Proc. Nat. Acad. Sci. USA
Vol. 73, No. 1, pp. 95–99, January 1976
Biochemistry

Free energy levels and entropy production associated with biochemical kinetic diagrams

(active transport/muscle contraction/enzyme kinetics)

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Contributed by Terrell L. Hill, October 29, 1975

ABSTRACT **"Basic" and "gross" free energy levels are defined for the discrete states of a macromolecular biochemical kinetic system such as a free energy transducing enzyme (e.g., myosin or Na,K-ATPase). Basic free energy level differences are related to the first-order rate constants for transitions between states while gross free energy differences, along with the corresponding fluxes, determine the rate of entropy production in the system. In muscle contraction the analysis is complicated by the possibility of the system doing external mechanical work. The question of the sign of the flux or of the gross free energy level change in a given transition is examined for both single-cycle and multi-cycle models. More definite statements can be made in single-cycle cases. Some numerical examples are included. The more complicated cases are reserved for a subsequent paper.**

We shall be concerned here with the thermodynamics and kinetics of biochemical systems in which a macromolecule (e.g., myosin, or a simple enzyme) or macromolecular complex (e.g., Na,K-ATPase) can exist in a number of discrete states with possible transitions between these states. Binding of ligands, substrates, etc., is included among the transitions. In the corresponding kinetic "diagram," the states are represented by points and each inverse pair of possible transitions between two states is represented by a line connecting the two states. The diagram may consist of only a single cycle but often it is more complicated than this. The cases of interest include one or more "chemical" driving forces (e.g., ATP $\rightarrow$ ADP + P_i, or a ligand concentration gradient) and the possibility of free energy transduction, as in active transport or muscle contraction.

There is a certain amount of confusion in the literature concerning the relative free energies of the states of such a diagram, and the connection between these free energies and the diagram kinetics. This confusion arises in part because several definitions of free energy levels of states are possible, and the precise choice is usually not made clear. Different definitions lead to different properties. Matters are further complicated in the biochemistry of active muscle by the dependence of the free energies of some myosin states on a positional variable x (1–3) that must be introduced in order to relate the myosin biochemistry to the mechanical work accomplished by muscle.

In an earlier paper (4), particular attention was paid to those free energy levels (referred to as "basic" in the next section) that are directly associated with the first-order rate constants of the kinetic diagram. The primary purpose of the present paper is to discuss properties of the "gross" free energy levels (see below) that govern, for an ensemble of macromolecular systems, the direction of spontaneous net reaction along any line of the diagram. These are also the free energy levels that are involved in the rate of entropy production in an ensemble of systems. Several numerical examples will be included for illustrative purposes.

Stochastic aspects of this problem will not be included here but they have been discussed elsewhere (4, 5).

We shall begin with a brief summary of notation and results from the previous work (4) in order to make the present paper self-contained. The discussion will be continued in a second paper concerned with more complicated cases (6).

Notation, definitions, and review

We consider a large ensemble of N independent and equivalent macromolecular systems, each of which has the same kinetic diagram (states, transitions, and rate constants). We let E represent the particular macromolecule of interest. Also, let L = ligand, S = substrate, and P = product. Possible states of a system are then, for example, E, ES, EP, LES, etc., depending on the special case. Fig. 1 provides, as an example, a diagram that we shall consider extensively in the second paper (6). In this case there are six states in the diagram; the enzymatic reaction $S \rightarrow P$ provides the thermodynamic drive or chemical force; and the ligand L modifies the enzyme kinetics. For example, E = myosin, S = ATP, P = ADP + P_i, L = actin.

For simplicity, in our examples, we shall assume that E has at most one site for any given ligand, substrate, etc. But this limitation is not at all necessary.

L, S, and P exist as separate species in solution as well as possibly bound to E. We write for their chemical potentials in solution,

$$\mu_L = \mu_L^\circ + kT \ln c_L, \text{ etc.,} \qquad [1]$$

where c_L = molar concentration (or activity, if necessary) and μ_L° = standard chemical potential. The concentrations c_L, etc., are constant over the time intervals we consider. In active or facilitated transport, one or more ligands may be at different concentrations on either side of a membrane (4).

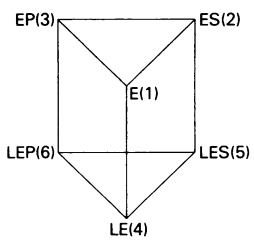

FIG. 1. Kinetic diagram for E = enzyme, S = substrate, P = product. L is a ligand that modifies the enzyme kinetics.

The N macromolecular systems may be immobilized (e.g., myosin in muscle, Na,K-ATPase in a membrane, an enzyme bound to a surface) or they may be free to move in solution. In either case, the macromolecules are assumed not to interact with each other.

Immobilized Macromolecular Systems. Imagine the ensemble frozen in composition at an arbitrary time t. Of the N systems in the ensemble, let N_i be the number in state i and define the probability or fraction $p_i = N_i/N$. From the point of view of statistical mechanics, this is an ideal solid solution (see ref. 7, p. 373) with components $i = 1, 2, \cdots$. Let $A_i = -kT\ln Q_i$ be the Helmholtz free energy of a single system in state i (see also ref. 2, Section IIA). Then the canonical partition function of the solid solution (ensemble) is

$$Q = \frac{N!\prod_i Q_i^{N_i}}{\prod_i N_i!} \qquad [2]$$

and the chemical potential of an arbitrary component j in the ensemble is

$$\mu_j = -kT\frac{\partial \ln Q}{\partial N_j} = A_j + kT \ln p_j. \qquad [3]$$

In taking the derivative, we use $N = \Sigma_i N_i$ and hold all N_i other than N_j constant. Note that if $p_j = 1$ (all systems in state j = standard state), $\mu_j = A_j$. We shall refer to A_j as the "free energy" of state j, in order to distinguish it from the chemical potential μ_j which depends on the population of state j in the ensemble. A_j may be regarded as either the free energy of a single isolated system in state j, or the free energy per system if the whole ensemble is in state j ($p_j = 1$). Incidentally, we shall use throughout the paper the approximation G_j (Gibbs free energy) = A_j since the pV_j term is negligible.

The single-system free energies A_i are fundamental in that they are directly connected to the first-order rate constants of the diagram which determine the kinetics of the ensemble (2, 4). For example, consider first the inverse transitions between two states i and j such that no ligand is bound or released in the transitions (e.g., a conformational change $E \rightleftarrows E^*$, or $ES \rightleftarrows EP$). That is, these are *isomeric* transitions. Let the first-order rate constants for $i \rightarrow j$ and $j \rightarrow i$ be α_{ij} and α_{ji}, respectively. A hypothetical equilibrium between the two states can be used to establish the connection between the A's and α's (4):

$$\alpha_{ij} p_i^e = \alpha_{ji} p_j^e \text{ (detailed balance)} \qquad [4]$$

$$\mu_i = A_i + kT \ln p_i^e = \mu_j = A_j + kT \ln p_j^e \qquad [5]$$

$$\alpha_{ij}/\alpha_{ji} = \exp[-(A_j - A_i)/kT] \equiv K_{ij}, \qquad [6]$$

where e = equilibrium and K_{ij} is a dimensionless equilibrium constant.

Now suppose, on the other hand, that a ligand L (or S, or P) is bound in the transition $i \rightarrow j$. In this case, if we consider the equilibrium between states i and j in the presence of L in solution at its *actual* concentration c_L (4), we have

$$\alpha'_{ij} c_L p_i^e = \alpha_{ji} p_j^e,$$

$$\alpha_{ij} p_i^e = \alpha_{ji} p_j^e, \qquad [7]$$

where the first-order rate constant $\alpha_{ij} \equiv \alpha'_{ij} c_L$, and for the equilibrium condition,

$$\mu_i + \mu_L(c_L) = A_i + kT \ln p_i^e$$
$$+ \mu_L = \mu_j = A_j + kT \ln p_j^e. \qquad [8]$$

Therefore

$$\frac{\alpha_{ij}}{\alpha_{ji}} = \exp\left\{-\frac{[A_j - (A_i + \mu_L)]}{kT}\right\} \equiv K_{ij}, \qquad [9]$$

where again K_{ij} is a dimensionless equilibrium constant. Thus, in binding transitions, μ_L (or μ_S, or μ_P) must be included in the free energy difference to establish the correct relationship to the first-order rate constant ratio α_{ij}/α_{ji} (ref. 2, pp. 278 and 327). Note that this is $\mu_L(c_L)$, *not* $\mu_L°$ (Eq. **1**).

Relative free energy levels of all the states in a diagram, when based on the free energies A_i and μ_L, μ_S, or μ_P (as appropriate), will be referred to below as "basic" free energy levels. On the other hand, relative free energy levels of the states when the free energies A_i are replaced by the chemical potentials μ_i (Eq. 3), again including μ_L, μ_S, or μ_P as required, will be called "gross" free energy levels. The latter levels are of course all equal when there is equilibrium among all states i, but not otherwise. From an operational point of view, in theoretical work, basic free energy levels are introduced *ab initio* as fixed parameters of a model, while the gross free energy levels emerge as calculated macroscopic properties of the ensemble that depend on the p_i and, therefore, on the initial conditions and on the time t, in general.

In its stochastic behavior, any *individual* system of the ensemble is governed entirely by the α's of the diagram (which are related to the basic free energy levels). The individual system has no knowledge of *ensemble* properties such as the p's, the gross free energy levels, transient versus steady-state, etc.—or even whether there *is* an ensemble ($N > 1$).

As already mentioned, a positional variable x must be introduced in the problem of muscle contraction. Some of the A_i and some or all of the α_{ij} (depending on the model) are functions of x (2). The above considerations still apply but, in effect, we have to consider a different ensemble of myosin molecules (= E) in each interval $x, x + dx$. This generalization is, however, not needed in studies of heavy meromyosin (or its subfragment S1) + ATP + actin in solution since there is no variable x in this case.

Macromolecular Systems in Solution. We have here a multicomponent mixture of macromolecules, in the various states i, that may be regarded as solutes in a mixed solvent (water, salt, ligands, etc.). The solution is necessarily dilute with respect to macromolecules since they are assumed to be kinetically independent. The chemical potential of systems in state i can then be written as

$$\mu_i = \mu_i° + kT \ln c_i, \qquad [10]$$

where c_i = molar concentration in solution. This is the analogue of Eq. 3. In order to be able to use the notation in Eq. 3 for both cases (immobile and free), let us introduce $c_i = bp_i$ in Eq. **10**, where b is the same constant for all states i and p_i is the fraction of macromolecular systems in state i. Thus Eqs. 3–9 apply to the present situation as well, if we understand A_i to mean here

$$A_i \equiv \mu_i° + kT \ln b. \qquad [11]$$

Since only free energy differences, such as $A_i - A_j$, have

physical significance in any case, introduction of the term $kT\ln b$ is a mere formality. Use of $\mu_i°$ as essentially the free energy of a single macromolecule (in state i) in solution is equivalent to a procedure previously introduced in the thermodynamics of small systems (ref. 8, pp. 50–58).

Direction of spontaneous transition

Most of the remainder of the paper will be based on special cases, as examples, but in this section we deal with one property that can easily be discussed in a completely general way. Consider an ensemble of N systems (immobilized or in solution) with an arbitrary kinetic diagram. At an arbitrary time t (this need not be a steady-state), let p_i be the probability of state i. For any transition‡ ij in the diagram, the net mean flux $i \to j$ is

$$J_{ij}(t) = N[\alpha_{ij}p_i(t) - \alpha_{ji}p_j(t)]. \quad [12]$$

Corresponding to Eqs. 6 and 9, we use the notation (note $\Delta \equiv$ *initial* − *final*)

$$\alpha_{ij}/\alpha_{ji} = e^{+\Delta A'_{ij}/kT} = K_{ij} \quad [13]$$

to express the relation between the α's and the basic free energy levels of states i and j for *any* kind of transition (conformational change, binding of ligand, release of ligand, etc.). The prime indicates that μ_L, μ_S, etc., are included as needed (compare ref. 2, p. 278). For example, from Eq. 9,

$$\begin{aligned} \Delta A'_{ij} &= (A_i + \mu_L) - A_j \quad (i \to j = \text{binding of } L) \\ \Delta A'_{ji} &= A_j - (A_i + \mu_L) \quad (j \to i = \text{release of } L) \end{aligned} \quad [14]$$

We use a similar notation for the gross free energy level difference between states i and j. For example,

$$\Delta\mu'_{ij} = (\mu_i + \mu_L) - \mu_j \quad (i \to j = \text{binding of } L). \quad [15]$$

In view of Eqs. 3 and 11, for an arbitrary transition ij, we have

$$\Delta\mu'_{ij} = \Delta A'_{ij} + kT \ln (p_i/p_j). \quad [16]$$

Then, on using Eq. 13,

$$\alpha_{ij}p_i/\alpha_{ji}p_j = e^{+\Delta\mu'_{ij}/kT}. \quad [17]$$

To summarize: if the transition $i \to j$ occurs, the *actual* free energy change in the ensemble is $-\Delta\mu'_{ij}$, and this includes a concentration term of entropic origin, $kT\ln(p_j/p_i)$; if this transition is isomeric, $-\Delta A'_{ij}$ is the corresponding *standard* free energy change; but if the transition is not isomeric, $-\Delta A'_{ij}$ is not the standard free energy change (because μ_L replaces $\mu_L°$, as in Eq. 9, where L = ligand).

On comparing Eqs. 12 and 17, we see that the net mean flux J_{ij} for any transition ij always has the same sign as the gross free energy level difference $\Delta\mu'_{ij}$ (at any time t). For example, if i has the higher gross free energy level, the net mean flux will be in the direction $i \to j$ (there are, of course, stochastic exceptions in single systems or in small groups of systems). This is just the second law of thermodynamics at work on individual elementary reactions of a complex reaction scheme (9).

‡ As will be clear from the context, for brevity "transition" sometimes refers to "inverse pair of transitions."

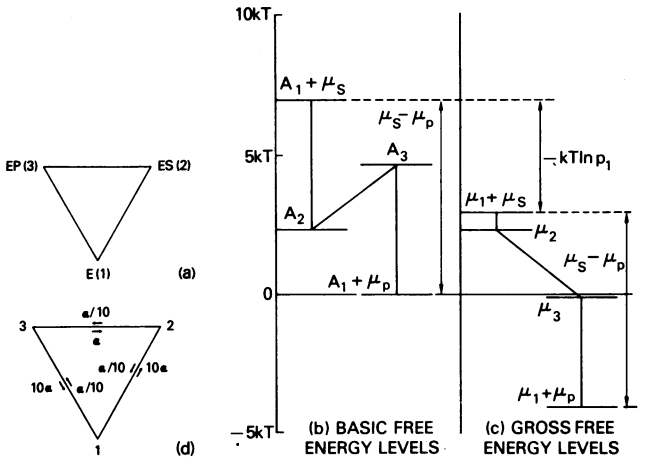

FIG. 2. (a) Kinetic diagram for E, S, P without ligand. (b) Basic free energy levels for numerical example. Vertical and slanting lines indicate possible transitions. (c) Gross free energy levels for example. (d) First-order rate constants for example.

Thus, net reaction (positive flux) always occurs in a *downhill* direction with reference to a set of gross free energy levels. This is *not* true of the invariant basic free energy levels. This point will be illustrated in the next two sections.

The product $J_{ij}\,\Delta\mu'_{ij}$ is always ≥ 0, with the equality holding only at equilibrium. This product is the contribution of the transition ij to the rate of entropy production in the ensemble. The total rate of entropy production, at any time, t, is (including a factor T for convenience)

$$T\frac{d_iS}{dt} = \sum_{ij} J_{ij}(t)\,\Delta\mu'_{ij}(t) \geq 0, \quad [18]$$

where the sum is over all lines in the diagram (the direction chosen along each line is immaterial). This is not to be confused with the phenomenological sum $\Sigma_i J_i X_i$ (4, 9, 10) in which the terms need not all be positive.

In muscle, with a different ensemble at each x, since each $J_{ij}(t,x)\Delta\mu'_{ij}(t,x) \geq 0$, we not only have a sum $\geq$ for each x when adding contributions from all transitions as in Eq. 18, but we also have an integral ≥ 0 for each transition ij on summing contributions from all intervals x, $x + dx$. The total rate of entropy production is obtained from both operations combined: Σ_{ij} and $\int dx$ (see the second paper, ref. 6).

Single cycle steady state: An example

A diagram consisting of only a single cycle is worth discussing separately because of its simplicity and frequent importance. We choose Fig. 2a as an example (see ref. 4 for further details). E can be either immobilized or free in solution. The extension of the properties found below to larger single cycles will be rather obvious from this example.

The drive or chemical force in this case (Fig. 2a) is associated with the reaction $S \to P$ for which, in solution at c_S and c_P, we assume $\mu_S(c_S) - \mu_P(c_P) > 0$ (but the reaction rate is negligible without E). Note that this is *not* the standard free energy change $\mu_S° - \mu_P°$ (4). Fig. 2b shows a hypothetical set of basic free energy levels, with nonhorizontal lines indicating possible transitions. From Eq. 13, applied around the cycle counterclockwise, we have

$$\frac{\alpha_{12}\alpha_{23}\alpha_{31}}{\alpha_{21}\alpha_{32}\alpha_{13}} = e^{(\mu_S - \mu_P)/kT} = K_{12}K_{23}K_{31}. \quad [19]$$

That is, $\mu_S - \mu_P$ is the total basic free energy drop, the sum of the $\Delta A'_{ij}$, for one circuit. Note that, in this example, $\Delta A'_{ij}$ is not positive in every counterclockwise step. In a particular model, any choice of α's must satisfy Eq. **19**. If we apply Eq. **17** around the cycle, the p's cancel and Eq. **19** is again obtained. Thus, as illustrated in Fig. 2c (which happens to be a steady-state example), the total gross free energy drop, the sum of the $\Delta\mu'_{ij}$, for one circuit is also $\mu_S - \mu_P$.

The above property of the gross free energies obtains even in a transient (i.e., at arbitrary t). It should be pointed out, however, that in transients $\Delta\mu'_{ij}$ is not necessarily positive for every counterclockwise step. For example, suppose that, at $t = 0$, $p_3 = 1$ and $p_1 = p_2 = 0$. Then for $t > 0$ but small, we would clearly have $J_{23} < 0$ and hence $\Delta\mu'_{23} < 0$.

We turn now to the steady-state situation. As there is only one cycle in the diagram, we must have $J_{12} = J_{23} = J_{31} \equiv J$ at steady state. Since

$$T\frac{d_iS}{dt} = \Sigma J_{ij}\Delta\mu'_{ij} = J\Sigma\Delta\mu'_{ij} = J(\mu_S - \mu_P) > 0 \quad [20]$$

and $\mu_S - \mu_P > 0$, we also have $J > 0$ (i.e., the net flux is in the direction of the force). Further, since $J\Delta\mu'_{ij}$ (ij = 12, 23, 31) > 0 (see above), we deduce that $\Delta\mu'_{ij}$ (ij = 12, 23, 31) > 0. Thus, at *steady state*, the gross free energy level must decrease (Fig. 2c) and the net flux must be positive (and equal) for each step in the direction of the force ($S \rightarrow P$).

For the model in Fig. 2a (or any single-cycle model), $\Delta\mu'_{ij}$ and J_{ij} are both positive in every step in the direction of the force, at steady state. This is true irrespective of the arrangement of the basic free energy levels (of course there is the restraint $\Sigma\Delta A'_{ij} = \mu_S - \mu_P > 0$, around the cycle). Thus it would seem that, say, even an up-hill sequence of basic free energy levels ($\Delta A'_{12}$ and $\Delta A'_{23}$ negative; Δ'_{31} positive) would provide a workable model. It is true that such a model would work in principle, but in practice the flux would be very small if $\mu_S - \mu_P$ is large and the up-hill steps are significant.

There is no fundamental complication when a single cycle contains more than one chemical force (e.g., $\mu_S - \mu_P$ above plus the force $\mu_A - \mu_B$ from the concentration gradient of a ligand, where A and B refer to the two sides of a membrane). Examples are included in ref. 4. The *net* force determines the direction of positive flux.

Numerical Example. Let us use the particular set of rate constants in Fig. 2d. These have been chosen to be consistent with Fig. 2b, which, therefore, shows the basic free energy levels for this case. We have $K_{12} = 100$, $K_{23} = 0.1$, $K_{31} = 100$, and $\mu_S - \mu_P = kT\ln 1000$. Given the rate constants, we can easily calculate the steady-state probabilities: $p_1 = 0.01855$, $p_2 = 0.97244$, and $p_3 = 0.00901$. Most of the enzyme accumulates in state 2 (= ES) because of the relatively small rate constants for transitions out of state 2. From Eq. **12**, the mean flux in each step of the cycle is $J/N = 0.08823\alpha$. The gross free energy levels (Fig. 2c) are obtained from the basic levels (Fig. 2b) by subtraction of $-kT\ln p_i$ in each case. The gross level drops in each counterclockwise step, as required. The rate of entropy production, $J(\mu_S - \mu_P)$, is $0.6095\, N\alpha kT$.

Multi-cycle diagram at steady state: An example

As we have just seen, it is possible to be explicit about directional properties of individual transitions in steady-state ensembles with single-cycle diagrams. Multi-cycle diagrams, especially those with two or more chemical forces, present a variety of possibilities. For many of these the ability to make categorical statements on steady-state directional properties is reduced to some extent. Rather than attempting a general analysis, we confine ourselves to a few examples, especially Fig. 1 (studied in ref. 6). These models may represent the kinetics of either immobilized or free macromolecules E.

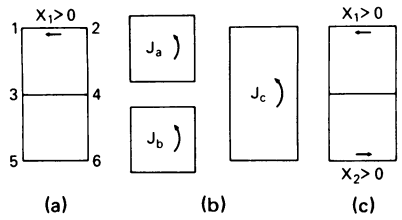

FIG. 3. (a) Diagram in a multi-cycle case with one thermodynamic force (X_1). (b) Cycles belonging to this diagram. Assigned direction of positive cycle fluxes is indicated by arrows. (c) Same diagram as (a) but with two forces.

As a prerequisite to further discussion, we need to recall that the steady-state net mean flux for any transition $i \rightarrow j$ of a diagram can be written as a sum of contributions (cycle fluxes) from each of the cycles of the diagram that include the transition ij (4, 10, 11). For example, the diagram in Fig. 3a has three cycles, a, b, and c, shown in Fig. 3b, with positive cycle fluxes arbitrarily assigned the directions indicated. Then we have, at steady state,

$$\begin{aligned} J_{42} &= J_{21} = J_{13} = J_a + J_c \\ J_{35} &= J_{56} = J_{64} = J_b + J_c \\ J_{34} &= J_a - J_b. \end{aligned} \quad [21]$$

In turn, each cycle flux (for any diagram) can be expressed in terms of all the α's of the diagram in the form (10, 11)

$$J_a = N(\Pi_{a+} - \Pi_{a-})\Sigma_a/\Sigma, \quad [22]$$

where Σ is the sum of directional diagrams for all states, Π_{a+} (Π_{a-}) is the product of rate constants around cycle a in the + (−) direction, and $(\Pi_{a+} - \Pi_{a-})\,\Sigma_a$ is the sum of cycle a flux diagrams (this defines Σ_a).

If a thermodynamic force (e.g., $\mu_S - \mu_P$) is included in a cycle, this will be reflected in the α's of the cycle. For example, with the single positive force X_1 as indicated in Fig. 3a (we need not be more explicit about the model than this),

$$\begin{aligned} &\Pi_{a+}/\Pi_{a-} = e^{X_1/kT}, \qquad \Pi_{b+}/\Pi_{b-} = 1, \\ &\Pi_{c+}/\Pi_{c-} = e^{X_1/kT}. \end{aligned} \quad [23]$$

Correspondingly, in Fig. 3a, in view of Eq. **22**,

$$J_a > 0, \qquad J_b = 0, \qquad J_c > 0. \quad [24]$$

Eqs. **23** are of the same form as Eq. **19** for a single-cycle diagram, and they follow from both Eqs. **13** and **17** (as does Eq. **19**).

For the model with two positive forces, as shown in Fig. 3c,

$$\begin{aligned} &\Pi_{a+}/\Pi_{a-} = e^{X_1/kT}, \qquad \Pi_{b+}/\Pi_{b-} = e^{X_2/kT}, \\ &\Pi_{c+}/\Pi_{c-} = e^{(X_1+X_2)/kT}, \end{aligned} \quad [25]$$

and

$$J_a > 0, \qquad J_b > 0, \qquad J_c > 0. \quad [26]$$

Continuing with these steady-state examples, what can we say about the sign of J_{ij} and $\Delta\mu'_{ij}$ (they necessarily have the same sign) for the seven separate transitions? In the case of Fig. 3a, it follows from Eqs. **21** and **24** that all seven of the J_{ij} (and $\Delta\mu'_{ij}$) in Eq. **21** must be positive. But for Fig. 3c, from Eqs. **21** and **26**, although the first six J_{ij} are surely positive, the sign of J_{34} (and $\Delta\mu'_{34}$) is uncertain (it depends on the particular set of α's). Note that if $X_1 > X_2$ in Fig. 3c, we do not necessarily have $J_{34} > 0$, because the rate constants for the pairs of transitions 42, 21, 13 could be relatively small, leading to $J_a < J_b$. This is possible because the fluxes are proportional to the differences $\Pi_{a+} - \Pi_{a-}$ and $\Pi_{b+} - \Pi_{b-}$ while the forces are related to the corresponding ratios (Eqs. **25**).

A further comment on Fig. 3c: since the rate of cycle completions *in a given direction* is given by (4, 5)

$$J_{a+} = N\Pi_{a+}\Sigma_a/\Sigma, \qquad J_{a-} = N\Pi_{a-}\Sigma_a/\Sigma, \text{ etc.,} \qquad [27]$$

we can conclude that the force alone (i.e., for *any* allowed set of α's) determines the *ratio* of the opposed cycle fluxes:

$$J_{a+}/J_{a-} = e^{X_1/kT}, \qquad J_{b+}/J_{b-} = e^{X_2/kT}, \text{ etc.} \qquad [28]$$

Cycle fluxes are not experimentally observable (except in the case of single-cycle diagrams); the operational combinations of cycle fluxes for the present example are given in Eqs. **21**.

It is easy to show, for Fig. 3c, using Eqs. **21**, that the total rate of entropy production is

$$T\frac{d_iS}{dt} = \sum_{ij} J_{ij}\Delta\mu'_{ij} = J_{21}X_1 + J_{56}X_2, \qquad [29]$$

where the ij sum is over the seven transitions in the diagram. The other sum is the phenomenological one.

Finally, we note that Eq. **29** can also be written as

$$T\frac{d_iS}{dt} = J_aX_1 + J_bX_2 + J_c(X_1 + X_2). \qquad [30]$$

This is a special case of a general result (easy to prove) for an arbitrary diagram at steady state:

$$T\frac{d_iS}{dt} = J_aX_a + J_bX_b + \cdots \geq 0, \qquad [31]$$

where X_a is the total (net) thermodynamic force in cycle a in the direction of J_a, etc. That is, the total rate of entropy production may be considered to be a sum of contributions from the separate cycles. Each term in the sum is ≥ 0 (see, for example, Eq. **23**).

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