CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

1

Fundamental concepts in biochemical reaction theory

Law of mass action

Consider a reaction in which a chemical A of concentration A combines reversibly with a chemical B of concentration B to yield **complex**, C, of concentration C. This reaction is symbolized by

$$\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{C}. \tag{1}$$

The **forward and backward rate constants** k_{+1} and k_{-1} are the proportionality factors in the **law of mass action** that is assumed to describe the process of the reaction. According to this law, the rate at which the species A reacts to form C is proportional to the mass of A, or equivalently, to the number of molecules of A available for reaction. In mathematical terms, the law takes the form of the following differential equations for the concentrations A, B, and C, at time t;

$$\mathrm{d}A/\mathrm{d}t = -k_{+1}AB + k_{-1}C, \qquad \mathrm{d}B/\mathrm{d}t = -k_{+1}AB + k_{-1}C, \quad (2a, b)$$

$$\mathrm{d}C/\mathrm{d}t = k_{+1}AB - k_{-1}C. \quad (2c)$$

In (2a), A is supposed to decrease at a rate jointly proportional to the concentrations of A and B. The idea behind this is again the law of mass action: doubling the concentrations of either A or B will double the rate of collision between these two molecules and hence will double the rate of 'successful' collisions that lead to the formation of C. Such an assumption is plausible as long as the concentrations are not too large. The break-up of an individual C molecule into its constituents is held to occur with a constant probability per unit time.

The phenomenological law of mass action can, in principle, be derived from statistical mechanics, or on a deeper level from quantum mechanics, but this law can be regarded as being well established because of experimental information on a wide variety of theories in the biological, chemical and physical sciences that assume it.

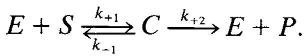
© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

Enzyme–substrate complex system

Enzymes are large molecules that speed up the conversion of a chemical to an altered form. According to the theory of enzymatic reactions of Michaelis & Menten (1913), the enzyme accomplishes this in two steps. First the enzyme (concentration E) reacts reversibly with the chemical, called a **substrate** in this context, to form a **complex** (concentration C). Secondly, the complex breaks apart into an altered substrate or **product** and the original enzyme. This last reaction is often assumed to be irreversible, in which case one writes



$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \xrightarrow{k_{+2}} E + P.$$

The law of mass action for the concentrations $E(t)$, $S(t)$, $C(t)$, and $S(t)$ takes the form

$$dE/dt = -k_{+1}ES + k_{-1}C + k_{+2}C, \quad (3a)$$

$$dS/dt = -k_{+1}ES + k_{-1}C, \quad (3b)$$

$$dC/dt = k_{+1}ES - k_{-1}C - k_{+2}C, \quad (3c)$$

$$dP/dt = k_{+2}C. \quad (3d)$$

The above **system of differential equations** representing the enzymatic conversion of substrates to product was first put forward by Briggs & Haldane (1925). The equation must be supplemented by **initial conditions** that describe the system at some reference time. This time is conveniently designated $t = 0$. The standard initial conditions, which conform to the usual investigation of enzymatically controlled reactions, prescribe starting concentrations of enzyme and substrate, and assume that complex and product have had no opportunity to form:

$$E(0) = E_0, \qquad S(0) = S_0, \qquad C(0) = 0, \qquad P(0) = 0. \quad (4)$$

Addition of (3a) and (3c) yields

$$d(E + C)/dt = 0. \quad (5)$$

Consequently $E + C$ must be a constant, reflecting the fact that at any time t all enzyme molecules are either in their original form or bound in a complex. Using the initial conditions, the constant can be determined, so that we can write the **conservation equation**

$$E(t) + C(t) = E_0. \quad (6a)$$

This equation may be used to eliminate E from (3b) and (3c), leaving two equations for the two unknown functions $S(t)$ and $C(t)$:

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

$$dS/dt = k_{+1}E_0S + C(k_{+1}S + k_{-1}), \tag{6b}$$

$$dC/dt = k_{+1}E_0S - k_{+1}C(S + K_m), \tag{6c}$$

$$K_m \equiv (k_{-1} + k_{+2})/k_{+1}. \tag{6d}$$

Pseudo-steady state: Michaelis–Menten equation

In laboratory experiments, it is typically the case that, at the start, many substrate molecules are present for each enzyme molecule. Under these circumstances one expects that after an initial short transient period there will be a balance between the formation of complex by the union of enzyme and substrate and the breaking apart of complex (either to enzyme and substrate, or to enzyme and product). Because there are so many substrate molecules, this balance will be achieved before there is perceptible transformation of substrate into product. One anticipates, therefore, that calculation of product formation can be carried out under the assumption that $dC/dt = 0$, or, from (3*c*),

$$k_{+1}ES = (k_{-1} + k_{+2})C. \tag{7}$$

This equation is said to result from a **quasi-** or **pseudo-steady state hypothesis**. If any quantity no longer changes with time it is said to be in a **steady state**. We add 'pseudo' or 'quasi' to the description of (7) as a steady state, since although C is fully adjusted to the instantaneous values of E and S, those values are changing slowly with time.

Upon substitution of (6*a*) and (7) into (3*b*), we obtain the following equation for S:

$$dS/dt = -k_2E_0S/(K_m + S). \tag{8}$$

The solution of (8) (by the method of separation of variables) subject to the initial condition $S(0) = S_0$, is

$$S + \frac{k_{-1} + k_{+2}}{k_{+1}} \ln \frac{S}{S_0} = S_0 - k_{+2}E_0t.$$

Of particular interest is the velocity of reaction $V(t)$ defined as the rate of appearance of product. In view of the steady state hypothesis, we have from (6*a*), (7) and (3*d*) that

$$V(t) = dP/dt = k_{+2}C = |dS/dt|. \tag{9}$$

Biochemists are usually interested in $V(t)$ at the beginning of the reaction. From (8) we can write for this **initial velocity** $V_0 \equiv V(0)$,

© Cambridge University Press

Cambridge

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

$$V_0 = \frac{VS_0}{K_m + S_0}, \tag{10a}$$

where

$$V \equiv k_{+2}E_0. \tag{10b}$$

Equation (10*a*) is called the **Michaelis–Menten** equation. Its graph starts from the origin, for the absence of substrate implies the absence of reaction, and approaches the asymptote $V_0 = V$ as S_0 becomes larger and larger (see Figure 1.1). Thus, when S_0 is large compared to K_m and $V_0 \approx V$, there is an abundance of substrate and the 'chemical factory' is working as fast as possible. In such cases the system is said to be **saturated**. Because the constant V is the maximum velocity that the reaction can attain, the term 'V-max' is used to describe it. (The term **Langmuir isotherm** is also associated with (10*a*), which is said to have the form of a **rectangular hyperbola**.)

The biochemical determination of the **Michaelis constant** K_m follows from the observation that when $S_0 = K_m$ then $V_0 = \frac{1}{2}V$. Thus K_m gives the concentration at which the reaction attains its half-maximal value. If this concentration is relatively low, then the reaction is said to be highly **specific**. A relatively low K_m means a relatively large k_{+1} and this in turn means that an enzyme–substrate collision is relatively likely to result in the formation of

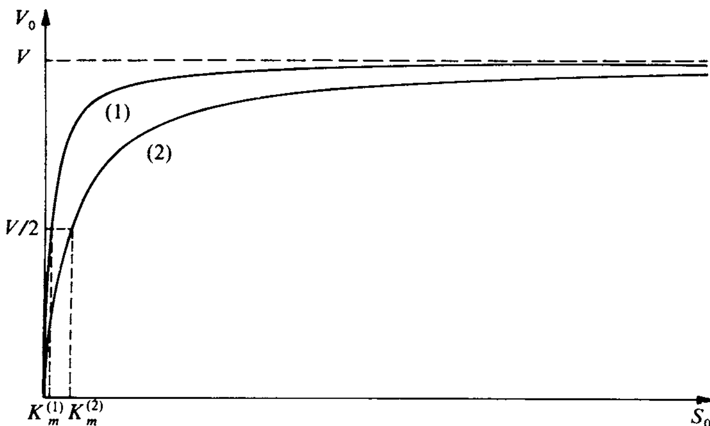


Figure 1.1. Graphs of the Michaelis–Menten equation (10*a*) in two situations with the same maximum velocity V. The reaction represented by curve (1) is more specific than that of curve (2) because the Michaelis constant for it is smaller:

$$K_m^{(1)} < K_m^{(2)}.$$

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

product, i.e. that the enzyme is specifically adapted to act on the particular substrate.

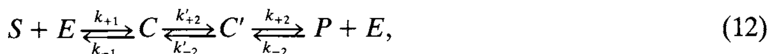
Biochemists frequently rearrange the Michaelis–Menten equation (10*a*) into the **Lineweaver–Burk** or **double-reciprocal** form

$$\frac{1}{V_0} = \frac{1}{V} + \left(\frac{K_m}{V}\right)\frac{1}{S_0}. \tag{11}$$

The graph $1/V_0$ versus $1/S_0$ is thus a straight line, which simplifies the problem of fitting the theory to data. Then $1/V$ and $-1/K_m$ can be found at once as the intersection of this line with the vertical and horizontal axes, respectively (Figure 1.2).

Note from (10*b*) that V depends on the product of the initial enzyme concentration E_0 and the product formation rate constant k_{+2}. This reflects the fact that at high substrate concentrations the speed of reaction depends only on how many reaction units there are, and on how fast they can transform complex into product. Under such circumstances one says that the enzyme is the **rate-limiting chemical** and the complex–product conversion is the **rate-limiting step** in the conversion of substrate to product.

The back reaction for the conversion of complex to product can also be included in the theory. Further, Haldane (1930) has indicated that the reaction of product and substrate should be viewed symmetrically so that the complete set of reactions presumed to take place is represented as



$$S + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \underset{k'_{-2}}{\overset{k'_{+2}}{\rightleftharpoons}} C' \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} P + E, \tag{12}$$

where C is an S–E complex, and C' is a P–E complex. The analysis of this reaction scheme does not alter the form of the Michaelis–Menten equation (10*a*), although the meanings of V and K_m in terms of fundamental rate constants are more complicated than indicated by (10*b*) and (6*d*).

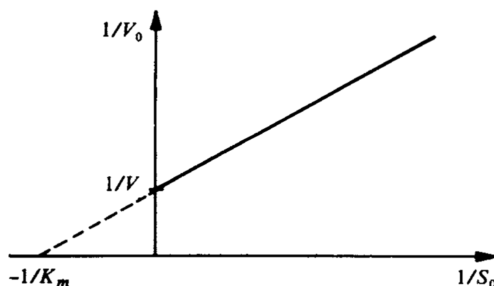


Figure 1.2. The Lineweaver–Burk plot, from which V and K_m can be readily determined. (The dashed part of the line corresponds to 'unphysical' negative substrate concentrations.)

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

The assumption of a pseudo-steady state can simplify a wide variety of kinetic problems. The most elementary application of this assumption yields the Michaelis–Menten equation (10*a*) that is a keystone of theoretical biochemistry. For both of these reasons it is worth carefully working out the conditions under which the pseudo-steady state assumption (7) is expected to be valid.

The key concept here is that of 'time scale', the order of magnitude of time that characterizes the duration of a process or subprocess. For example, what is the time scale of the fast transient process during which the complex concentration changes from its initial value of zero to a pseudo-state condition? Does it take microseconds, milliseconds or seconds? To *estimate* the duration of this period we can make the approximation $S = S_0$ in (6*c*). This transforms (6*c*) into a linear equation, with the solution

$$C(t) = \bar{C}[1 - \exp(-\mu t)], \qquad \mu \equiv k_{+1}(S_0 + K_m),$$
$$\bar{C} \equiv E_0 S_0/(K_m + S_0). \qquad (13a, b, c)$$

Thus the *complex* (*fast*) *time scale* is given by $t_C = \mu^{-1}$:

$$t_C = [k_{+1}(S_0 + K_m)]^{-1}. \qquad (14)$$

Now let us estimate the *substrate* (*slow*) *time scale* t_S, namely how long it takes for a significant change to occur in the substrate concentration. We employ the characterization (Segel 1984, p. 56).

$$t_S \approx \frac{\text{total change in } S \text{ after fast transient}}{\max |\mathrm{d}S/\mathrm{d}t| \text{ after fast transient}}. \qquad (15)$$

The numerator of (15) is approximately S_0. Assuming the validity of the steady state assumption, we observe that the denominator is given by (8) with $S = S_0$. Thus $t_S \approx S_0/[k_{+2}E_0S_0/(K_m + S_0)]$, i.e.

$$t_S = (K_m + S_0)/k_{+2}E_0. \qquad (16)$$

One necessary criterion for the validity of the pseudo-steady state assumption is that the 'fast transient' is indeed brief compared to the time during which the substrate changes appreciably. This criterion is $t_C \ll t_S$ or, from (14) and (16)

$$\frac{E_0}{K_m + S_0} \ll \left(1 + \frac{k_{-1}}{k_{+2}}\right)\left(1 + \frac{S_0}{K_m}\right). \qquad (17)$$

A second criterion concerns the 'initial' condition $S(0) = S_0$ that is imposed on (8). For this condition to be approximately valid there must be only a negligible decrease in substrate concentration during the duration t_C

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

of the brief transient. This decrease, which we denote by ΔS, is certainly less than the product of the time duration t_C and the initial (maximal) rate of substrate consumption. 'Initial' in the previous sentence refers to the very beginning of the experiment, so that the desired rate is obtained by setting $t = 0$ in (6*b*). This yields

$$\left|\frac{\Delta S}{S_0}\right| = \frac{1}{S_0}\left|\frac{dS}{dt}\right|_{\max} \cdot t_C = \frac{E_0}{K_m + S_0}. \tag{18}$$

The requirement that $|\Delta S/S_0|$ be small compared to unity is thus expressed by

$$\varepsilon \ll 1, \qquad \text{where } \varepsilon \equiv \frac{E_0}{K_m + S_0}. \tag{19}$$

If (19) holds then (17) holds. Thus $\varepsilon \ll 1$ is a simple criterion for the validity of the pseudo-steady state assumption.

For considerable further discussion along the above lines see Segel (1988) and Segel & Slemrod (1989).

References

Briggs, G. E. & Haldane, J. B. S. (1925). A note on the kinetics of enzyme action. *Biochem. J.* **19**, 338–9.

Haldane, J. B. S. (1930). *Enzymes*, 2nd edn, London, Longmans, Green (reprinted by MIT Press, Cambridge, Mass. 1965), Chapter 5.

Michaelis, L. & Menten, M. L. (1913). Die kinetik der Invertinwirkung. *Biochem. Z.* **49**, 333–69.

Segel, L. A. (1984). *Modeling dynamic phenomena in molecular and cellular biology*, Cambridge, Cambridge University Press.

Segel, L. A. (1988). On the validity of the steady state assumption of enzyme kinetics. *Bull. Math. Biol.* **50**, 579–93.

Segel, L. A. & Slemrod, M. (1989). The quasi-steady state assumption: a case study in perturbation. *SIAM Rev.* **31**, 446–77.

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

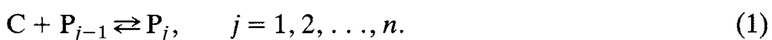
2

Equilibrium binding of macromolecules with ligands

Theory of equilibrium dialysis

A basic and important method for studying the reaction of a protein P with a small molecule or ion C is **equilibrium dialysis**. In it, a known amount of the macromolecular protein is placed in solution inside a membrane bag that is suspended in a solution of the small molecule with which it is capable of reacting (the **ligand**). The membrane is permeable to the ligand, but impermeable to the macromolecule. Available membranes possessing such a permeability property require the molecular weight of the macromolecule to be greater than 10 000. The solution is allowed to stand for a sufficiently long time, of the order of one or two days, for the ligand to permeate the membrane and react with the protein so that equilibrium is established. At equilibrium, there exists both bound and unbound components of the ligand. Furthermore, the unbound concentrations on both sides of the membrane are equal to each other (if the ligand is an ion, electrical effects must be neutralized for the equality to hold true). By measuring the equilibrium concentrations outside the bag at the beginning and end of the equilibrium dialysis experiment, the bound ligand concentration is readily determined as the difference of those two quantities.

We present here the theory of this simple experimental procedure. We shall asume that the protein possesses n binding sites for the ligand, where n is an integer greater than unity in usual cases of interest. Let us denote by P_j the complex of a protein molecule with j ligand molecules attached, where $j = 0, 1, 2, \ldots, n$ (P_0 represents the bare protein). Then the reactions leading to equilibrium are as follows,



$$C + P_{j-1} \rightleftarrows P_j, \qquad j = 1, 2, \ldots, n. \qquad (1)$$

Note that we have tacitly assumed that all complexes consisting of exactly j ligands attached are the same, regardless of the set of j attachment sites. We denote by italic lower case letters the concentrations of quantities rep-

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

resented by capital letters. Let us consider mathematically the first reaction above for $j = 1$. Employing the law of mass action, we express the time-dependent behavior of the concentration of one of these reactants, say p_0, as

$$\mathrm{d}p_0/\mathrm{d}t = -k_{+1}p_0c + k_{-1}p_1. \tag{2}$$

At equilibrium, p_0, c, and p_1 are no longer time dependent and attain constant values which are interrelated, according to (2), by the relation

$$0 = -k_{+1}p_0c + k_{-1}p_1. \tag{3}$$

It is customary to define the **association constant** K_a as

$$K_a \equiv k_{+1}/k_{-1}, \tag{4}$$

although for some purposes, it is found more convenient to utilize its inverse, the **dissociation constant** $K_d = 1/K_a = k_{-1}/k_{+1}$. Either one of these may be referred to as the **equilibrium constant**, although a certain ambiguity is thereby introduced by this usage if no further clarification is made. According to equations (3) and (4), at equilibrium

$$K_a = p_1/p_0c. \tag{5}$$

To describe now the full set of reactions (1) at equilibrium, we introduce, for uniformity of notation, the n association constants $K_j, j = 1, 2, \ldots, n$, by definitions analogous to (4). Generalizing (5)

$$K_1 = \frac{p_1}{cp_0}, K_2 = \frac{p_2}{cp_1}, \ldots, \qquad K_j = \frac{p_j}{cp_{j-1}}, \ldots, K_n = \frac{p_n}{cp_{n-1}}. \tag{6}$$

The quantities $p_0, p_1, \ldots, p_n$ are not usually experimentally determinable, but it is possible to find the average number of molecules of C associated with each macromolecule. This number is denoted by r and is defined as

$$r \equiv \frac{\text{total number of molecules of C combined with P}}{\text{total number of molecules of P}}. \tag{7}$$

We shall call r the **mean association function**. The numerator and denominator above are experimentally measurable quantities, as already indicated. Because there are j ligand molecules attached to each molecule P_j, r is expressible as

© Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
0521391849 - Biological Kinetics
Edited by Lee A. Segel
Excerpt
More information

$$r = \frac{p_1 + 2p_2 + 3p_3 + \ldots np_n}{p_0 + p_1 + p_2 + \ldots + p_n}, \tag{8}$$

or, using (6),

◆◆
$$r = \frac{K_1c + 2K_1K_2c^2 + 3K_1K_2K_3c^3 + \ldots + nK_1K_2\ldots K_nc^n}{1 + K_1c + K_1K_2c^2 + \ldots + K_1K_2\ldots K_nc^n}. \tag{9}$$

The above result is known as **Adair's equation** (Adair, 1925). A related quantity frequently utilized is the **saturation function** Y defined as the mean fraction of sites per protein molecule that are occupied, or

$$Y = r/n. \tag{10}$$

Identical independent sites

A significant simplification occurs when the binding sites in the protein are identical. Further, assume that binding at a given site is independent of the state of binding of all other sites. That is to say, let k_+ be the forward rate constant for attachment of a ligand molecule at a particular binding site, and let k_- be the associated backward rate constant. In terms of these rate constants, (3) assumes the form

$$0 = -nk_+p_0c + k_-p_1. \tag{11}$$

The factor n appears because there are n possible ways to form the state P_1 from the state P_0 (n available sites of attachment of the ligand molecule). Contrariwise, there is only one way for the ligand to be removed from the state P_1 to form the state P_0 (the ligand molecule is removed at its site of attachment). By a similar argument, we see that equilibrium between the states P_1 and P_2 is described as

$$0 = -(n-1)k_+p_1c + 2k_-p_2, \tag{12}$$

i.e. there are $n - 1$ empty sites available for ligand binding in the state P_1, and two ways to remove a ligand molecule from the state P_2. Hence, with the **intrinsic association constant** K defined as

$$K \equiv k_+/k_-, \tag{13}$$

we infer that $K_1 = nK$, $K_2 = (n-1)K/2$, and, in general (Bjerrum, 1941),

$$K_j = (n - j + 1)K/j, \qquad j = 1, 2, \ldots, n. \tag{14}$$

Thus, the assumption that the binding sites of the protein are identical and independent is equivalent to the assertion that the intrinsic binding constant at one site is the same as at any other site, and moreover is unaffected by the

© Cambridge University Press www.cambridge.org