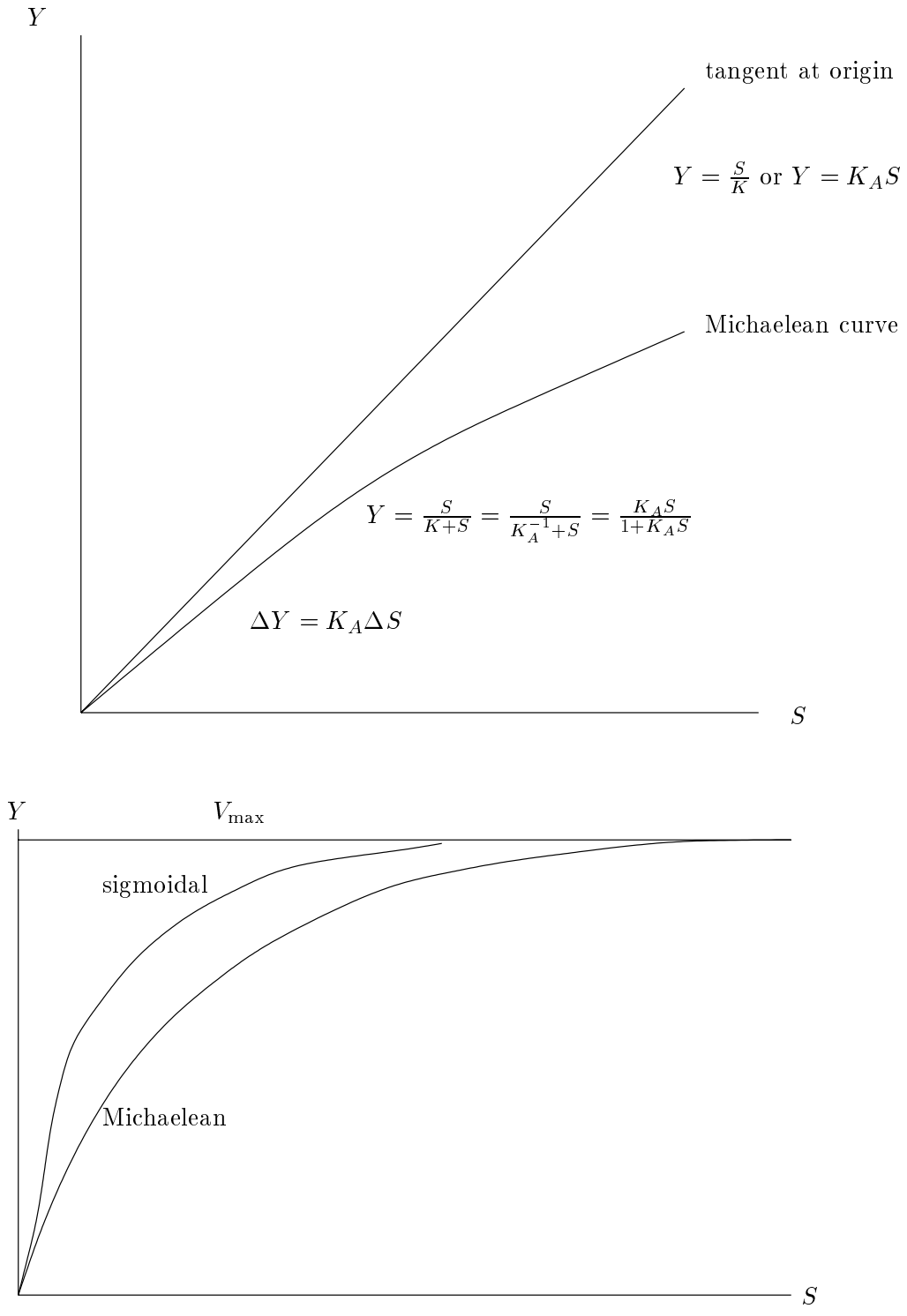

Figure 7.14: BA. Michaelean binding for small values of the ligand concentration S. Approximate calculations, using Taylor approximation, of increases (ΔY) in the fraction of sites bound Y, owing to increasing ligand concentration by ΔS. B. Plot of sites bound Y according to (7.13) together with the Michaelean expression for Y with the same initial slope. [Shows positive and neg cooperativity for different parameters?]. [fig716lb7]

As suggested in [38], let us examine the difference Y_{diff} between Y_{th} and a Michaelean binding fraction whose initial slope is $1/K_{th}$:

$$Y_{\text{diff}}(S) = Y_{th}(S) - \frac{S}{K_{th} + S} \ . \tag{7.28}$$

For reasons that will be given immediately it make sense to characterize the cooperativity of $Y_{th(}S)$ as follows.

$$\begin{aligned} &Y_{\text{diff}}(S) > 0 \text{ for small } S\text{:} \quad \text{positive cooperativity} \ , \\ &Y_{\text{diff}}(S) < 0 \text{ for small } S\text{:} \quad \text{negative cooperativity} \ . \end{aligned} \tag{7.29}$$

This **comparison method for determining cooperativity** is sensible because, for sites with affinity $1/K_{th}$, the Michaelean binding fraction $S/(K_{th}+S)$ describes the slowing increase in binding fraction as S increases. The kinetics that yield Y_{th} also have an (effective) affinity $1/K_{th}$ when the substrate concentration is small. Given this, if the increase of Y_{th} is faster than that of $S/(K_{th} + S)$ then there is some positive influence on binding that is partially overcoming the effect of saturation (positive cooperativity). If the increase of Y_{th} is slower than that of $S/(K_{th} + S)$ then there is a negative influence on binding that augments saturation in decreasing $\Delta Y/\Delta S$ as S increases (negative cooperativity). Note that our characterization of cooperativity applies to small values of S. There could in principle be numerous shifts between positive and negative cooperativity as S increases, but such relatively rare occurences will not be dealt with here. As will now be shown, the comparison method of (7.28) and (7.29) yields a result for cooperativity that is in accord with our definitions of positive and negative binding cooperativity. This is shown in the following Example. **Example.** Use (7.28) and (7.29) to characterize the cooperativity implied by (7.25). **Solution.** Since, from (7.27), $K_{th} = K$ for (7.25)

$$\begin{aligned} Y_{\text{diff}}(S) &\equiv Y_{th}(S) - \tfrac{S}{K_{th}+S} \\ &= \tfrac{S(\beta K+S)}{K^2\beta+2\beta KS+S^2} - \tfrac{S}{K+S} \\ &= \tfrac{KS^2(1-\beta)}{(K+S)(K^2\beta+2\beta KS+S^2)} \ . \end{aligned}$$

Thus, in agreement with one's intuitive expectation, when $\beta < 1$, $Y_{diff} > 0$ and the cooperativity implied by (7.25) is positive. When $\beta > 1$ then

$Y_{\text{diff}} < 0$ and the cooperativity implied by (7.25) is negative. Let us consider the relationship between positive cooperativity and sigmoidality. **Example.** Show that the comparison method of (7.28) and (7.29) implies that if the graph of $Y_{th}(S)$ is sigmoidal for small S then cooperativity is positive. **Solution.** From the definition (7.28) and the facts that Y_{th} and $S/(K_{th}+S)$ are both zero at $S=0$, and that both have the same derivative $(1/K_{th})$ at $S=0$, it follows from the Taylor approximation (Appendix 2) that

$$Y_{\text{diff}}(S) \approx \left[\frac{d^2Y_{th}}{dS^2}|_{S=0} + \frac{2}{K_{th}}\frac{S^2}{2}\right] , \tag{7.30a}$$

where we have used (7.23) when $S=0$. When $[d^2Y_{th}/ds^2] > 0$ for $S=0$ then $Y_{\text{diff}} > 0$ and cooperativity is positive. Note $d^2Y_{\text{diff}}/dS^2 > 0$ when S is small, and the graph of Y is concave upward for small S. (Here we use the theorem that if a continuous function of S is positive at a point S_0 then it is positive when S is sufficiently near S_0.) *Thus sigmoidality implies positive cooperativity in binding; but the converse is not true.* That the converse need not be true is illustrated by the example of (7.24) that we have been studying. Here $d^2Y/dS^2 > 0$ for $Y=0$ if and only if $\beta < 0.5$ (and d^2Y/dS^2 is always negative for large S); remember that the weaker condition $\beta < 1$ implies positive cooperativity. **Cooperativity in enzyme action** The comparison method of (7.28) and (7.29) is also suitable for examining enzyme cooperativity. Here, instead of the binding fraction Y one considers the reaction velocity V. The constant K_{th} of (7.28) is replaced by $K_{m:th}$, the effective Michaelis constant at low substrate levels. Thus for enzyme cooperativity one examines

$$V_{\text{diff}}(S) \equiv V_{th}(S) - \frac{S}{K_{m:th}+S} \tag{7.30b}$$

and makes the definitions

$$\begin{aligned} &V_{\text{diff}}(S) > 0 \text{ for small } S\text{:} \quad \text{positive cooperativity} , \\ &V_{\text{diff}}(S) < 0 \text{ for small } S\text{:} \quad \text{negative cooperativity} . \end{aligned} \tag{7.30c}$$

If this method is applied to case (7.10) of enzyme-substrate interaction, it is found (Exercise 8) that cooperativity is positive (negative) if

$$\alpha + \beta\alpha^{-1} > 2\beta . \tag{7.30d}$$

We have here a particular case of the rule that positive cooperativity implies sigmoidality. The condition for the former, $\alpha > 2\beta$, indeed implies (7.30d). **The MWC theory of cooperativity** There are several theories

that can account for the appearance of cooperativity in binding and enzyme activity. Perhaps the earliest, and still in intensive use, is the "MWC theory" of Monod, Wyman and Changeaux [29]. The MWC approach offers an alternative to the theory based on the scheme of (7.1). We now sketch the MWC theory, with details left to Exercises. In the course of our analysis we encounter a major pillar of kinetics, the principle of microscopic reversibility. As will be seen, the reason why the MWC theory works is somewhat subtle. Once again, we restrict ourselves for simplicity to dimers. We will consider the binding of a ligand S to a protein, and we will calculate the fraction of sites that are bound *at steady state*. It will prove easy to generalize our results to the MWC theory of cooperativity for enzymes. According to MWC, each monomer can exist in one of two configurations. These are termed R (for "relaxed") and T (for "tight") in accord with the assumption that binding to the R configuration is of higher affinity than binding to the T configuration. MWC postulate that **spontaneous concerted transitions** characterize shifts between dimer conformations, in that either both states are R or both are T. (In the model of Fig. 7.13, concerted transitions also occur, but only when they are induced by substrate binding or dissociation.) Figure 7.15 depicts the various states of the dimer. For example, R_j is an RR dimer with j sites bound with S. The kinetic coefficients are given. Note the statistical coefficients 2 when two sites are available for the transition in question. *For the moment we will assume that the dashed arrows can be ignored*. Assuming that these transitions are negligible greatly simplifies the calculations. Exercise 5 requests the reader to fill in the omitted details of the following derivation. We first write out the kinetic equations corresponding to the scheme of Fig. 7.15 — ignoring the dashed arrows. We will consider only the steady state version of these equations. (Our derivation is also appropriate for quasi-steady state situations where the substrate concentration changes sufficiently slowly.) Setting the time rates of change equal to zero, and introducing the dimensionless parameters

$$K \equiv \frac{k_-}{k_+} \ , \qquad M = \frac{m_-}{m_+} \ , \qquad L = \frac{b}{f} \tag{7.31a,b,c}$$

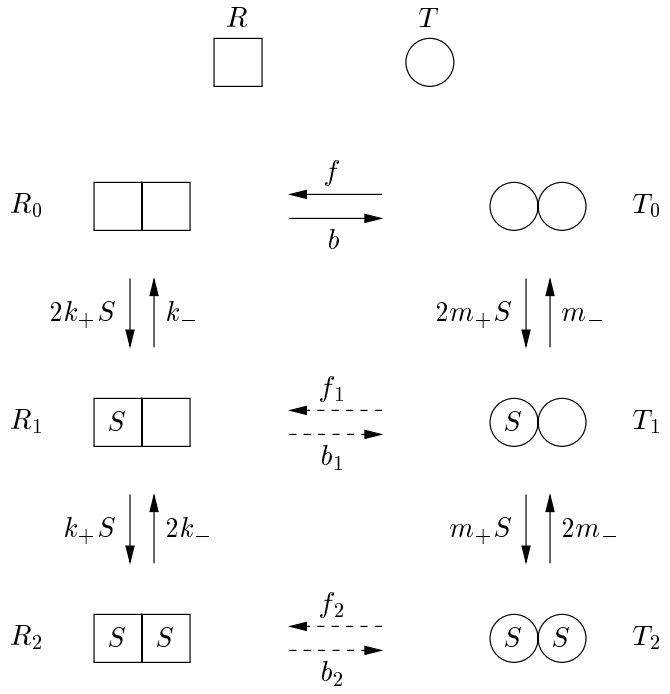

Figure 7.15: Diagram of the MWC model for binding to a dimer. There are concerted transitions between situations where both subunits are in the "relaxed" state R and situations where both subunits are in the "tense" state T. [fig717lb7]

one obtains

$$\begin{aligned} R_2 &= \frac{SR_1}{2K} \ , \qquad R_1 = \frac{2SR_0}{K} \ , \\ T_2 &= \frac{ST_1}{2M} \ , \qquad T_1 = \frac{2ST_0}{M} \ , \qquad T_0 = R_0 L \ . \end{aligned} \tag{7.32a-e}$$

For example (7.32a) comes from the steady state version of

$$\frac{dR_2}{dt} = -2k_- R_2 + k_+ S R_1 \ .$$

It is important to obtain the equations in the order given in (7.32), for then already determined steady state conditions provide simplifications for succeeding steady state conditions. For example, given that $dR_2/dt = 0$, the condition for $dR_1/dt = 0$ is simply $2k_+ S R_0 = k_- R_1$. There is a conservation law

$$R_0 + R_1 + R_2 + T_0 + T_1 + T_2 = P \ , \tag{7.33}$$

where P is the total protein concentration. With this, one obtains from (7.32)

$$R_0 = \frac{P}{(1+s)^2 + L(1+\theta s)^2} \ , \qquad s = \frac{S}{K} \ , \quad \theta = \frac{K}{M} \ . \tag{7.34}$$

Formulas for R_1, R_2, T_0, T_1 and T_2 can now be obtained straighforwardly from (7.32). Note that because R is of higher affinity than T, $\theta < 1$. The occupancy fraction is defined by

$$Y \equiv \frac{R_1 + 2R_2 + T_1 + 2T_2}{2P} \ . \tag{7.35}$$

A little further calculation yields the result we seek

$$Y(s) = \frac{s(1+s) + L\theta s(1+\theta s)}{(1+s)^2 + L(1+\theta s)^2} \ . \tag{7.36}$$

Detailed balance/microscopic reversibility Before examining the consequences of (7.36) let us consider the possibility that the dashed transitions in Fig. 7.15, heretofore ignored, are in fact present. For general values of the transition rates, the problem of determining steady states would now be far more difficult. However from thermodynamic considerations there follows the extremely important **principle of detailed balance**, also known as the **principle of microscopic reversibility**. (See Hill [16]). This principle requires that *for every closed loop in a kinetic scheme the product of rate constants in the clockwise direction around the loop must equal the product of the rate constants in the counterclockwise direction.* For the top loop in Fig. 7.15 this gives

$$b_1 m_- f(2k_+ s) = f_1 k_- b(2m_+ s) \ . \tag{7.37a}$$

From (7.37a) and the comparable result for the bottom loop one finds formulae for the equilibrium constants of the "extra" transitions

$$\frac{b_1}{f_1} = \frac{KL}{M} \ , \qquad \frac{b_2}{f_2} = \frac{b_1}{f_1}\frac{K}{M} = \frac{K^2 L}{M^2} \ . \tag{7.37b,c}$$

[There is also a "big loop", a circuit including $R_0, R_1, R_2, T_2, T_1, T_0$. This big loop is in a sense the "sum" of the top loop and the bottom loop. By Exercise 5(d), microscopic reversibility for the big loop is assured by (7.37).] Let us examine the principle of microscopic reversibility

a little more closely. This thermodynamic principle asserts that at equilibrium the transition between any two states occurs with equal frequency in either direction. Thus, for example, at equilibrium the scheme

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \tag{7.38}$$

implies

$$k_1 A = k_{-1} B \ . \tag{7.39}$$

But if in addition to (7.38) there are also the transitions

$$B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \ , \qquad C \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} A \ , \tag{7.40a, b}$$

as in the diagram

$$\begin{array}{ccc} & C & \\ k_{-3} \swarrow & & \searrow k_{-2} \\ A & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & B \end{array} \tag{7.41}$$

then there is an additional indirect way for a molecule to shift from A to B, via C. At equilibrium the rates of the indirect transitions

$$A \overset{k_{-3}}{\longrightarrow} C \overset{k_{-2}}{\longrightarrow} B \qquad \text{and} \qquad B \overset{k_2}{\longrightarrow} C \overset{k_3}{\longrightarrow} A \tag{7.42}$$

must also be in balance:

$$k_{-3} A k_{-2} C = k_2 B k_3 C \ . \tag{7.43}$$

Together, (7.43) and (7.39) imply

$$\frac{k_{-3} k_{-2}}{k_3 k_2} = \frac{B}{A} = \frac{k_1}{k_{-1}} \ , \quad \text{i.e.} \quad k_{-1} k_{-2} k_{-3} = k_1 k_2 k_3 \ . \tag{7.44a, b}$$

Equation (7.44b) illustrates the consequences of microscopic reversibility, on the closed "triangle" of transitions among states A, B, and C. We note that in "far from equilibrium" cases where energy is consumed, approximate reaction schemes exist wherein the principle of detailed balance appears to be violated (Segel *et al.* [42]), although such schemes must be employed with considerable care (Walz and Caplan [49, 50]). Let us now reconsider the steady state calculations for the scheme of Fig. 7.15, this time including the "extra" transitions indicated by the dashed arrows. In the equations for

dR_2/dt and dT_2/dt, and dR_1/dt and dT_1/dt, there now appear the additional terms, respectively

$$f_2T_2 - b_2R_2 \quad \text{and} \quad f_1T_1 - b_1R_1 \ . \tag{7.45}$$

However, as the reader can verify, if the steady state expressions for R_2, T_2 and R_1, T_1 are employed then the microscopic reversibility conditions (7.37) imply that the additional terms in (7.45) in fact equal zero [Exercise 5(c)]. In general *because of microscopic reversibility extra transitions* such as the dashed transitions in Fig. 7.14 *do not change the steady state results*. On the other hand, kinetic results *are* dependent on the additional forward and backward rate constants. Thus the kinetics provide an opportunity to determine these constants — whose ratio is fixed by (7.44b) and analogues of (7.44b) for more complex kinetic schemes (see Hayashi and Sakamoto [15]; Chapter 4). Suitable versions of the results of the previous paragraph are true in general. That is, for steady state calculations, pruning of complex kinetic diagrams is permitted by the "loop relations" of the principle of microscopic reversibility [which require the vanishing of terms such as those in (7.45)]. As we have seen, for the case of Fig. 7.15 the loop relations allow the removal of the dashed arrows in the full diagram. Hence the equilibrium relations (7.32) are valid in spite of the complexity of the full diagram in Fig. 7.15. There is a mathematical subtlety connected with the proof that the "extra" transitions do not change the steady state results. Consider the steady state equations *with* the extra transitions. We have seen that *one* solution of these equations is the solution obtained when the extra transitions are ignored. But we expect that equations of chemical kinetics such as those encountered here have a unique solution. If so, the one solution that we have obtained is the *only* solution. Why do we expect a unique solution to the steady-state version of the kinetic equations for the transition between n states? Because for fixed S the $n-1$ steady-state rate equations plus a conservation law comprise a system of linear inhomogeneous equations. "Generically" such equations have a unique solution. Problems arise only when the determinant of the coefficients is non-zero. Of course, this determinant condition must be checked to obtain a rigorous result. *MWC cooperativity for enzymes* We record here the counterpart of (7.36) for an n-mer:

$$Y = \frac{s(1+s)^{n-1} + L\theta s(1+\theta s)^{n-1}}{(1+s)^n + L(1+\theta s)^n} \ . \tag{7.46}$$

Next we point out that it is easy to generalize our MWC results so that they apply to the velocity of an enzyme-catalyzed reaction. Providing that we make quasi steady-state assumptions on all the various complexes, adding steps for the production of product (at rate $k_{_R}$ from R and $k_{_T}$ from T) merely generalizes the dissociation constants to the appropriate Michaelis constants. The statistical factors in the numerator of formula (7.35) for the occupancy fraction Y are precisely those needed to calculate the rate of product formation when several sites on a complex are bound. Let us change P, the total amount of protein, to E, the total amount of enzyme. As the reader is requested to verify in Exercise 7(a), by appropriately altering (7.36) one thus obtains for the (dimensional) reaction velocity V

$$V = \frac{2E\left[k_{_R} s_m(1+s_m) + k_{_T}\theta s_m(1+\theta s_m)\right]}{(1+s_m)^2 + L(1+\theta s_m)^2} \ , \tag{7.47}$$

where

$$s_m \equiv \frac{S}{K_m} \ , \qquad K_m \equiv \frac{k_- + k_{_R}}{k_+} \ , \qquad M_m \equiv \frac{m_- + k_{_T}}{m_+} \ , \quad \theta \equiv \frac{K_m}{M_m} \ . \tag{7.48$a-d$}$$

Discussion What is the nature of the cooperativity afforded by the MWC theory for binding? To use the "comparison method", we note that for the binding fraction Y defined in (7.46) we have

$$Y \approx \frac{s(1+L\theta)}{1+L} \quad \text{for small } s \ . \tag{7.49}$$

We thus examine

$$Y_{\text{diff}} \equiv \frac{s(1+s)^{n-1} + L\theta s(1+\theta s)^{n-1}}{(1+s)^n + L(1+\theta s)^n} - \frac{s}{\frac{1+L\theta}{1+L} + s} \ . \tag{7.50}$$

It turns out that $Y_{\text{diff}} > 0$ [Exercise 7(b)]. Thus the MWC theory can account for positive cooperativity in binding but not negative cooperativity. Let us now tackle the question of why the MWC model yields (positive) cooperativity. In doing so it is especially instructive to consider the special case of (7.36) wherein θ approaches zero. Small θ means $M \gg K$, i.e. the affinity of binding to a T site is much less than the affinity of binding to an R site. In the limit $\theta \to 0$ there is no binding to the T site at all, or in other words

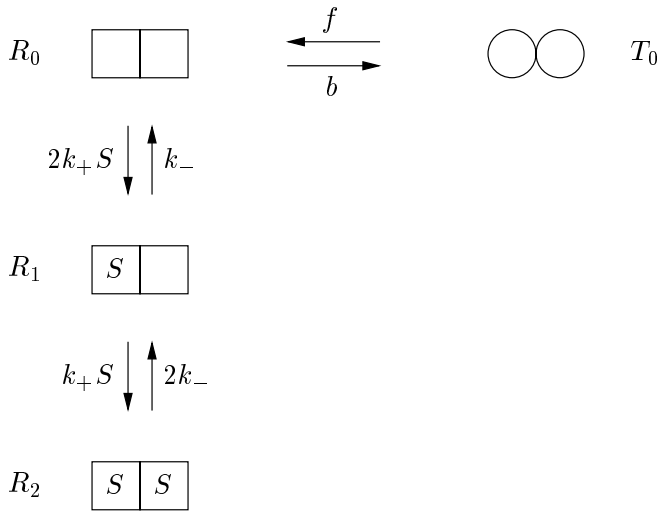

Figure 7.16: Diagram of the MWC model for binding to a dimer — for exclusive binding to the R state. [fig717alb7]

there is *exclusive binding to the R state* (Fig 7.16). In this situation (7.36) becomes

$$Y_{_R} = \frac{s(1+s)}{L+(1+s)^2} = \frac{S(S+K)}{K^2L+(S+K)^2} \ . \tag{7.51}$$

Applying the comparison method to (7.51) we find

$$Y_{\rm diff} = \frac{S(S+K)}{K^2L+(S+K)^2} - \frac{S}{K(L+1)+S}$$

i.e.

$$Y_{\rm diff} = \frac{S^2KL}{[K^2L+(S+K)^2][K(L+1)+S]} > 0 \ . \tag{7.52}$$

Cooperativity is thus positive. This is expected; (7.53) is a special case of (7.46), for which the comparison method always gives positive cooperativity. But how can any form of positive cooperativity arise in a situation such as this where all of the binding is to the R state? It is certainly not true here, as it can be for the model of Fig. 7.13, that positive cooperativity arises because bindings at higher substrate levels occur more frequently at high affinity sites. When $L > 1$, the second derivative of $Y_{_R}$ is positive at $S = 0$

(and hence for sufficiently small positive S), since (Exercise 10a)

$$\text{when } S = 0\colon \frac{d^2Y_{_R}}{dS^2} = \frac{2(L-1)K^2}{(L+1)^2} \ . \qquad (7.53a,b)$$

Consequently, not only is there cooperativity for $L > 1$, but for small S there is an increase in $\Delta Y/\Delta S$ — and hence sigmoidality. Let us attempt to discern what the mechanism can be that can so strongly overcome the tendency of saturation so as to increase $\Delta Y/\Delta S$ as S increases. The first point to bear in mind is that adding substrate decreases the concentration of T_0. To help see this intuitively, note that as $S\to\infty$ all the sites will become bound, so that $R_2\to P$ and all the other concentrations approach zero (Exercise 10b). In particular, $T_0\to 0$, i.e. all T states become R states. Why will adding substrate induce a $T\to R$ transition, since the rate constants that govern transitions into and out of state T_0, namely b and f, are independent of S? The reason is that the addition of substrate will convert R_0 into R_1. The consequent decrease of R_0 decreases the transition rate bR_0 into T_0. Hence, at steady state, there must be a lower value of the balancing transition rate fT_0 out of T_0, i.e. T_0 must decrease. (Kineticists attribute the phenomenon just described to a **shift in equilbrium** from R_0 toward R_1 that is caused by the addition of substrate. This in turn shifts the $T_0 - R_{_O}$ equilbrium toward R_0.) The decrease in T_0 concentration contributes to an increase in R_0 concentration, and every $T_0\to R_0$ transition contributes two new possible binding sites. But adding substrate not only adds possible binding sites by means of the $T_0\to R_0$ transition but also decreases binding sites by means of the transitions $R_0\to R_1$ and $R_1\to R_2$. Which effect dominates? To see, let us examine the change as S increases in the total number of free sites available for binding. This number is $2R_0 + R_1$. We find from (7.32) and (7.34) that (when $\theta = 0$)

$$2R_0 + R_1 = \frac{2PK(K+S)}{K^2L + (K+S)^2} \ , \qquad (7.54)$$

and

$$\frac{d(2R_0 + R_1)}{dS} = \frac{2PK[K^2L - (K+S)^2]}{[K^2L + (K+S)^2]^2} \ . \qquad (7.55)$$

Thus as S increases the number of available binding sites $2R_0 + R_1$ increases (sigmoidality) as long as $(K+S)^2 < K^2L$, i.e. as long as $S < K\sqrt{L-1}$ (provided $L > 1$). Now we see the source of sigmoidality. If $L > 1$ and if S is sufficiently low, then adding more S can induce enough $T_0\to R_0$ transitions

to outweigh the transitions $R_0 \rightarrow R_1$ and $R_1 \rightarrow R_2$. Adding substrate causes a net creation of new binding sites when non-bindable T_0 molecules shift conformation to T_1. Sigmoidality implies positive cooperativity, so that the above discussion accounts for positive cooperativity when $L > 1$. When $L < 1$, adding substrate S (at low levels of S) does not lead to net creation of new binding sites. Nonetheless, for all values of L the $T_0 \rightarrow R_0$ transition slows the saturating effect of transitions $R_0 \rightarrow R_1$ and $R_1 \rightarrow R_2$, thereby bringing about positive cooperativity for all (positive) values of L. Let us summarize some salient conclusions that can be gleaned from our study of two types of model for positive cooperativity. If binding sites operate independently, increasing the substrate concentration will decrease the number of sites available for further binding (saturation), until all sites are bound (Michaelaen binding). We have identified two ways to make this saturation process occur more slowly than it does for Michaelean binding (positive cooperativity). One possibility, illustrated by the scheme in Fig. 7.13, is that binding to one site of a multimer can induce a conformational change that increases the affinity of binding to the remaining unbound states of the multimer. Another possibility, illustrated by the MWC model with exclusive binding to the R state, is that binding can indirectly favor transitions that add to the number of free binding sites. (The general MWC model combines both sources of cooperativity.) **Hill plots for estimating cooperativity** Here is a rough

and ready way to arrive at a procedure often used by kineticists to represent, estimate and compare cooperativities. Assume that the fraction of bound sites is given by

$$Y = \frac{S^n}{K^n + S^n} \ . \tag{7.56}$$

As seen in Fig. 7.17, this **Hill equation** for Y exhibits saturation toward $Y = 1$ (all sites bound) for large S, with half-saturation at $S = K$. What about cooperativity? Figure 7.17 shows that when $n \neq 1$, the behavior of (7.56) for small S is completely different from the Michaelean case $n = 1$. Pursuing this matter analyticaly, one sees from (7.56) that when $S \ll K$, $Y \approx (S/K)^n$. As $S \rightarrow 0$, $dY/dS \rightarrow \infty$ if $n < 1$ and $dY/dS \rightarrow 0$ for $n > 1$. Hence we cannot use our previous characterization of cooperativity that compares Y with the Michaelean curve that has the same slope for small S. Nonetheless, it will be seen that binding according to the Hill equation can be said to exhibit positive or negative cooperativity, depending on whether n is greater or less than unity. Let us calculate the first and second derivatives

as the initial step in understanding the behavior of (7.56). We find

$$\frac{dY}{dS} = \frac{nK^nS^{n-1}}{(K^n + S^n)^2} , \qquad \frac{d^2Y}{dS^2} = \frac{nK^n[(n-1)K^nS^{n-2} - (n+1)S^{2n-2}]}{(K^n + S^n)^3} . \tag{7.57a, b}$$

There are significant differences in behavior when $n > 1$ and when $n < 1$. When $n > 1$ the second derivative changes sign from positive to negative as S increases (Exercise 10c). Thus at smaller values of S the graph of Y is concave upward — the slope increases as S increases. We consequently *identify* $n > 1$ *with sigmoidality and hence with positive cooperativity*, for there is an increase in effective binding affinity as S increases that is strong enough to outweigh the decrease in the number of binding sites. By contrast, when $n < 1$ we see that the slope dY/dS is infinite at $S = 0$; there is a vertical tangent. Moreover $d^2Y/dS^2 \to -\infty$ as $S \to 0$, compared to the finite value $(-2/K^2)$ when $n = 1$. Thus for small S, when $n < 1$ the slope dY/dS decreases much faster than in the Michaelean case, when the decrease in slope occurs because of the decrease in available binding sites. This faster decrease must be due to some sort of effect that makes successive bindings more difficult. Consequently $n < 1$ *is identified with negative cooperativity*. Thus the simple expression for Y in (7.56) not only saturates correctly ($Y \to 1$ as $S \to \infty$) but it also exhibits behavior that one can identify with positive cooperativity ($n > 1$) and negative cooperativity ($n < 1$). We now go through the standard exercise of trying to rearrange a formula so that its graph is a straight line. Solving (7.56) for S^n, one obtains

$$S^n = \frac{YK^n}{1-Y} \quad \text{so that} \quad \log\left(\frac{Y}{1-Y}\right) = n\log S - n\log K . \tag{7.58}$$

From (7.58) it follows that logarithmic plot of $\log\big[Y/(1-Y)\big]$ vs. $\log S$ should yield a straight line with slope n. This is the **Hill plot**. The **Hill number** is defined by

$$n_{_H} = \frac{d\log[Y/(1-Y)]}{d\log S} . \tag{7.59a}$$

Alternatively (Exercise 11, Appendix 2)

$$n_{_H} = \frac{S}{Y(1-Y)}\frac{dY}{dS} . \tag{7.59b}$$

It is common practice to make a Hill plot of $\log[Y/(1-Y)]$ vs $\log S$ when confronted with new experimental results concerning $Y(S)$. The degree of

cooperativity is typically identified with the maximal slope. Of course if the mechanism for cooperativity were known then parameters could be fit with the appropriate model. The advantage of the Hill plot is that cooperativity can be revealed without commitment to any particular model. Often modelers "need" to assume that some little understood process is cooperative in order to produce an observed effect. We see examples of this in Chapters ? and ? where some form of cooperativity is assumed in order that models of physiological systems exhibit observed oscillating and/or excitable behavior. Here too, in the absence of knowledge concerning mechanism the simple phenomenological form of the Hill equation is often used to represent the required cooperativity. One more point needs to be made. Our investigations in this section can be regarded as exploring non-Michaelean molecular stimulus-response curves, where the stimulus is the presence of some molecule and the response is protein binding or product formation via enzyme catalysis. We have seen how some form of cooperative binding of the stimulating molecule can explain the observations. There are however, other molecular stimulus-response curves that exhibit a non-Michaelean character in the absence of any cooperative binding. An example is binding of neurotransmitter to post-synaptic receptors. These receptors are typically multimers that function as ion channels. To take a specific case, the receptor of the transmitter acetylcholine has two binding sites. When *both* sites bind transmitter, then the receptor-channel switches to an open state, permitting the influx of calcium. Binding is not cooperative, but the response curve exhibits sigmoidality. (MORE) *Characterizing cooperativity in 14-mer chaperones* As

more experimental information becomes available about protein binding and enzyme action, more sophisticated modeling becomes required. One good example of strong interaction between detailed modeling and careful experimental work concerns the GroEL-GroES system for facilitating protein folding in bacteria. (Similar "chaperone" systems are present in higher organisms.) GroEL contains 14 identical subunits that form a double-heptameric "sandwich". GroES, a heptamer of identical subunits, modulates the activity of GroEL. Yifrach and Horovitz [52] developed a model for GroEL wherein each heptameric ring follows the MWC model in an equilibrium between T and R states. Between the rings there are more general transitions between the TT, TR, and RR states. These transitions are assumed to occur via the "KNF theory" of Koshland, Nemethy and Filmer [22], a generalization of the MWC theory. Theoretical and experimental work by Inbar and Horovitz [19]

demonstrated how groES promotes the T to R transition in GroEL. Other experiments have directly demonstrated the concerted transitions postulated for the heptameric rings [28]. Inbar and Horovitz [19] illustrate an interesting methodology, the characterization of a satisfactory fit between theory and experiment by a random distribution *about zero* of the differences between the theory and and many measurements of the initial velocity of ATP hydrolysis by GroEL as a function of ATP concentration. (Since there is no bias in comparing theory and experiment, it is reasonable to assume that the error is not due to a deficient theory.) Note that this work is yet another illustration of how theories for macroscopic concentration variables can give information on microscopic events, i.e. on molecular mechanism. See White *et al.* [51] for characterization via electron cryo-microscopy of the different molecular states predicted by the macroscopic theory.

Exercises

1. (a) Write down the substrate-product conservation law for scheme (7.1) and use this law to check whether the equations (7.2) have been written correctly.
   [Hint: Make sure that you don't forget a factor of "2" in one of the terms in the conservation law.]
   (b) Verify (7.6) and (7.10).
   (c) Show that in fact it is not a "coincidence" that (7.13) is a special case of (7.10) for $\alpha = 1$.
   (d) Verify that the kinetic scheme (7.16) implies that the fraction of F molecules bound at steady state is given by (7.15).
   (e) From (7.10), express $1/V$ as a function of $1/S$ (Lineweaver-Burk plot). Sketch the graph when $\alpha = 1$.

2. (a) Show from (7.13) that there is at most one positive value of s at which dY/ds vanishes. What is the geometric significance of this result?
   (b) Is it possible that the second derivative defined in (7.13) has three sign changes? If so, sketch the corresponding graph of $V(s)$.
   [Hint: Use "Descartes' rule of signs". See for example [30, Appendix 2].]

3. Discuss the true maximal velocity for scheme (7.1) (maximum of dP/dt as a function of S); (a) when $\alpha > 1$; (b) when $\alpha < 1$.

4. Take the following steps toward deriving conditions for the validity of the QSSA (7.5). (This problem requires knowledge of how to solve a pair of linear ordinary differential equations with constant coefficients.)
   (a) Find t_s by applying (6.15a). Use the fact that $dS/dt \approx -dP/dt$ when the QSSA holds.
   (b) Show that determination of t_C requires study of a certain quadratic equation.
   (c) Study the quadratic equation for "sufficiently small" K'_m. Use Example 2 of Appendix 2 to find an explicit and relatively simple expression for t_C. Thus obtain explicit expressions for the requirement $t_c \ll t_s$.

5. (a) Verify (7.32), (7.34), and (7.36).
   (b) From (7.34) and (7.32), calculate steady state values for all the states. Show that as $S \to \infty$, all concentrations approach zero except R_2, which approaches P.
   (c) Verify that the microscopic reversibility conditions imply that the expressions in (7.45) vanish at steady state.
   (d) Verify the text's assertion concerning the "big loop" in Fig. 7.16.

6. Construct the kinetic scheme, write the relevant equations, and thereby verify (7.46) for $n = 3$.

7. (a) Verify (7.47) by adapting the text's calculations of (7.32) with an enzyme in mind from the beginning. That is, start with a kinetic scheme for enzyme-product formation, write the relevant equations, etc.
   (b) Show that Y_{diff} of (7.50) is positive and thereby verify that the MWC model always yields positive cooperativiy.
   (c) **Project.** Discuss conditions for the validity of the quasi-steady state assumptions that are necessary to obtain (7.47).

8. (a) Verify (7.54).
   (b) Verify (7.57a,b).
   (c) Sketch graphs for (7.57a,b) for $n < 1$ and for $n > 1$.

9. Let B_0 be the concentration of a molecule B when it is free (not bound) and let B_1 be the concentration of a complex of B and a ligand S. The

kinetic scheme is

$$B_0 + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B_1 \ . \tag{7.60}$$

(a) Write the equation for dB_0/dt. Assuming the conservation law

$$B_0 + B_1 = T \ , \tag{7.61}$$

where T is a constant, show that at *steady state*

$$\frac{B_1}{T} = \frac{S}{K+S} \qquad \text{where} \qquad K = \frac{k_{-1}}{k_1} \ . \tag{7.62}$$

Suppose that S also binds to a molecule C such that

$$C_0 + S \underset{\ell_{-1}}{\overset{\ell_1}{\rightleftarrows}} C_1 \ , \qquad C_0 + C_1 = T \ . \tag{7.63}$$

Then there is an analogous steady state result for C_1:

$$\frac{C_1}{T} = \frac{S}{L+S} \qquad \text{where} \qquad L = \frac{\ell_{-1}}{\ell_1} \ . \tag{7.64}$$

If a solution contains an equal concentration T of B and of C, then the fraction of B and C that are bound to S is

$$Y \equiv \frac{B_1 + C_1}{2T} = \frac{1}{2}\left[\frac{S}{K+S} + \frac{S}{L+S}\right] \ . \tag{7.65}$$

Let Q be defined by

$$Y \approx \frac{S}{Q} \text{ for small } S \ . \tag{7.66}$$

(b) Show that

$$Y - \frac{S}{Q+S} = \frac{-\frac{1}{2}(K-L)^2 S^2}{(S+K)(S+L)(S+\frac{2LK}{L+K})} \ . \tag{7.67}$$

(c) Give an intuitive explanation for the result of (7.67) that "negative cooperativity" seems to occur when S is added to an equal mixture of B and C. Why is it biochemically "obvious" that the right side of (7.67) will be zero when $K = L$. Figure 7.18 gives the kinetic scheme for the binding of a ligand S to a protein molecule with two binding

sites. Each site has a fixed binding affinity, i.e., the kinetic coefficients for a given site are the same whether or not the other site is bound.

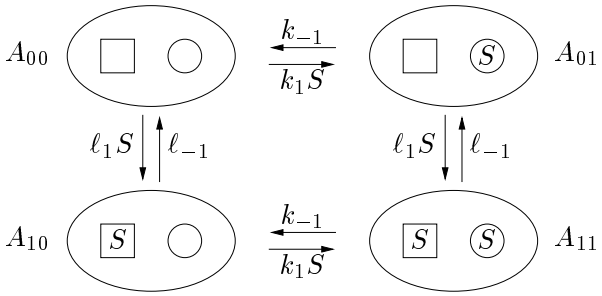

Figure 7.18:

(d) Write the equations for dA_{00}/dt and dA_{10}/dt. By adding these equations, obtain a differential equation that involves B_0 and B_1 where

$$B_0 \equiv A_{00} + A_{10} \ , \qquad B_1 \equiv A_{01} + A_{11} \ . \tag{7.68}$$

If you did the calculations correctly, you will get the same equation for dB_0/dt as you got in part (a). Why is this result "obvious"? What can you conclude about the type of cooperativity that will be seen for binding of a ligand to a protein with two binding sites of different fixed affinities?

10. What intuitive sense can be made of the relation between sigmoidality and cooperativity for (7.10)?

8 Ultrasensitivity in covalent protein modification

Introduction This section can be regarded as continuing the exploration of cooperativity that was begun in Section 9. The focus here is the regulation of the activity of various proteins by covalent binding of a regulatory group and subsequent alteration in the activity of the protein. Covalent modification has been shown to provide control in a variety of activities including metabolism, sensory transduction, muscular contraction and protein synthesis. The most widely occurring type of covalent modification involves binding or removal of a phosphate group. The resulting **phosphorylation** and **dephosphorylation** are catalyzed by enzymes respectively called **kinases** and **phosphatases**. In bacteria, regulation can be mediated by a methyl group. Here the corresponding enzymes are methyltransferase and methylesterase. Other groups can also be involved, but in most of our discussion we will refer to phosphorylation for definiteness. It appears that in many instances evolution has provided controls that are very precise in that processes can be almost completely "turned on" or "turned off" by small changes in the concentration of some effector molecule. (As we shall see later (REF), such switch-like behavior can have far reaching consequences in addition to affording precision of control.) Cooperativity of the type we discussed in Section 9 can provide a fairly steep increase in enzyme activity in response to a moderate increase in substrate level. As we saw, analogous increases in activity are obtained when ligands bind to multi-subunit protein effector molecules. We demonstrate here that covalent modification can in principle provide arbitrarily steep switching. This occurs when the kinases and the phosphotases operate with "zero order kinetics". By this is meant that the velocity of the reaction is proportional to the zeroth-power of the substrate — i.e. the reaction velocity is independent of the substrate concentration. (This occurs when the enzyme is saturated. See for example Eq. (7.10a) when $S \gg K_m$.) Consequently, the phenomenon was termed **zero order ultra-sensitivity** by its discoverers Goldbeter and Koshland [13, 14]. Huang and Ferrell [18] and Ferrell [6] have provided evidence that zero order ultra-sensitivity indeed finds application in biological control. The context is the mitogen-activated protein (MAP) cascade, a controller that plays important roles in living systems ranging from protists to animals. Another

reference reporting experimental confirmation that zero order ultrasensitivity occurs in biological systems is that of LaPorte and Koshland [25]. Here is how one of the authors describes how the idea of zero order ultra-sensitivity arose (A. Goldbeter, private communication). Dan Koshland and I were working on a simple model of a bacterial chemoreceptor which undergoes methylation and demethylation. The parameters were the amount of receptor, and the K_m and $V_{\max}$ (including the total amount) of each of the two enzymes. We derived the expression for the steady state amount of methylated receptor, and found by numerical simulations, to our amazement, that this fraction could sometimes vary from less than 1 per cent to more than 99 per cent upon slightly varying the natural control parameter which is the ratio of maximum rates of methyltransferase to methylesterase. We immediately realized that such a switch-like behavior, which occured when the K_m's of the enzymes were lower than the total amount of receptor (i.e. the enzymes operated under saturated conditions) could be of general significance for regulation through covalent modification of proteins. With this motivation, let us examine Goldbeter and Koshland's [13] model for covalent modification. We shall follow their line of development with a few minor alterations and the addition of some interpretation and some analytical results.

Formulation Let W denote the concentration of the unmodified protein, and W^* the concentration of the modified form. The respective converter enzymes (e.g. kinase and phosphatase) will be denoted by E and F, with C and C^* denoting the concentrations of the corresponding complexes. This gives the kinetic scheme

$$W + E \underset{b}{\overset{f}{\rightleftarrows}} C \xrightarrow{k} W^* + E \ , \tag{8.1a}$$

$$W^* + F \underset{b_*}{\overset{f^*}{\rightleftarrows}} C^* \xrightarrow{k^*} W + F \ , \tag{8.1b}$$

with the corresponding equations

$$dW/dt = -fWE + bC + k^*C^* \ , \tag{8.2a}$$

$$dC/dt = fWE - (b+k)C \ , \tag{8.2b}$$

$$dW^*/dt = -f^*W^*F + b^*C^* + kC \ , \tag{8.2c}$$

$$dC^*/dt = f^*W^*F - (b^*+k^*)C^* \ . \tag{8.2d}$$

Conservation laws for the enzymes are

$$E_T = E + C \ , \tag{8.3a}$$
$$F_T = F + C^* \ , \tag{8.3b}$$

and for the protein

$$W_T = W + W^* + C + C^* \ . \tag{8.4a}$$

An important simplification is the assumption that various other substrates and products that are involved in the modification-demodification process are not appreciably altered during this process and therefore can be regarded as constants. These constants appear implicitly in the rate constants of (8.2). The details of the kinase and phosphotase action are still being worked out. It has long been known that the phosphate group for the phosphorylation comes from the energy-rich molecule ATP. It now appears that the molecule to be phosphorylated can first form a complex with the kinase, to be joined later by an ATP molecule or, alternatively, the first step to the ternary complex can be the binding of ATP to the kinase (Shaltiel, PNAS, Jan 1998). The scheme (8.1) of course does not capture such details, but the essence of the phenomenon under investigation can be explored via this scheme. Another simplification is the assumption that the concentration of the total amount of protein, W_T, is large compared to the enzyme concentrations E_T and F_T. Since $C \leq E_T$, $C^* \leq F_T$ [by (8.3)] (8.4a) can be approximated by

$$W_T = W + W^* \ . \tag{8.4b}$$

We now shall examine conditions at steady state. Consequently, until further notice the left sides of equations (8.2a–d) will be regarded as zero.

Steady-state solution By adding (8.2a) and (8.2b) [or (8.2b) and (8.2c)] we obtain

$$k^*C^* = kC \ . \tag{8.5}$$

Employing (8.5) and (8.4b) we obtain from (8.2a) and (8.2b) respectively (Exercise 1)

$$\frac{C}{E_{_T}} = \frac{1-m}{K_{_E}+1-m} \ , \qquad m = \frac{\alpha C}{E_{_T}}(m+K_{_F}) \ . \tag{8.6a,b}$$

Here

$$m \equiv \frac{W^*}{W_{_T}} \tag{8.7}$$

denotes the fraction of modified protein, the analysis of which is the goal of our theory. [Keep in mind that $m \leq 1$, which is implied by (8.4a).] We have employed the abreviations

$$K_{_E} \equiv \frac{K_{_{ME}}}{W_{_T}} \ , \quad K_{_F} \equiv \frac{K_{_{MF}}}{W_{_T}} \ , \quad K_{_{ME}} \equiv \frac{b+k}{f} \ , \quad K_{_{MF}} \equiv \frac{b^*+k^*}{f^*} \ . \tag{8.8}$$

Note that $K_{_E}$ and $K_{_F}$ are the ratios of the respective Michaelis constants for the two enzymes to the total protein concentration $W_{_T}$. Another parameter in (8.6) is the ratio α of the maximum velocities, $V_{_E}$ and $V_{_F}$, of the two enzymes:

$$\alpha \equiv \frac{V_{_E}}{V_{_F}} \ ; \qquad V_{_E} \equiv kE_{_T} \ , \qquad V_{_F} \equiv k^*F_{_T} \ . \tag{8.9a,b,c}$$

We will regard the fraction of modified protein m as the quantity to be controlled and the ratio α as the instrument of control. Modifying the ratio $E_{_T}/F_{_T}$ of phosphotase to kinase is a biologically straightforward way to alter α. Normally there is a stimulus s that modifies α; s might be a substrate of some reaction, a hormone or a neurotransmitter. As we discuss further below, the overall sensitivity of control is compounded of the sensitivity of m to α and the sensitivity of α to s. If we now employ (8.6a) to replace $C/E_{_T}$ in (8.6b) we obtain the following equation relating α to the fraction of modified protein m:

$$\alpha = \frac{m(K_{_E}+1-m)}{(m+K_{_F})(1-m)} \ . \tag{8.10}$$

Upon rearrangement (8.10) becomes (Exercise 1)

$$(\alpha - 1)m^2 + m\left[1 - \alpha + K_{_F}\left(\frac{K_{_E}}{K_{_F}} + \alpha\right)\right] - K_{_F}\alpha = 0 \ . \tag{8.11}$$

The quadratic equation (8.11) for m can be solved explicitly and the results graphed for various values of the parameters $K_{_E}$, $K_{_F}$ and α. (Exactly one of the two roots satisfies the biological constraints $0 \leq m \leq 1$. See Exercise 4.) This was done by Goldbeter and Koshland [13]. They found that as a function of α, m increased monotonically from 0 to 1. When $K_{_E}$ and $K_{_F}$ were of order unity, the increase was gradual. When both $K_{_E}$ and $K_{_F}$ were small (for example $K_{_E} = K_{_F} = 0.01$) the increase was very sharp, and confined entirely to the vicinity of $\alpha = 1$ ("ultrasensitivity"). See Fig. 7.19. From (8.8) we see that the assumed smallness of $K_{_E}$ and $K_{_F}$ means that when W^* and W are of magnitude $W_{_T}$, both "substrate" concentrations W and W^* are large compared to the relevant Michaelis constants $K_{_{ME}}$ and $K_{_{MF}}$. Thus both enzymes are in the saturating range ("zero order"). As a quantitative measure of steepness in transition Goldbeter and Koshland [13] employed the ratio R of the value of α that gives $m = 0.9$ (protein 90% modified) to the value of α that gives $m = 0.1$. From (8.10)

$$R = \frac{81(K_{_E} + 0.1)(K_{_F} + 0.1)}{(K_{_E} + 0.9)(K_{_F} + 0.9)} \ . \tag{8.12}$$

When $K_{_E}$ and $K_{_F}$ are both large compared to unity, $R = 81$, as in ordinary Michaelean binding (Exercise 2). When $K_{_E}$ and $K_{_F}$ are both small compared to 0.1, however, $R \sim 1$, i.e. only a tiny change in the ratio $V_{_E}/V_{_F}$ "turns on" the protein. *Explaining the ultrasensitivity* Having observed the striking

phenomenon of zero-order ultrasensitivity, the question obviously arises, How can we explain it? In answering this question, it turns out to be useful to return to the time-varying equations, and thereby to see how the solution evolves to the step-like steady states shown in Fig. 7.19 when $K_{_E}$ and $K_{_F}$ are small compared to unity. We first observe that the assumption that the total protein concentration $W_{_T}$ is large compared to both enzyme concentrations suggests that (after a transient) quasi-steady state conditions will obtain. *Provided that the "substrate" concentrations W and W^* are of magnitude $W_{_T}$*, this follows from the sufficient condition of Section 7 for quasi-steady

state: "enzyme concentration $\ll$ substrate concentration". One or two lines of algebra show that the resulting approximation

$$\frac{dC}{dt} \approx 0 \ , \qquad \frac{dC^*}{dt} \approx 0 \tag{8.13a,b}$$

simplifies (8.2a) and (8.2c) to

$$\frac{dW}{dt} = k^*C^* - kC \ , \qquad \frac{dW^*}{dt} = kC - k^*C^* \ . \tag{8.14a,b}$$

Under saturating conditions, only a small fraction of the enzymes will be free; most of E and F will be complexed with W or W^*, respectively. Hence it is to be expected from the conservation laws (8.3) that $C \sim E_{_T}$, $C^* \sim F_{_T}$ [see Exercise 1(b) for a more formal derivation of this approximation]. By (6.9b,c), (8.14) is thus approximated by

$$\frac{dW}{dt} \approx V_{_F} - V_{_E} \ , \qquad \frac{dW^*}{dt} \approx V_{_E} - V_{_F} \ . \tag{8.15a,b}$$

Note that the right hand sides in (8.15) are constants. The key equations (8.15) can be represented by the special kinetic diagram in Fig. 7.20A. The unusually heavy arrows are employed to emphasize the fact that saturated enzymes are responsible for the interchange (catalyzed by enzyme E) between the substrate W and its product W^*, and the reverse interchange (catalyzed by enzyme F) between the substrate W^* and the product W. The consequences of (8.15a) are diagrammed in Fig. 7.20B,C. When $V_{_F} > V_{_E}$, W increases (at a constant rate $V_{_F} - V_{_E}$). When $V_{_F} < V_{_E}$, W decreases. No valid conclusions can be drawn from the "saturated equation" (8.15a) in the shaded areas of the diagram. In these areas, $W \ll W_{_T}$, or $W \approx W_{_T}$ so that $W^* \ll W_{_T}$. When $W \ll W_{_T}$, then enzyme E is not saturated and when $W^* \ll W_{_T}$, then enzyme F is not saturated. Ignoring the shaded areas where no conclusions can be drawn, we can conclude from Figs. 7.20B and 7.20C that the steady state dependence of $W/W_{_T}$ on $V_{_E}/V_{_F}$ is as shown in Fig. 7.20D — a jump from $W/W_{_T} \approx 1$ to $W/W_{_T} \ll 1$ at $V_{_E}/V_{_F} \approx 1$. There is of course a corresponding result for $W^*/W_{_T}$. Thus the essence of zero-order ultra-sensitivity can be deduced from the "saturated equations" (8.15), a rapid switch from $W^* \ll W_{_T}$ to $W^* \approx W_{_T}$ as $V_{_E}/V_{_F}$ passes through unity. The qualitative reasoning based on (8.15) is of course completely in accord with the exact results that were obtained from (8.11). A shaded area

of uncertainty appears in Fig. 7.20D when $\alpha \approx 1$. This is because (8.15) is not expected to be valid when $V_{_E} \sim V_{_F}$, for then the small right side of (8.15) is expected to be of the same magnitude as the small errors made in our various aproximations. *Analytic approximations* As will now be shown,

it is rather easy to derive analytically the qualitative behavior that has been observed. The approach can be used for more complex models when the quadratic (8.11) is replaced by equations that can not be solved explicitly. For simplicity let us consider (8.11) in the special case where

$$K_{_E} = K_{_F} = K \ . \tag{8.16}$$

Since rapid changes are expected near $\alpha = 1$ we write

$$\alpha = 1 + \epsilon \ . \tag{8.17}$$

With the notation of (8.16) and (8.17), the quadratic (8.11) becomes

$$\epsilon m^2 + m\big[K(2+\epsilon) - \epsilon\big] - K(1+\epsilon) = 0 \ . \tag{8.18}$$

Of most interest will be situations wherein $\epsilon \ll 1$. We now derive analytic approximations for appropriate solutions m of (8.18). It is when $K \ll 1$ that ultrasensitivity occurs. Let us therefore approximate (8.18) by setting $K = 0$. We obtain

$$\epsilon m^2 - \epsilon m = 0 \ , \qquad \text{i.e.} \quad m = 0 \text{ or } m = 1 \ . \tag{8.19}$$

¿From Fig. 7.19 we expect $m = 0$ to be an appropriate approximation for certain ranges of ϵ and $m = 1$ for others. There is as yet no clue of this behavior. Let us therefore seek a better approximation. To do this, we shall calculate dm/dK and use the Taylor approximation

$$m(K) = m(0) + \left.\frac{dm}{dK}\right|_{k=0} K + \cdots \ . \tag{8.20}$$

(Reference). Using an approach that is often helpful, *we calculate the required derivative directly from the governing equation* (8.18). We differentiate both sides with respect to K. The right side of (8.18) is zero, so its derivative is zero. We thus obtain

$$2\epsilon m \frac{dm}{dK} + \frac{dm}{dK}\big[K(2+\epsilon) - \epsilon\big] + m(2+\epsilon) - (1+\epsilon) = 0 \tag{8.21a}$$

which yields

$$\left.\frac{dm}{dK}\right|_{K=0} = \frac{1+\epsilon-(2+\epsilon)m(0)}{\epsilon\big[2m(0)-1\big]} \ . \tag{8.21b}$$

Hence

$$\text{when} \quad m(0)=0\text{:} \quad \left.\frac{dm}{dK}\right|_{K=0} = -\frac{1+\epsilon}{\epsilon} \ ; \tag{8.22a}$$

$$\text{when} \quad m(0)=1\text{:} \quad \left.\frac{dm}{dK}\right|_{K=0} = -\frac{1}{\epsilon} \ . \tag{8.22b}$$

¿From the Taylor approximation (8.20), for small K we thus have

$$m \approx \Big(\frac{1+\epsilon}{\epsilon}\Big)K + \cdots \ , \qquad m \approx 1 - \Big(\frac{1}{\epsilon}\Big)K + \cdots \ . \tag{8.23a,b}$$

If $\epsilon > 0$ in (8.23a) then $m < 0$, which is inadmissible since m is the ratio of concentrations. Similarly if $\epsilon < 0$ in (8.23b) then $m > 1$ — which is impossible by the conservation law (8.4b). Thus (8.23a) holds for $\epsilon < 0$ and (8.23b) for $\epsilon > 0$. But however small K is, neither formula can hold when ϵ is very small. To be more precise, for fixed small K the approximations (8.23) cease to satisfy the constraint $0 < m < 1$ when ϵ is smaller than K. This already gives us the overall picture of the function m; it must have a small value approximated by (8.23a) when ϵ is negative ($V_{_E} < V_{_F}$) and a value near unity approximated by (8.23b) when ϵ is positive ($V_{_E} > V_{_F}$). There is a rapid increase from $m \approx 0$ to $m \approx 1$ near $\epsilon = 0$ ($V_{_E} \approx V_{_F}$). To estimate the rapidity of the transition near $\alpha = 1$ let us consider small values of ϵ, for arbitrary values of K. From (8.18)

$$\text{when} \quad \epsilon = 0 \ , \qquad m = \frac{1}{2} \ .$$

Since $\alpha = 1 + \epsilon$, $dm/d\alpha = dm/d\epsilon$. When $\alpha = 1$, $\epsilon = 0$. Thus to find the steepness of the graph of m given implicitly in (8.18), we calculate the derivative with respect to ϵ from (8.18) and then set $\epsilon = 0$:

$$2m\epsilon\frac{dm}{d\epsilon} + m^2 + \frac{dm}{d\epsilon}\big[K(2+\epsilon)-\epsilon\big] + m(K-1) - K = 0 \ , \tag{8.24a}$$

$$\left.\frac{dm}{d\epsilon}\right|_{\epsilon=0} = \frac{-\frac{1}{4} - \frac{1}{2}(K-1) + K}{2K} = \frac{1+2K}{8K} \ . \tag{8.24b}$$

Equivalently

$$\left.\frac{dm}{d\alpha}\right|_{\alpha=1} = \frac{1+2K}{8K} \ . \tag{8.25}$$

According to the present simple model, then, the sensitivity of the ratio m to a change in parameter α (as measured by the derivative) can be arbitrarily large if K is sufficiently small. If α itself is a function of a stimulus s then the overall sensitivity is given by

$$\frac{dm}{ds} = \frac{dm}{d\alpha}\frac{d\alpha}{ds} \ . \tag{8.26}$$

The sensitivity factor $d\alpha/ds$ can thus increase or decrease the overall sensitivity dm/ds. See Goldbeter and Koshland [14] or Exercise ? for examples of the role of $d\alpha/ds$. **Example.** Use (8.24b) to provide a check of the calculations that were used to obtain Fig. 7.19: compare the computed slope at $\alpha = 1$ for $K_{_E} = K_{_F} = 1$ with the calculated slope of the figure. **Solution.** In the original graph, the horizontal axis was labelled at equal intervals with powers of 10: a logarithmic scale was employed. This means that in fact m has been plotted as a function of $\log\alpha$, where by "log" we mean $\log_{10}$, "log to the base 10". Different labelling of the horizontal axis is required when m is regarded as a function of $\log\alpha$. This has been added to the graph, below the original labelling. A straight line has been drawn by eye to be tangent to the curve for $K_{_E} = K_{_F} = 1$ at $\alpha = 1$. The slope of this line is the ratio of the "rise" to the "run". This can be estimated from the right triangle of which the tangent line is the hypotenuse. A ruler gives the ratio "rise/run" — but this is the wrong ratio! A correction must be made to take account of the fact that different units are used for the vertical and horizontal axes. When this is done, a value of approximately 5/6 is obtained for the slope:

$$\left.\frac{dm}{d\log\alpha}\right|_{\alpha=1} \approx 0.83 \ . \tag{8.27}$$

By chain rule

$$\frac{dm}{d\log\alpha} = \frac{dm}{d\alpha}\frac{d\alpha}{d\log\alpha} = \frac{dm}{d\alpha}\Big/\frac{d\log\alpha}{d\alpha} \ . \tag{8.28}$$

We now must recall that it is $\ln\alpha$, not $\log\alpha$, whose derivative is $1/\alpha$. Recalling further that

$$\log_{10}\alpha = \log_e\alpha\frac{\log_{10} e}{\log_{10} 10} \ , \quad \text{i.e.} \quad \log\alpha = \ln\alpha\log_{10} e \ , \tag{8.29a, b}$$

$$\frac{d\log\alpha}{d\alpha} = \frac{\log_{10} e}{\alpha} = \frac{0.434}{\alpha} \ , \tag{8.29c}$$

we obtain

$$\left.\frac{dm}{d\log\alpha}\right|_{\alpha=1} = \frac{\alpha}{0.434}\left.\frac{dm}{d\alpha}\right|_{\alpha=1} = 0.86\ , \tag{8.30}$$

upon employing (8.25) and (8.24) with $K = 1$. There is a satisfactory agreement with (8.27). However, agreement with Fig. 1 of Ref [14] is not satisfactory, and indeed there are quantitative errors in that figure. (This error has no effect on the conclusions of Ref [14].

Exercises

1. (a) Verify (8.6) and (8.11).
   (b) Show that $C \sim E_{_T}$ follows from the quasi-steady state assumption (8.13a) and the assumption $K_{_E} \ll 1$, provided that W is of magnitude $W_{_T}$.

2. Consider the Hill equation (7.39) for the velocity of a maximally cooperative enzymatic reaction, divided by $U_{\max}$. Analogously to the definition used in (8.12), the **response coefficient**, R is defined as the ratio of the values of S required to give, respectively, $V = 0.9$ and $V = 0.1$. Find a formula for R. In particular, show that $R = 81$ in the non-cooperative Michaelean case where $n = 1$.

3. By differentiating (8.21a) find a formula for d^2m/dK^2 when $K = 0$. Thereby improve the approximations in (8.24b).

4. This problem concerns the solutions m to the quadratic equation (8.11). We wish to know which of the pair of solutions of (8.11) satisfy restrictions that make the roots biologically significant, that m is non-negative and is less than or equal to 1.
   To help in our algebraic manipulations we write (8.11) in the abbreviated form
   $$am^2 + bm - c = 0 \tag{8.31}$$
   where $c > 0$ but a and b can be of either sign. Let m_+ and m_- be the two roots of (8.31), respectively taking the + and − signs preceding the square root in the quadratic formula.
   First let us consider the case $a > 0$.
   (a) Show, using the quadratic formula, that $m_- < 0$ but $m+ > 0$. This result is obvious when $b > 0$, but you should also demonstrate this

result when $b < 0$.
(b) Show that $m_+ < 1$ if and only if

$$c < a + b \ . \tag{8.32}$$

(c) Show that (8.32) holds for Eq. (8.11).
Consider $a < 0$.
(d) Show that if $a < 0$ then $b > 0$.
(e) Show that $b^2 + 4ac > 0$ so that both roots of the quadratic are real.
[Hint: Express b in terms of a and c, together with an additional constant.]
(f) Show that both roots are positive but that only $m_+ < 1$.
In summary, precisely one of the two roots, m_+, satisfies $0 < m < 1$.
[Additional hints: It is not true that $r < s$ implies $r^2 < s^2$ (consider $r = -4$, $s = 3$), but it is true if r (and hence s) is positive. This fact can be used to "get rid" of the awkward square root in the various conditions that you derive.
Also, remember that multiplying (or dividing) both sides of an inequality by some constant preserves the direction of the inequality if the constant is positive but reverses the direction of the inequality if the constant is negative.]

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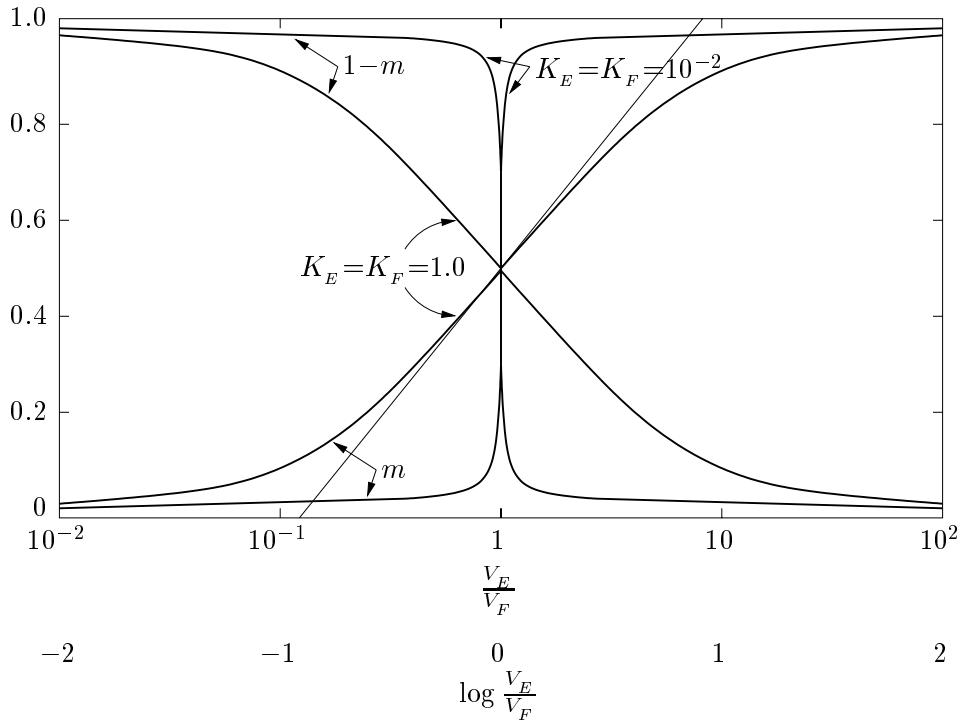

Figure 7.19: Graph of m $(= W^*/W_T)$ and $1 - m$ $(= W/W_T)$ as a function of α $(= V_E/V_F)$ for two sets of parameters. Revised version of Fig. 1 in Goldbeter and Koshland [13]. [fig720lb7]

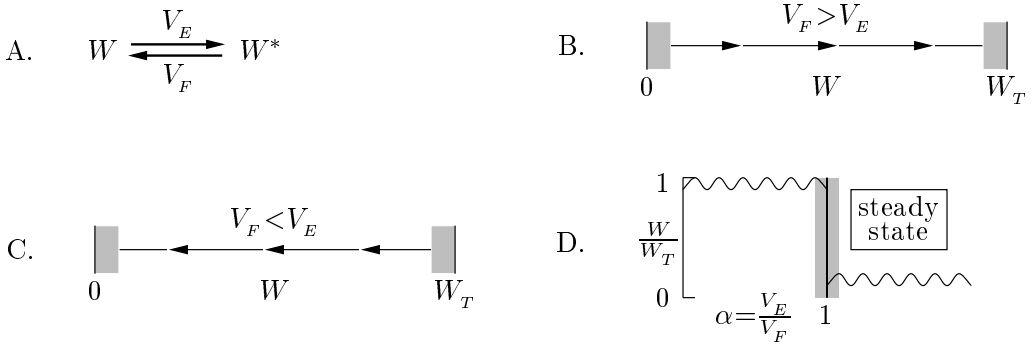

Figure 7.20: A. Diagram representing the essence of zero-order ultrasensitivity, saturated interchange between W and its phosphorylated state W^*. B and C. Consequences of A for W. Arrows indicate direction that W changes. Here and in D, shaded regions indicate regions where A is invalid. D. Implications of B and C for the steady state of W. The wiggly lines indicate uncertainty. [fig721lb7]