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Analysis of a Model for Active Transport*

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Abstract. A particular model of active transport is analyzed, at steady state, by means of a diagram method for the kinetics, and supplemented by use of a free energy diagram. It is assumed that there is cyclic adsorption of adenosine triphosphate (ATP), chemical reaction to adenosine diphosphate (ADP), and desorption of ADP, on a two-state protein (the "carrier"). ATP is the "effector" for the change in state of the protein.

One of the most fundamental molecular processes in biology is translocation.[1] Examples occur in active transport, muscle contraction, protein synthesis, etc. It is essential that the molecular transporting step in the translocation mechanism be capable of steady repetition. That is, the mechanism must be cyclic. Furthermore, a free-energy source is needed to "drive" the steady-state cyclic mechanism, and the free energy source itself must be able to participate in a repetitive or cyclic manner.

For example, suppose that the transport is actually accomplished by a conformational change in a protein (the "carrier"), and that the conformational change is induced by binding ATP on the protein. Then it is clearly essential that the protein molecule be able quickly to rid itself of the ATP so that the process may be repeated. That is, binding of the "effector" molecule, ATP, is a necessary step but is not sufficient for a workable system. Disposal of the ATP can be accomplished, however, if the protein is also an enzyme for a chemical reaction (ATP $\rightarrow$ ADP + P) the products of which are relatively weakly bound to the protein.

Furthermore, if the concentrations of ATP, ADP, and P in the surrounding medium (where the reaction is negligibly slow) are maintained at suitable values far out of equilibrium with each other, the effector of the conformational change (ATP) can also serve as the free energy source for the steady-state cycling. The net effect of the whole process, then, is molecular translocation at the expense of conversion of ATP in the medium into ADP and P.

Everything considered, this cyclic repetition of adsorption, chemical reaction, and desorption would seem to be an admirably simple method for promoting translocation, using an enzyme molecule as a vehicle or carrier. Of course, it remains to be seen whether this scheme is actually used or not.

Our object in the present paper is to analyze a simplified model of active transport as an example. One generally assigns certain properties to a model. It is important then to examine the implications of the assumed properties in suf-

ficient detail to avoid inconsistencies and possibly to discover implicit characteristics which may or may not be in agreement with experiment. We shall illustrate considerations of this sort below.

The Model. Let us start with the rather complicated scheme[1] in Figure 1, and then simplify to a stripped-down version which, however, retains the essential features.

We assume that the membrane contains an ensemble of independent and equivalent protein molecules, each of which can be in conformational state I or II. The membrane separates bath A from bath B. When in state I(II), adsorption or desorption of the molecule to be transported (designated by O) and of T (abbreviation for ATP) or D (i.e , ADP) may occur from or into bath $A(B)$. There is one site for O per protein molecule, and one site for either T or D.

Actually, the kind of steady-state analysis used below (based on the diagram method) is still applicable when the protein molecules interact with each other (i.e., are not independent)—provided the Bragg-Williams approximation[2] is used. Cooperative behavior, resulting from interactions, may possibly be involved in "triggered" membrane phenomena (e.g., in nerve). But this topic will be reserved for another paper.

For simplicity, we assume that T (also D) has the same concentration in baths A and B. But in general O has different concentrations in the two baths. We shall assume that ATP splitting is coupled in such a way as to aid the transport of O *from* bath A *to* B.

Figure 1 shows the 12 possible states of a protein molecule in the membrane. The notation is rather obvious: for example, ${}_{T}{}^{O}\mathrm{I}$ represents a protein molecule in conformation I with one molecule of O and one of T bound to it. The fact that O and T are on the left side of I indicates access to bath A (similarly, right side $\leftrightarrow$ bath B). A line in Figure 1 indicates a possible transition between two states in either direction. At steady state, there must be an over-all counterclockwise circulation[1] around the diagram (Fig. 1) in order to transport O from bath A to B (at the expense of $T \rightarrow D + P$ in solution).

Figure 1, however, is too complex for brief analysis. We therefore simplify this to Figure 2. In the latter figure we are concerned only with the machinery of the transport and not with the objects being transported (i.e., the molecules O). These "passengers" introduce complications without altering the fundamental properties of the transport system itself.

The state ${}_{D}\mathrm{I}$ is omitted from Figure 2 because of two assumed properties of

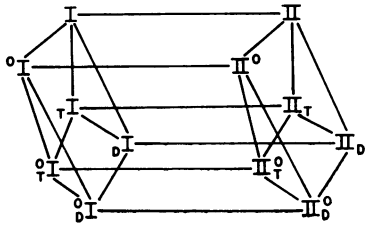


FIG. 1.—States and transitions for protein molecule in a membrane.

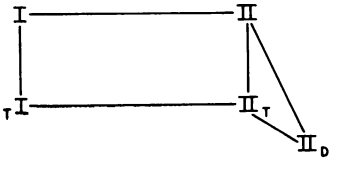


FIG. 2.—Simplified version of Fig. 1.

the model: (1) D does not bind well on either I or II; and (2) II is a good enzyme for $T \rightarrow D + P$, but I is not. Hence ${}_D\mathrm{I}$ will have a negligible probability at steady state. We do not omit the state II_D from Figure 2 because this state is readily reached via the transition $\mathrm{II}_T \rightarrow \mathrm{II}_D$.

There are additional assumed properties of the model: (3) I is more stable (thermodynamically) than II; (4) II_T is more stable than ${}_T\mathrm{I}$ (i.e., T is the "effector" for the transition from conformational state I to II); and (5) T binds well on I. The binding of T on II requires special discussion (see below).

The above properties have been chosen so that the cycle

$$\rightarrow \mathrm{I} \rightarrow {}_T\mathrm{I} \rightarrow \mathrm{II}_T \rightarrow \mathrm{II}_D \rightarrow \mathrm{II} \rightarrow \tag{1}$$

will be particularly effective in the active transport.

Further Properties of the Model. We assume that the concentrations of T, D, and P, under biological steady-state conditions, are roughly[3] $c_T = 2.5$ mM, $c_D = 0.2$ mM, and $c_P = 5$ mM. We designate the corresponding so-called absolute activities ($\lambda \equiv e^{\mu/kT}$) by λ_T, λ_D, and λ_P. The λ's are proportional to concentrations at low concentrations; they are proportional to concentration activities at any concentration. If D were in equilibrium with T and P at their concentrations as just specified, we would have $\lambda_D{}^e = \lambda_T/\lambda_P$. As we shall see, $\lambda_D{}^e \gg \lambda_D$.

The ratio c_D/c_T at steady state is about 0.08. Using c_P above and[4] ΔG° ($T \rightarrow D + P$) $\cong -7.4$ kcal mole^{-1}, this ratio[5] at equilibrium would be about 3×10^7. Thus, $\lambda_D{}^e/\lambda_D \cong 3 \times 10^7/0.08 = 4 \times 10^8$. The fact that, at steady state, D is so far out of equilibrium (for the given c_T and c_P) is, of course, what provides the steady-state "drive" of the active transport.

Because $\lambda_D \ll \lambda_D{}^e$ and because of the assumption mentioned above of poor binding of D on II, we shall consider below that the rate constant for adsorption of D onto II at steady state ($c_D = 0.2$ mM) is negligibly small, though at equilibrium this rate constant would be very large.

Let us denote the equilibrium partition functions of a protein molecule (including a bound molecule of T or D, when applicable) in the different states in Figure 2 by Q_1, Q_2, Q_{1T}, Q_{2T}, and Q_{2D}. The equilibrium probabilities of the different states would then be[6] (omitting normalization and writing $\lambda_D{}^e = \lambda_T\lambda_P{}^{-1}$)

$$\begin{aligned} &p_1{}^e \sim Q_1,\ p_2{}^e \sim Q_2,\ p_{1T}{}^e \sim Q_{1T}\lambda_T \\ &p_{2T}{}^e \sim Q_{2T}\lambda_T,\ p_{2D}{}^e \sim Q_{2D}\lambda_T\lambda_P{}^{-1}. \end{aligned} \tag{2}$$

In view of the assumptions and discussion above, the relative probabilities or populations of the different states at equilibrium would be

$$p_{2D}{}^e \gg p_{2T}{}^e \gg p_{1T}{}^e > p_1{}^e \gg p_2{}^e. \tag{3}$$

That is; because of[7] assumption (3), $Q_1 \gg Q_2$; because of assumption (4), $Q_{2T} \gg Q_{1T}$; because of assumption (5),[8] $Q_{1T}\lambda_T > Q_1$; and because $D + P$ is presumably much more stable than T, even when D and T are on the enzyme, $Q_{2D} \gg Q_{2T}\lambda_P$. Note that this last inequality refers, formally, to "binding" of P onto II_D. In the usual vernacular of surface chemistry this is "chemisorption," whereas the binding of T and D is "physical" adsorption.

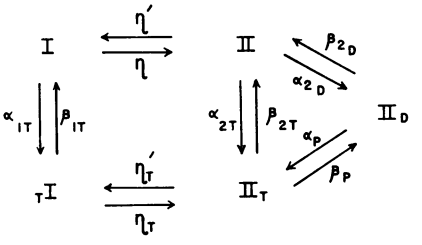


FIG. 3.—Assignment of rate constants for Fig. 2.

Figure 3 shows the rate-constant notation to be adopted here. These are all unimolecular rate constants (with units $\sec^{-1}$). Detailed balance at equilibrium provides useful relations. For example, $\eta' p_2^e = \eta p_1^e$. Hence, from equations (2), $\eta' Q_2 = \eta Q_1$. Thus we obtain the following:

$$\begin{aligned} \eta' Q_2 &= \eta Q_1 & \alpha_{2T} Q_2 &= \beta_{2T} Q_{2T} \lambda_T \\ \alpha_{1T} Q_1 &= \beta_{1T} Q_{1T} \lambda_T & \alpha_{2D}{}^e Q_2 &= \beta_{2D} Q_{2D} \lambda_D{}^e \\ \eta_T Q_{1T} &= \eta'_T Q_{2T} & \alpha_P Q_{2D} &= \beta_P Q_{2T} \lambda_P. \end{aligned} \tag{4}$$

The η's and β's are temperature dependent; the α's are concentration dependent as well. In fact, as we see in equations (4), each α is proportional to the appropriate λ. Not all of the rate constants are independent of each other because of the interrelations

$$\begin{aligned} \alpha_{2D}{}^e \alpha_P \beta_{2T} &= \alpha_{2T} \beta_P \beta_{2D} \\ \eta \alpha'_{1T} \eta_T \beta_{2T} &= \alpha_{2T} \eta'_T \beta_{1T} \eta. \end{aligned} \tag{5}$$

These follow from the triangular and square cycles in Figure 3, respectively.

Equations (4) apply at equilibrium. They also apply at steady state ($\lambda_D \neq \lambda_D{}^e$) if we omit the superscript on $\alpha_{2D}{}^e$ and on $\lambda_D{}^e$. In fact, the steady state of primary interest (see above) is $\lambda_D \cong 0$ and hence $\alpha_{2D} \cong 0$.

Implications of the Model. Figure 4 is a standard free-energy diagram showing the relative free-energy levels of the various states we have discussed. The quantitative features of this particular diagram are somewhat arbitrary, but it should serve as a reasonable illustration.

The standard states used in Figure 4 are: T in solution, a_T (concentration activity) = 1 M; D in solution, a_D = 1 M; T or D bound on I or II, half-saturation[9] ($\theta = 1/2$). The phosphate concentration is taken throughout as c_P = 5 mM.

The standard free energies for the protein species are, of course, closely related to the Q's in equation (2). For example, the top few pairs of free energy differences in Figure 4 can be written:

$$G_2^\circ - G_1^\circ = RT \ln (Q_1/Q_2), \tag{6}$$

$$(G_T^\circ + G_1^\circ) - G_{1T}^\circ = G_T^\circ + RT \ln (Q_{1T}/Q_1), \tag{7}$$

$$G_{1T}^\circ - G_{2T}^\circ = RT \ln (Q_{2T}/Q_{1T}), \tag{8}$$

$$(G_P + G_{2D}^\circ) - G_{2T}^\circ = G_P + RT \ln (Q_{2T}/Q_{2D}), \quad (9)$$

where G_T°, G_D°, and G_P refer to $T(a_T = 1\ \text{M})$, $D(a_D = 1\ \text{M})$, and $P(c_P = 5\ \text{mM})$ in solution.

The following facts or assumptions have been used in constructing Figure 4:

(1) ΔG° for $T \rightarrow D + P$ in solution, all species at $a = 1$ M, is[4] -7.4 kcal mole^{-1} (37°C, pH 7, Mg^{++}). With $c_P = 5$ mM, the "corrected" $\Delta G^\circ = -10.7$ kcal mole^{-1}.

(2) We assume, rather arbitrarily, that ΔG° for both II $\rightarrow$ I and ${}_T\text{I} \rightarrow \text{II}_T$ is -2.0 kcal mole^{-1}. The exact value is not crucial.

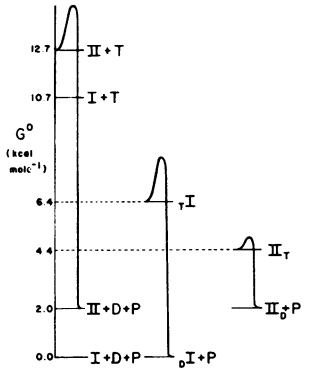


FIG. 4.—Standard free-energy level diagram.

(3) Binding of D on I or II is supposed to be weak (see above). We assume, in both cases, that half saturation ($\theta = 1/2$) occurs at $a_D = 1$ M. Then, at $c_D = 0.2$ mM, we would have[6] $\theta = 2 \times 10^{-4}$ (almost no binding).

(4) Binding of T on I is supposed to be strong. We assume in Figure 4 that $c_T = 1$ mM gives half saturation. The (standard) free energy of T at $a_T = 1$ M would then be 4.3 kcal mole^{-1} higher than at $c_T = 1$ mM (37°C). Also at $c_T = 2.5$ mM (see above), we would have[6, 8] $\theta = 2.5/3.5 = 0.71$.

The precise meaning of "half saturation," as used in Figure 4, is most readily made clear in terms of the *process* of binding. For example:

$$2 \text{ moles of I} + 1 \text{ mole of } T(a_T = 1\ \text{M}) \rightarrow 2 \text{ moles of equimolar mixture } (\text{I}, {}_T\text{I}); \quad \Delta G^\circ = -4.3 \text{ kcal}. \quad (10)$$

Figure 4 is based on a "package" of desirable properties (consistent with a mechanism which is dominated by cycle (1)). However, an undesirable thermodynamic consequence is apparent from the figure: the binding of T on II is very strong ($\Delta G^\circ = -8.3$ kcal mole^{-1}). The objectionable aspect of this property is that the cycle

$$\rightarrow \text{II} \rightarrow \text{II}_T \rightarrow \text{II}_D \rightarrow \quad (11)$$

is wasteful (i.e., it uses T but performs no transport) and would compete seriously with cycle (1). We are therefore forced, in the interests of a realistic efficiency, to assume that kinetic (rather than thermodynamic) considerations greatly reduce or rule out the use of cycle (11). This would be the case, for example, if T is somewhat embedded in II (but not in I). Thus, though T adsorbed on II would be very stable thermodynamically, there would be a significant activation free energy for adsorption as well as for desorption—and both rate constants would be small. For this reason we shall assume below that $\beta_{2T} \cong 0$ and $\alpha_{2T} \cong 0$, though this is probably an oversimplification.

The three activation free energy curves shown in Figure 4 are strictly schematic. They are meant to indicate that the reaction $T \rightarrow D + P$ is very slow in

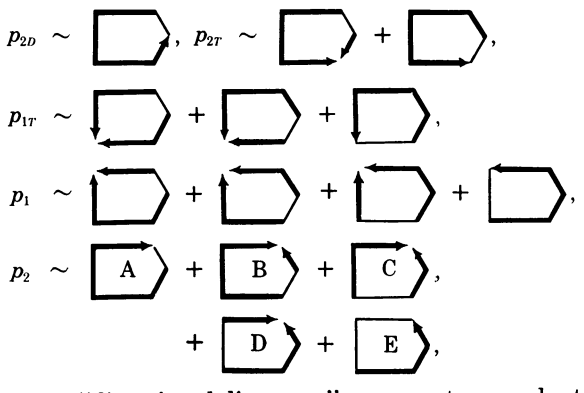
solution and on I, but that II serves as an enzyme for this reaction (i.e., the free energy of activation is greatly reduced on II).

The kinetic properties mentioned in the two preceding paragraphs might possibly originate in the following plausible way. The site for T on I is quite open (i.e., at the surface) and the fit is rather good, except that the enzymatically active region of the protein molecule does not make contact with the terminal P-P portion of T. Binding is probably partly due to electrostatic forces (negative P-P-P; positive site). D is bound less well on the site than T because D does not fit as well as T and because D is less negatively charged. The conformational change ${}_T\mathrm{I} \rightarrow \mathrm{II}_T$ may well be triggered by electrostatic interactions. In any case, the result of the conformational change is to: (1) shift the access of T on the site from bath A to B; (2) partially bury or embed the T molecule in the protein in such a way that the passage of T into or out of the site is slow, while the somewhat smaller D molecule is not seriously impeded;[10] (3) increase the contact and improve the fit (van der Waals forces, etc.) of T in the site—in particular, the active region of the enzyme comes into close contact with the terminal P-P of T; and (4) produce little or no effect on the strength of binding of D.

Kinetic Analysis. For reasons previously mentioned, we would expect α_{2D}, α_{2T}, and β_{2T}, in Figure 3, to be relatively small quantities. We therefore simplify Figure 3 considerably by setting these three rate constants equal to zero. The result is Figure 5.

Our object here is to derive equations for the relative population of the five states in Figure 5, at steady state, and for the steady-state flux around the cycle.

Let p_1, p_2, etc., be the steady-state probabilities. Then[11]



(12)

where each of these "directional diagrams" represents a product of four rate constants which can be read off of Figure 3. For example, the last diagram (E) represents $\alpha_{1T}\eta_T\beta_P\beta_{2D}$. If we use the detailed-balanced relations

$$\eta' p_2^e = \eta p_1^e \qquad \eta_T p_{1T}^e = \eta'_T p_{2T}^e$$
$$\alpha_{1T} p_1^e = \beta_{1T} p_{1T}^e \qquad \beta_P p_{2T}^e = \alpha_P p_{2D}^e \tag{13}$$

to "reverse the direction" of (i.e., to eliminate) η', α_{1T}, η_T, and β_P in the first four of equations (12), we find

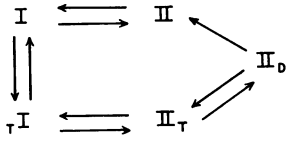


Fig. 5.—Simplified version of Fig. 3.

the alternative expressions

$$\begin{aligned} p_{2D} &\sim p_{2D}^{e}(A), \; p_{2T} \sim p_{2T}^{e}(A + B), \\ p_{1T} &\sim p_{1T}^{e}(A + B + C), \\ p_1 &\sim p_1^{e}(A + B + C + D), \\ p_2 &= p_2^{e}(A + B + C + D + E). \end{aligned} \tag{14}$$

The p^e's here occur in decreasing order, according to equation (3). However, this order is probably "neutralized" and even to some extent reversed for the steady-state p's by the factors in parentheses (see eq. (16)). For not only is a new term added in each successive parenthetical expression, but there is a strong tendency for the terms to increase in the order A, B, C, D, E, in view of equations (3) and (13). That is, the counterclockwise rate constant is the larger one of each pair.

Although it is difficult to make precise statements about the relative magnitudes of the steady-state p's without a complete specification of the rate constants, the following is a useful approximation. Because counterclockwise rate constants are larger, the last diagram in each of equations (12) dominates. If we neglect all other diagrams (i.e., "back-flow" is negligible), we have

$$\begin{aligned} &p_{2D} \sim \beta_{2D}^{-1}, \; p_{2T} \sim \beta_P^{-1}, \; p_{1T} \sim \eta_T^{-1} \\ &p_1 \sim \alpha_{1T}^{-1}, \; p_2 \sim \eta'^{-1}. \end{aligned} \tag{15}$$

Of these rate constants, we might expect β_P (chemical reaction) to be smallest, η_T and η' (conformational change) to be intermediate, and β_{2D} and α_{1T} (desorption and adsorption) to be largest. Then we would have

$$p_{2T} > (p_{1T}, p_2) > (p_{2D}, p_1). \tag{16}$$

Incidentally, a general result of the form (14), at steady state, would be found for any sequence of states in a single cycle with one rate constant absent (i.e., with value zero) and with all other rate constants having their equilibrium values,[12] as in Figure 5.

The steady-state flux around the cycle (for example, the net number of T molecules hydrolyzed per second) is simply

$$J = \beta_{2D} p_{2D} N, \tag{17}$$

where N is the number of protein molecules in the membrane and p_{2D} (normalized) is given by equations (12) or (14). In the approximation (15), we have the simple result

$$J \cong N/(\beta_{2D}^{-1} + \beta_P^{-1} + \eta_T^{-1} + \alpha_{1T}^{-1} + \eta'^{-1}). \tag{18}$$

The author is indebted to Professor Cedric Davern for his helpful suggestions.

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[1] The present paper is in some respects a sequel to Hill, T. L., these PROCEEDINGS, **64**, 267 (1969).

[2] See, for example, R. Gordon, *J. Chem. Phys.*, **49**, 570 (1968); Hill, T. L., and Y. Chen, these PROCEEDINGS (in press).

[3] Burton, K., and H. A. Krebs, *Biochem. J.*, **54**, 94 (1953).

[4] Mahler, H. R., and E. H. Cordes, *Biological Chemistry* (New York: Harper and Row, 1966), p. 201.

[5] Actually, this would be the ratio of concentration *activities* a_D/a_T (where $a \to c$ as $c \to 0$).

[6] See Hill, T. L., *Statistical Thermodynamics* (Reading, Mass.: Addison-Wesley, 1960), chapter 7.

[7] For example, if the free-energy difference is 2 kcal mole^{-1}, $Q_1/Q_2 = e^{3.24} = 26$. See equation (6).

[8] This inequality implies[6] that there is more than half saturation of I with T at λ_T (at equilibrium).

[9] See, for example, equations (7-8) and (7-9) in reference 6: when $\theta = 1/2$, $\mu = -kT \ln q =$ standard free energy per bound molecule.

[10] However, the simplification $\alpha_{2D} = 0$, used below in the kinetic analysis, would be further justified if the adsorption of D is hindered somewhat. Although β_{2D} would also be reduced in value by the same free energy barrier, this would not have a serious effect on the flux J, according to the approximation (18).

[11] Hill, T. L., *Thermodynamics for Chemists and Biologists* (Reading, Mass.: Addison-Wesley, 1968), section 7.5; Hill, T. L., *J. Theoret. Biol.*, **10**, 442 (1966).

[12] Actually, the steady-state value of the unpaired rate constant (β_{2D} here) could also be changed without altering the *form* of equation (14), because the equilibrium state is completely determined by the paired rate constants (four pairs here).