Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4432–4436, December 1976
Biochemistry

Steady-state kinetic formalism applied to multienzyme complexes, oxidative phosphorylation, and interacting enzymes

(free energy transduction/membranes/interlocking transitions/linear complexes)

TERRELL L. HILL

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

Contributed by Terrell L. Hill, September 21, 1976

ABSTRACT **A kinetic formalism, quite generally valid for free energy transducing, steady-state, macromolecular systems in biology, is applied here to multienzyme complexes, oxidative phosphorylation, and interacting enzymes. Systems of this type, comprising several interacting subunits, each with its own discrete set of states, present no new features in principle. Hence, they may be handled by the earlier kinetic formalism without modification. However, the kinetic diagram can become quite complicated because the state of *each* subunit (enzyme) must be specified in order to specify any one state of the system (complex) as a whole. Cycles, forces, fluxes, free energy levels, and state probabilities are considered.**

In recent papers (1–7) and a book (8), a kinetic formalism for steady-state systems (9–11) has been applied and extended in several ways to various kinds of ensembles of independent and equivalent macromolecular units. Ref. 7 and chapter 7 of ref. 8 deal with multienzyme complexes, oxidative phosphorylation, and interacting enzymes in some detail. The present paper is a preliminary and very much abbreviated version of these two works.

We use previous notation, definitions (1–6, 9–11), etc. Our object is to examine a few selected cases in which each unit (or system) consists of two or more distinct enzyme molecules or subunits (or subsystems). Each subunit has its own discrete set of states, but the subunits (within a unit) interact with each other because of proximity: the kinetic properties of any one subunit depend on the states of the other subunits.

Units or systems of this type do not differ in principle from those already studied (1–6, 9–11)—the same methods and theorems are applicable. But they do differ in the degree of complexity of the kinetic diagram. This complexity arises because the state of a unit depends on the state of *each* of the subunits.

Multienzyme complexes (12–14) are a special case of systems with interacting subunits. In its simplest form, the interaction in such cases might be confined to *interlocking* transitions in which two enzymes of the complex necessarily undergo *simultaneous* transitions because a ligand, substrate, electron pair, molecular fragment, etc. is transferred *directly* from one enzyme (plus prosthetic group, usually) to the other. We shall use the term "multienzyme complex" here, in a rather narrow sense, to refer to a system that makes use of interlocking transitions (for whatever reason) between neighboring pairs of enzymes of the complex. Besides well-known biochemical cases (12–14), it seems likely that many free energy transducing complexes in membranes are also multienzyme complexes in this sense [e.g., Na,K-ATPase (15), mitochondrial respiratory chain, etc.].

Two-enzyme complex

We begin the discussion with Fig. 1a, which does *not* represent a multienzyme complex. Here a unit comprises two two-state subunits (α and β) that interact with each other. Each subunit undergoes its own cyclic steady-state activity (reduced to two states for simplicity), and has its own flux, but in general the rate constants in one subunit depend on the state of the other subunit. The basic free energy drop (2, 4–6) around each cycle is equal to the corresponding thermodynamic force, X_α or X_β. These forces are determined only by *bath* concentrations of ligands, substrates, etc., and are therefore *independent* of the state of the opposite subunit.

The state of a *unit* must be specified by *two* indices ij: i = state of subunit α; j = state of subunit β; and $i,j = 1,2$. Fig. 1b shows the kinetic diagram for this system. Because some cycles in Fig. 1b contain both forces (10, 11) there will in general be some degree of thermodynamic coupling and free energy transduction between the α and β cyclic processes.

Now instead of the quite general interaction between subunits α and β that can be accommodated by Fig. 1, suppose α and β form a two-enzyme complex, as indicated in Fig. 2a. Here the "interior" (Fig. 1a) transitions $1 \rightleftarrows 2$ in *both* enzymes can only occur *simultaneously* (Fig. 2a). Ligands, substrates, etc. may enter or leave the scheme in Fig. 2a at any transition (2, 4), including $11 \rightleftarrows 22$, but this feature need not be made more explicit for present purposes. The new diagram is shown in Fig. 2b, which is somewhat simpler than Fig. 1b.

Because of the interlocking reaction $11 \rightleftarrows 22$ in Fig. 2a, completion of α and β cycles must now go hand in hand: there is complete coupling between these two fluxes. At the same time, there can be only a single net or effective thermodynamic force X driving the system. If X is in fact a composite of two or more thermodynamic forces (i.e., there are two or more over-all chemical or physical processes occurring in the bath or baths), free energy transduction is possible—just as in a single cycle system with two or more forces (10, 11). In the well-known

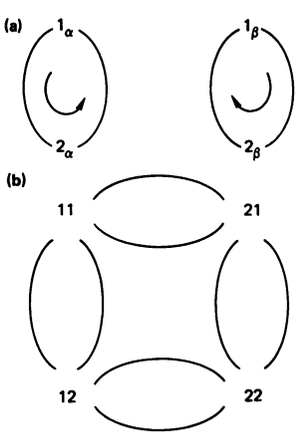

FIG. 1. (a) One unit = two two-state enzymes or subunits (α and β) that interact with each other. Arrows indicate dominant direction. (b) Diagram for the system.

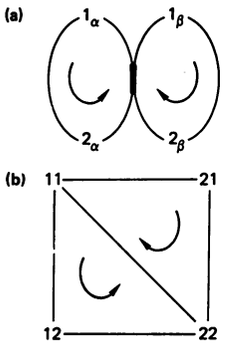

FIG. 2. (a) Two-enzyme complex constructed from enzymes in Fig. 1, with simultaneous transition (heavy bar) in the two enzymes. (b) Diagram for the system.

biochemical examples (12–14), this transduction takes the form of one chemical reaction driving another.

Let us now pursue further the model in Fig. 2. Fig. 3a introduces the rate constant notation used for the transitions. There are two cycles, *a* and *b* (Fig. 3b). The large (combined) cycle has zero force and can be ignored. The same force acts in both cycles:

$$\frac{\Pi_{a+}}{\Pi_{a-}} = \frac{\Pi_{b+}}{\Pi_{b-}} e^{X/kT}, \qquad [1]$$

where Π_{a+} is the product of rate constants around cycle *a* in the + (arrow) direction, etc. This leads to a required relation between α and β rate constants:

$$\beta_{12}\alpha^\dagger{}_{12}/\beta_{21}\alpha^\dagger{}_{21} = \alpha_{12}\beta^\dagger{}_{12}/\alpha_{21}\beta^\dagger{}_{21}. \qquad [2]$$

The physical significance of this relation can be seen from the basic free energy levels (2, 4) in Fig. 4: the basic free energy difference between states 11 and 22 must be independent of the path. That is, Eq. 2 is equivalent to

$$\Delta A'_\beta + \Delta A'^\dagger_\alpha = \Delta A'_\alpha + \Delta A'^\dagger_\beta \qquad [3]$$

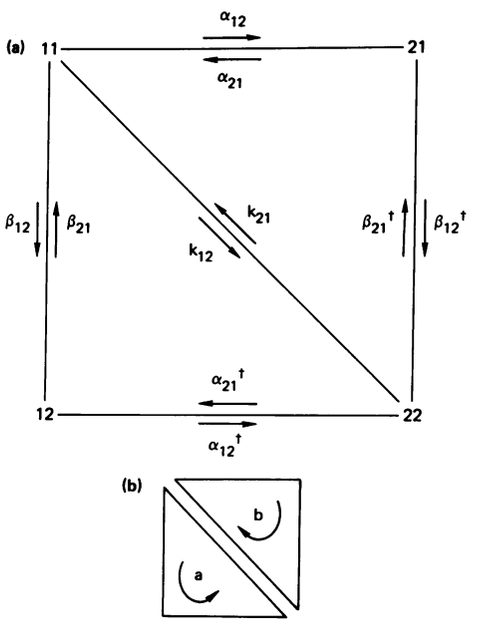

FIG. 3. (a) Rate constant notation for Fig. 2. (b) Significant cycles for the model. The combined (square) cycle has no force, no net flux, and accomplishes nothing.

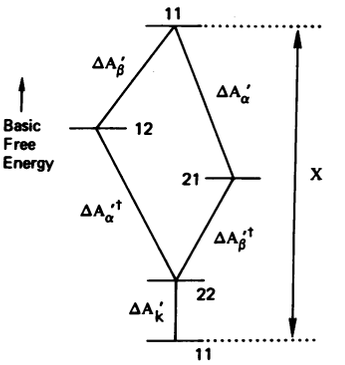

FIG. 4. Basic free energy levels for Fig. 3.

where $\Delta A'_\beta \equiv A'_{11} - A'_{12}$, etc., and

$$\beta_{12}/\beta_{21} = \exp(\Delta A'_\beta/kT), \qquad \alpha^\dagger{}_{12}/\alpha^\dagger{}_{21} = \exp(\Delta A'^\dagger_\alpha/kT),$$
$$k_{21}/k_{12} = \exp(\Delta A'_k/kT), \qquad [4]$$

etc. It should be recalled (2, 4) that it is not necessary for *all* of the basic free energy steps to be downhill (as shown in the figure).

The fact that we are using two pairs of α rate constants (α and $\alpha^\dagger$) and two pairs of β rate constants implies that there are two kinds of interaction between enzymes α and β in this model: (*a*) there are interlocking transitions $11 \rightleftarrows 22$; and (*b*) in the other transitions the state of one enzyme influences the kinetics of the other. Explicitly, when α is in state 1, β has rate constants β_{12} and β_{21}, but when α is in state 2, β has rate constants $\beta^\dagger{}_{12}$ and $\beta^\dagger{}_{21}$; etc.

If interaction (*b*) is not present, as could quite plausibly be the case, then

$$\alpha_{12} = \alpha^\dagger{}_{12}, \quad \alpha_{21} = \alpha^\dagger{}_{21}, \quad \beta_{12} = \beta^\dagger{}_{12}, \quad \beta_{21} = \beta^\dagger{}_{21}$$
$$\Delta A'_\alpha = \Delta A'^\dagger_\alpha, \quad \Delta A'_\beta = \Delta A'^\dagger_\beta,$$
$$\Delta A'_\alpha + \Delta A'_\beta + \Delta A'_k = X \qquad [5]$$
$$\Pi_{a+} = \Pi_{b+} = \beta_{12}\alpha_{12}k_{21}, \qquad \Pi_{a-} = \Pi_{b-} = \beta_{21}\alpha_{21}k_{12}.$$

In this event, the basic free energy differences $\Delta A'_\alpha$ and $\Delta A'_\beta$ can be attributed to the *individual* subunits. But in general, with interaction (*b*) present, all basic free energy differences refer to entire $\alpha\beta$ units or complexes and they cannot be decomposed into α and β contributions.

From the two flux diagrams (10, 11) for each cycle, we find for the cycle fluxes, in an ensemble of N units,

$$J_a = N(\Pi_{a+} - \Pi_{a-})(\alpha_{21} + \beta^\dagger{}_{12})/\Sigma$$
$$J_b = N(\Pi_{b+} - \Pi_{b-})(\alpha^\dagger{}_{12} + \beta_{21})/\Sigma. \qquad [6]$$

where Σ = sum of directional diagrams (10, 11). Despite Eq. 1, these cycle fluxes need not be equal. The total steady-state flux is, of course, $J = J_a + J_b$.

The total rate of free energy dissipation is JX. If, say, $X = X_1 + X_2$, where some overall reaction or transport process 1 ($X_1 > 0$) drives a second reaction or process 2 ($X_2 < 0$; $X_1 > -X_2$) uphill, by means of this model (Fig. 2), then the efficiency of free energy transduction is $\eta = -X_2/X_1$. This is seemingly unrelated to the kinetics but, in fact, X_2,X_1, and some of the rate constants would all be functions of bath concentrations.

It is easy but tedious to find Σ and the four steady-state probabilities p_{ij} either from the four flux diagrams (10, 11) or from the 4×8, 32 directional diagrams (10, 11).

Three-enzyme complex

One of the best known multienzyme complexes is the three-

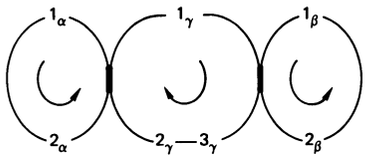

FIG. 5. Reaction scheme, in abstract notation, for the three-enzyme pyruvate dehydrogenase complex.

enzyme pyruvate dehydrogenase complex (12–14, 16). The reaction scheme, in abstract notation, is shown in Fig. 5. Enzymes α and β have two states each; enzyme γ has three states. The *complex* has $2 \times 3 \times 2 = 12$ states. The arrows in Fig. 5 indicate the dominant directions for transitions. The interlocking reactions are $1_\alpha 1_\gamma \rightleftarrows 2_\alpha 2_\gamma$ and $1_\gamma 1_\beta \rightleftarrows 3_\gamma 2_\beta$.

Because of the interlocking transitions, this system, as well as Fig. 2a, behaves in some respects like a single-cycle system with a single net effective thermodynamic force X. The same would be true if Fig. 5 were extended linearly to include 4, 5, $\cdots$ interlocking reaction loops (enzymes), and irrespective of the number of states per loop (2, 3, $\cdots$).

Respiratory chain enzymes

The respiratory chain enzyme complex of the mitochondrial inner membrane plays a crucial role in oxidative phosphorylation. This complex is represented in Fig. 6 in a very simplified manner as a linear array of M two-state enzymes with indices, from the left, $r = 1(I), 2(II), \cdots, M$. M is probably of order 10 or more (16). Each enzyme can be in an oxidized (O) or reduced (R) state. The complex has 2^M states. Enzymes $r = 1$ and $r = M$ are coupled with external half-reactions, reducing on the left and oxidizing on the right. In the transition $I_R II_O \rightarrow I_O II_R$, two electrons are transferred from enzyme $r = 1$ to $r = 2$, and similarly for the other interlocking transitions within the complex (excluding the end reactions). The overall chemical reaction is

$$\mathrm{NADH} + \tfrac{1}{2}\mathrm{O_2} + \mathrm{H^+} \longrightarrow \mathrm{NAD^+} + \mathrm{H_2O}.$$

Actually, this is only part of the story (16–18): proton transport and possibly ATP synthesis are also involved in some of the "interior" two-state cycles of the linear chain in Fig. 6.

Many reviews of oxidative phosphorylation are available (16, 19, 20).

One-Way Reactions. In this subsection we summarize results found (7) in the very much simplified case of one-way reactions with *all* rate constants taken equal to k (Fig. 7b shows the $M = 3$ kinetic scheme). We have calculated (7) exact steady-state results for $M = 1$ to 7; from these, general formulas for arbitrary M can be surmised.

Fig. 7 contains properties for $M = 3$, as an example. The diagram, states, and state probabilities are shown in Fig. 7a, while Fig. 7b gives values of $P_O{}^{(r)}$ and $P_R{}^{(r)}$ ($r = 1,2,3$), where $P_O{}^{(r)}$ is the probability that enzyme r is in state O, etc. It is easy to see that the flux J_M is equal to $NkP_O{}^{(1)}$ or to $NkP_R{}^{(r)}$.

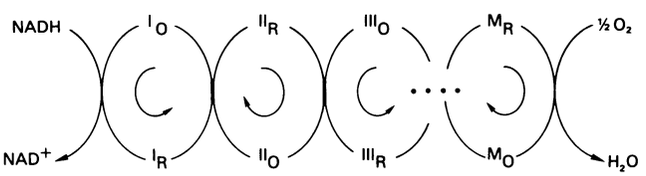

FIG. 6. Linear respiratory chain complex of M two-state enzymes. O = oxidized; R = reduced. The heavy bars indicate "interlocking" reactions.

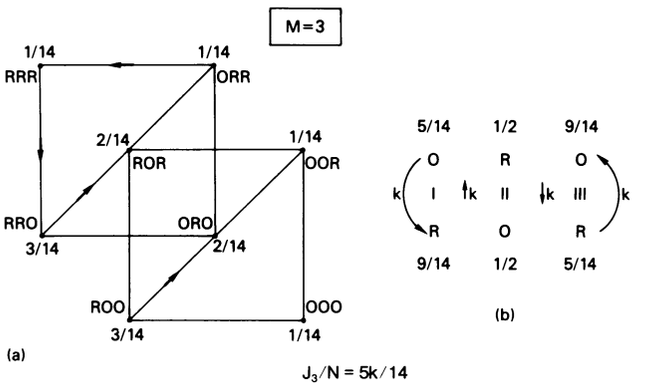

FIG. 7. (a) Steady-state probabilities of the states of an $M = 3$ complex, when all rate constants (one-way) are equal to k. Arrows show the directions of horizontal, vertical, and diagonal transitions. (b) Steady-state probabilities of the states of individual subunits, found by combining probabilities in (a).

Table 1 contains $P_O{}^{(r)}$ and J_M values implicitly, in the form (compare Fig. 7b)

$$P_O{}^{(r)} = W_{r,M}/\Sigma_M, \qquad J_M/Nk = W_{1,M}/\Sigma_M. \qquad [7]$$

The products in parentheses are differences. Note that the factors comprising the products are the same numbers that appear in the Σ_M and $W_{1,M}$ columns.

The general formula for Σ_M in Table 1 is apparently

$$\Sigma_M = 2(2M+1)!/M!(M+2)! \qquad [8]$$

and for $W_{1,M}$,

$$W_{1,M} = \Sigma_{M-1} = 2(2M-1)!/(M-1)!(M+1)!. \qquad [9]$$

Therefore, the flux is

$$J_M/Nk = P_O{}^{(1)} = \Sigma_{M-1}/\Sigma_M = (M+2)/2(2M+1). \qquad [10]$$

This approaches the finite value ¼ for $M \rightarrow \infty$. J_M is simply proportional to k for any M.

It is clear from Table 1 and Eq. 7 that the extent of oxidation $P_O{}^{(r)}$ increases along the enzyme chain from left to right ($r = 1$ to $r = M$) symmetrically but not linearly. $P_O{}^{(1)}$ is given by Eq. 10 and approaches ¼ as $M \rightarrow \infty$. Similarly, $P_O{}^{(M)} = 1 - P_O{}^{(1)} \rightarrow \frac{3}{4}$ as $M \rightarrow \infty$.

Arbitrary entries $W_{r,M}$ in Table 1 can be expressed as $W_{1,M}$ plus the sum of the differences in the Mth row, up to $W_{r,M}$. Thus,

$$W_{r,m} = \frac{2(2M+1)!}{(M-1)!(M+1)!} + 4\sum_{m=1}^{r-1} \frac{(2m-1)!}{(m-1)!(m+1)!} \times \frac{[2(M-m)-1]!}{(M-m-1)!(M-m+1)!}. \qquad [11]$$

Two-Way Reactions. In this subsection we generalize the above problem as follows: every transition and rate constant k, above, now has, in addition, an inverse transition with rate constant k'. We denote the ratio k/k' by α. Equilibrium corresponds to the choice $k = k'$ or $\alpha = 1$. From detailed balance at equilibrium we see that $p^e = 1/2^M$ for all states. The preceding subsection deals, in effect, with the case $\alpha \gg 1$. The case $\alpha \ll 1$ is not really different, because of symmetry: the system runs backwards (the flux is negative). This is indeed possible experimentally, in oxidative phosphorylation (16).

As might be expected, for arbitrary α the problem is rather formidable. Near equilibrium we take $\alpha = 1 + \epsilon$, where ϵ is small, $|\epsilon| \ll 1$. This is in the domain of linear irreversible

Table 1. Values of $W_{r,M}$ and Σ_M

M	Σ_M	$W_{1,M}$		$W_{2,M}$		$W_{3,M}$		$W_{4,M}$		$W_{5,M}$		$W_{6,M}$		$W_{7,M}$
1	2	1												
2	5	2	(1 × 1)	3										
3	14	5	(1 × 2)	7	(2 × 1)	9								
4	42	14	(1 × 5)	19	(2 × 2)	23	(5 × 1)	28						
5	132	42	(1 × 14)	56	(2 × 5)	66	(5 × 2)	76	(14 × 1)	90				
6	429	132	(1 × 42)	174	(2 × 14)	202	(5 × 5)	227	(14 × 2)	225	(42 × 1)	297		
7	1430	429	(1 × 132)	561	(2 × 42)	645	(5 × 14)	715	(14 × 5)	785	(42 × 2)	869	(132 × 1)	1001

thermodynamics. Omitting details (7), we find for the flux

$$\frac{J_M}{Nk'} = \frac{(M+1)\epsilon}{4M} = \frac{(M+1)(\alpha-1)}{4M} \quad (\alpha \longrightarrow 1). \quad [12]$$

The thermodynamic force per reaction step in this system is $k_BT\ln\alpha$ while the overall force is

$$X = (M+1)k_BT\ \ln\alpha \longrightarrow (M+1)k_BT\epsilon \quad (\alpha \longrightarrow 1) \quad [13]$$

where k_B = Boltzmann constant. Hence we also have

$$J_M/Nk' = X/4Mk_BT \quad (X \longrightarrow 0). \quad [14]$$

This is the linear flux–force relationship for the present model. Also, near equilibrium, there is a linear gradient in $P_O^{(r)}$, specified by the end values

$$P_O^{(1)} = \frac{1}{2} - \frac{(M-1)\epsilon}{8M}$$
$$P_O^{(M)} = P_R^{(1)} = \frac{1}{2} + \frac{(M-1)\epsilon}{8M}. \quad [15]$$

For arbitrary α, we have deduced (7) the state probabilities, $P_O^{(r)}$, and J_M for M = 1,2,3,4. Other cases can be handled numerically, for example by Monte Carlo methods (3). We give only the J_M results here:

$$J_1/Nk' = (\alpha-1)/2$$
$$J_2/Nk' = 2(\alpha^3-1)/(5\alpha^2+6\alpha+5)$$
$$J_3/Nk' = [5(\alpha^4-1)+6\alpha(\alpha^2-1)]/D_3 \quad [16]$$
$$J_4/Nk' = \{14[(\alpha^{10}-1)+\alpha^2(\alpha^6-1)+\alpha^3(\alpha^4-1)] + 20[\alpha(\alpha^8-1)+\alpha^4(\alpha^2-1)]\}/D_4$$

where

$$D_3 = 14\alpha^3+34\alpha^2+34\alpha+14$$
$$D_4 = 42\alpha^9+111\alpha^8+154\alpha^7+196\alpha^6+265\alpha^5 + 265\alpha^4+196\alpha^3+154\alpha^2+111\alpha+42. \quad [17]$$

Incidentally, the exact values of J_M (as above) for $\alpha \geq 1$ are bracketed surprisingly well by the asymptotic expressions (Eqs. **10** and **12**)

$$J_M/Nk' \longrightarrow (M+2)\alpha/2(2M+1) \quad (\alpha \longrightarrow \infty) \quad [18]$$
$$\longrightarrow (M+1)(\alpha-1)/4M \quad (\alpha \longrightarrow 1). \quad [19]$$

Monte Carlo methods would, in fact, allow quite arbitrary choices of rate constants.

Analogy to Multisite Diffusion Models. It is helpful conceptually and mathematically (see below and ref. 21, section 6) to recognize that there is an exact formal analogy between the above respiratory chain model and the diffusion of a ligand across a membrane, from one bath to another, by means of jumping from site to site along a row of M sites (21). A given site may be empty or occupied by one ligand molecule. Ligand adsorption and desorption between each of the baths and sites 1 and M, respectively, are analogous to the two end reactions in Fig. 6; the jumping of the ligand from site i to site $i+1$ is analogous to the interlocking transition $RO \rightarrow OR$ between enzymes i, $i+1$; a site occupied by a ligand is equivalent to state R; and an empty site is equivalent to state O. In fact, a ligand molecule is equivalent to a pair of electrons. As is indeed self-evident, the respiratory chain model may thus be regarded as a model for single-file "adsorption, diffusion, and desorption" of electron pairs from one external electron pool (NADH) to another (H_2O; Fig. 6).

Recognition of this analogy does not solve the steady-state mathematical problem but it makes equilibrium special cases trivial (even if all rate constants are different). For example, if, say, the $i \rightleftarrows i+1$ transitions mentioned above are blocked by an inhibitor (16, 22), the subsystems 1,2, $\cdots$, i and $i+1$, $i+2$, $\cdots$, M will separately come to *equilibrium* at $t = \infty$ (each with its own external pool). All enzymes (sites) are independent at equilibrium, each is in equilibrium with its external pool, and each will have a "Langmuir adsorption isotherm" of the form (23)

$$P_R^{(r)} = x_r/(1+x_r), \quad P_O^{(r)} = 1/(1+x_r) \quad (r = 1,2,\ldots,M)$$

where it is easy to relate x_r to rate constant ratios via detailed balance.

It should be added that the treatment given in Sections 6C and 6F of ref. 21 is approximate, not exact (7).

Two interacting enzymes

We return here to Fig. 1 and make a few additional comments. We consider two enzymes or macromolecules α and β, in con-

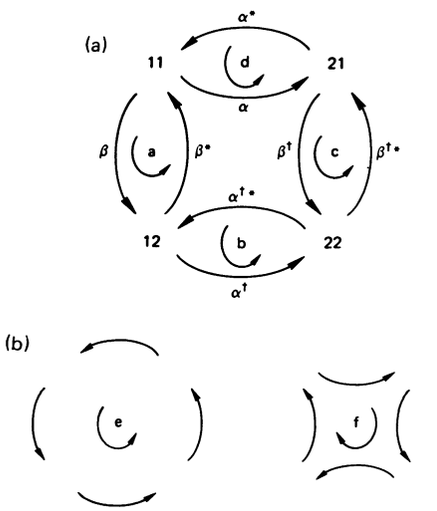

FIG. 8. (a) Diagram (with rate constant notation, back reactions omitted) based on Fig. 1. Cycles *a*, *b*, *c*, *d* are indicated. (b) Cycles *e* and *f* for this system.

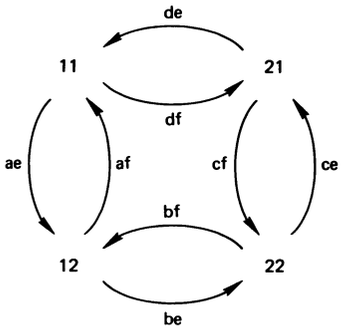

FIG. 9. Listing of cycles that contribute to each transition flux in Fig. 8a.

tact, that influence each other's kinetics—though there are no interlocking reactions as in our definition of an "enzyme complex." The thermodynamic forces are X_α and X_β and the fluxes, J_α and J_β. In deriving kinetic properties, we again assume one-way reactions for simplicity.

The diagram is shown in Fig. 8a. Note that the transitions with an asterisk ($2 \rightarrow 1$) replace the k transition ($22 \rightarrow 11$) in Fig. 3 (back reactions omitted). That is, each enzyme can now *separately* undergo the transition $2 \rightarrow 1$, thus completing a cycle. The asterisk transitions are *not* inverses of the other transitions shown (rather, β_{21} is inverse to $\beta \equiv \beta_{12}$, etc.).

This system has six cycles with non-zero force (if back reactions are included, there are 16 such cycles). These are labeled $a,b,\cdots,f$ in Fig. 8a and b. The force in cycles a and c is X_β, in b and d it is X_α, and in e and f it is $X_\alpha + X_\beta$. The mere fact that there is at least one cycle that contains both forces guarantees that there will, in general, be coupling between the fluxes and, therefore, free energy transduction (10, 11). But this coupling will not be *complete*, as it is in the linear multienzyme complexes we have been considering above. The coupling will, of course, disappear if the two enzymes do not interact with each other at all, that is, if the symbol † can be dropped from Fig. 8a ($\beta = \beta^\dagger$, $\alpha^* = \alpha^\dagger{}^*$, etc.). If back reactions are included, four of the 16 non-zero-force cycles contain both forces.

Each of the cycles a through d has three flux diagrams, while cycles e and f have only one each. Hence (10, 11)

$$\begin{aligned} J_a &= N\beta\beta^*(\alpha^*\beta^{\dagger *} + \beta^\dagger\alpha^{\dagger *} + \alpha^*\alpha^{\dagger *})/\Sigma, \\ J_b &= N\alpha^\dagger\alpha^{\dagger *}(\alpha^*\beta + \alpha\beta^\dagger + \beta\beta^\dagger)/\Sigma \\ J_c &= N\beta^\dagger\beta^{\dagger *}(\alpha\beta^* + \alpha^\dagger\beta + \alpha\alpha^\dagger)/\Sigma, \\ J_d &= N\alpha\alpha^*(\alpha^{\dagger *}\beta^* + \alpha^\dagger\beta^{\dagger *} + \beta^*\beta^{\dagger *})/\Sigma \\ J_e &= N\beta\alpha^\dagger\beta^{\dagger *}\alpha^*/\Sigma, \\ J_f &= N\alpha\beta^\dagger\alpha^{\dagger *}\beta^*/\Sigma, \end{aligned} \qquad [20]$$

where we omit the complicated expression for Σ.

Fig. 9 shows the composition of each transition flux in terms of cycles. The separate α and β steady-state fluxes are then

$$\begin{aligned} J_\alpha &= J_b + J_d + J_e + J_f \\ J_\beta &= J_a + J_c + J_e + J_f. \end{aligned} \qquad [21]$$

As a special case (an extreme form of interaction), suppose enzyme β can operate only if enzyme α as in state 1. That is, $\beta^\dagger = 0$ and $\beta^\dagger{}^* = 0$ in Fig. 8a. The diagram now has only the three cycles a, b, and d. No thermodynamic coupling or free energy transduction is possible between α and β because no cycle contains both X_α and X_β. The fluxes are

$$J_\alpha = J_b + J_d = N\alpha^{\dagger *}\alpha^*(\alpha^\dagger\beta + \alpha\beta^*)/\Sigma',$$

$$J_\beta = J_a = N\beta\beta^*\alpha^*\alpha^{\dagger *}/\Sigma' \qquad [22]$$

where Σ' is the appropriately simplified version of Σ. Although the enzymes influence each other, as seen in these equations, one cannot "drive" the other. For example (with back reactions included), if $X_\alpha = 0$, a force $X_\beta \neq 0$ cannot induce a flux $J_\alpha \neq 0$.

Note Added in Proof. Some properties of the above model of the respiratory chain have already been discussed by Holmes (24), especially the accuracy for $M = 2$ and $M = 3$, of the approximation used in ref. 21 (see above).

I am indebted to Dr. Britton Chance for very stimulating comments.

1. Hill, T. L. (1974) *Prog. Biophys. Mol. Biol.* **28,** 267–340.
2. Hill, T. L. (1975) *Biochemistry* **14,** 2127–2137.
3. Hill, T. L. & Chen, Y. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1291–1295.
4. Hill, T. L. & Simmons, R. M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 95–99.
5. Hill, T. L. & Simmons, R. M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 336–340, 2165 (correction).
6. Simmons, R. M. & Hill, T. L. (1976) *Nature* **263,** 615–618.
7. Hill, T. L. (1977) in *Statistical Mechanics and Statistical Methods in Theory and Application,* ed. Landman, U. (Plenum Press, New York), in press.
8. Hill, T. L. (1977) *Free Energy Transduction in Biology* (Academic Press, New York), in press.
9. King, E. L. & Altman, C. (1956) *J. Phys. Chem.* **60,** 1375–1378.
10. Hill, T. L. (1966) *J. Theor. Biol.* **10,** 442–459.
11. Hill, T. L. (1968) *Thermodynamics for Chemists and Biologists* (Addison-Wesley, Reading, Mass.), pp. 119–177.
12. Reed, L. J. & Cox, D. J. (1970) in *The Enzymes,* ed. Boyer, P. D. (Academic Press, New York), 3rd ed., pp. 213–240.
13. Ginsburg, A. & Stadtman, E. R. (1970) *Annu. Rev. Biochem.* **39,** 429–472.
14. Reed, L. J. (1974) *Acc. Chem. Res.* **7,** 40–46.
15. Stone, A. J. (1968) *Biochim. Biophys. Acta* **150,** 578–586.
16. Lehninger, A. L. (1975) *Biochemistry* (Worth, New York), 2nd ed., pp. 450–453.
17. Mitchell, P. (1973) *J. Bioenerg.* **3,** 5–24.
18. Mitchell, P. (1974) *J. Bioenerg.* **4,** 63–91.
19. Stryer, L. (1975) *Biochemistry* (W. H. Freeman, San Francisco).
20. Boyer, P. D., Chance, B., Ernster, L., Mitchell, P., Racker, E. & Slater, E. C. (1977) *Annu. Rev. Biochem.,* in press.
21. Hill, T. L. & Chen, Y. (1971) *Biophys. J.* **11,** 685–710.
22. Chance, B. & Williams, G. R. (1956) *Adv. Enzymol.* **17,** 65–134.
23. Hill, T. L. (1960) *Statistical Thermodynamics* (Addison-Wesley, Reading, Mass.), pp. 129–133.
24. Holmes, W. F. (1960) Ph. D. Dissertation, University of Pennsylvania, Philadelphia.