Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 2922–2925, May 1983
Biophysics

Some general principles in free energy transduction

(ligand chemical potentials/free energy subdivision/kinetics and mechanism/cycles)

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ABSTRACT **Chemical potentials or standard chemical potentials of bound ligands cannot be used to follow the step-by-step transfer of free energy from one ligand to another in a free energy transducing cycle. The basic difficulty is that, in most states of the cycle, separate ligand free energies are not even defined because, when ligands are bound on the enzyme, the interaction free energy of the complex cannot simply be assigned to ligands nor in general even be divided between two ligands if both are bound. This is a mutual, indivisible free energy among enzyme and ligands. Separate ligand free energies are well defined only at the complete cycle level, where the enzyme drops out of consideration (returns to its original state). Other types of free energy are also considered in order to discuss recent work of Tanford. In principle, the kinetics and mechanism can be followed in molecular or atomic detail through the steps of a transduction cycle, but the transfer of free energy from one ligand to another cannot be so followed.**

In 1981 Tanford (1) advocated the use of the chemical potentials or standard chemical potentials of bound ligands in order to follow the step-by-step transfer of free energy from one ligand to another through the free energy transduction cycle of the associated enzyme. In the same year, Hill and Eisenberg (2) presented a detailed analysis that showed explicitly in two examples that this point of view is untenable. Briefly, this follows because the free energy of the system at intermediate stages of the cycle is inextricably mixed up among the enzyme and the two ligands and cannot be assigned to the separate ligands. The enzyme drops out and the ligands tell the whole story only at the complete cycle level. Our objection was not to the use of these chemical potentials as thermodynamic quantities but rather to the unjustified molecular interpretation they were given.

In a recent paper (3), Tanford applied the same principles to the Ca-ATPase system as an illustration, without meeting or even referring to the objections we raised (2). Also, in several places Tanford attributed to me views that I do not hold and have never stated. In a current paper (4), a different such misstatement is made. The purpose of the present note is to deal with these matters, using a previous example (2) to illustrate a number of points.

THE MODEL

We consider a hypothetical membrane system in which a chemical reaction $S \rightarrow P$ (e.g., $ATP \rightarrow ADP + P_i$) can drive a ligand L (e.g., Ca^{2+}) "uphill" from inside to outside ($L_i \rightarrow L_o$), catalyzed by an enzyme E. The mechanism (the only kinetically important cycle in the diagram) is specified by the successive states in column 1 of Table 1. There are seven steps in the cycle, designated by Roman numerals at the left of the table. The net change in one cycle is $S + L_i \rightarrow P + L_o$. The enzyme shifts access of the L binding site from inside to outside by the conformation change $E \rightarrow E^*$. The two thermodynamic forces are (we omit the membrane potential for simplicity)

$$X_S = \mu_S - \mu_P,\ X_L = \mu_{L_i} - \mu_{L_o}, \qquad [1]$$

where the chemical potentials are related to concentrations by

$$\mu_L = \mu_L^\circ + RT \ln c_L, \text{ etc.} \qquad [2]$$

The force X_S is large and positive whereas X_L is large and negative (i.e., $c_{L_o} \gg c_{L_i}$). The sum $X = X_S + X_L$ is the total force acting in the cycle; this is small and positive (in the usual case). That is, X_S is a little larger than $-X_L$. Per cycle, an amount of free energy X_S is produced by $S \rightarrow P$, of which $-X_L$ is used to transport L from in to out and the remainder, X, is dissipated as heat. The efficiency is simply $\eta = -X_L/X_S$, because of the complete coupling in the only important cycle.

DEFINITIONS

The enzyme can exist in the membrane in seven different states (Table 1), E, LE, ..., EP. For an arbitrary state j, in this list, the enzyme chemical potential can be written (2, 5)

$$\mu_j = G_j + RT \ln p_j, \qquad [3]$$

where p_j is the fraction of enzyme molecules in state j and G_j is the standard chemical potential of the enzyme in state j (standard state, $p_j = 1$).

There are three classes of transition in the cycle. For an *isomeric transition* (III, V, or VI) from state j to state k, the relation between the first-order rate constants α_{jk} and α_{kj}, the standard free energy changes, and the dimensionless isomeric equilibrium constant K_{jk} is (2, 5)

$$G_j - G_k = RT \ln(\alpha_{jk}/\alpha_{kj}) = RT \ln K_{jk}. \qquad [4]$$

For a *binding transition*—e.g., $E + L \rightleftarrows LE$—with L at concentration c_L, the corresponding equation is (2, 5)

$$(\mu_L + G_E) - G_{LE} = RT \ln(\alpha_f/\alpha_r) = RT \ln K_b = RT \ln(K_b^* c_L). \qquad [5]$$

Here μ_L is given in Eq. 2, α_r is the first-order reverse rate constant, $\alpha_f = \alpha_f^* c_L$ is the pseudo-first-order forward rate constant, α_f^* is the second-order forward rate constant, K_b^* is the conventional binding constant, and $K_b = K_b^* c_L$ is a dimensionless "isomeric" binding constant. By including the term RT ln c_L in the various expressions in Eq. 5, this binding transition has been recast in the form of an effective isomeric transition. For a *release transition*, Eq. 5, or the equivalent, is simply multiplied through by -1.

The free energy changes in the successive steps of the cycle, when all steps are put in the isomeric form as in Eqs. 4 and 5, are called *basic free energy* changes (2, 5) and denoted by $\Delta G'$.

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Table 1. Subdivision of free energy of enzyme states

	1 State	2 S	3 E,S	4 L	5 E,L	6 E	7 E,L,S	8 Basic free energy = total†
I	(*a*) $E + S + L_i$	μ_S	—	μ_{L_i}	—	G_E	—	G_0
II	(*b*) $LE + S$	μ_S	—	—	$\mu_{L_i} - I$	G_E	—	$G_0 - I$
III	(*c*) LES	—	$\mu_S - II - w_S$	—	$\mu_{L_i} - I$	G_E	w_S	$G_0 - I - II$
IV	(*d*) LE*S	—	$\mu_S - X_S + V + VI + VII - A$	—	$\mu_{L_i} - X_L + IV - w_S^*$	$G_E + A$	w_S^*	$G_0 - I - II - III$
V	(*e*) $E^*S + L_o$	—	$\mu_S - X_S + V + VI + VII - A$	$\mu_{L_i} - X_L$	—	$G_E + A$	—	$G_0 - I - \ldots - IV$
VI	(*f*) $E^*P + L_o$	—	$\mu_S - X_S + VI + VII - A$	$\mu_{L_i} - X_L$	—	$G_E + A$	—	$G_0 - I - \ldots - V$
VII	(*g*) $EP + L_o$	—	$\mu_S - X_S + VII$	$\mu_{L_i} - X_L$	—	G_E	—	$G_0 - I - \ldots - VI$
	(*h*) $E + P + L_o$	$\mu_S - X_S$	—	$\mu_{L_i} - X_L$	—	G_E	—	$G_0 - I - \ldots - VII$

†$G_0 \equiv \mu_S + \mu_{L_i} + G_E$.

For example, for transitions I, III, and IV in Table 1,

$$\Delta G'_I = (\mu_{L_i} + G_E) - G_{LE} \quad [6]$$

$$\Delta G'_{III} = G_{LES} - G_{LE^*S} \quad [7]$$

$$\Delta G'_{IV} = G_{LE^*S} - (\mu_{L_o} + G_{E^*S}). \quad [8]$$

Note that Δ here means *initial − final*.

The *gross free energy* changes (5) in successive steps of the cycle simply include the p_j terms, as in Eq. 3. For example, at steady state, the steady-state p_j are used, as calculated from all the rate constants of the cycle (5). Thus, for a transition (of any type) from state j to state k, with basic free energy change $\Delta G'_{jk}$, the gross free energy change is

$$\Delta\mu'_{jk} = \Delta G'_{jk} + RT \ln(p_j/p_k). \quad [9]$$

The sum of either $\Delta G'_{jk}$ or $\Delta\mu'_{jk}$ over all steps of the cycle is X, which depends only on the ligand solution chemical potentials (Eq. 1) and not in any way on the enzyme or on the p_j. Gross free energy changes are related to the dissipation of free energy and to the second law of thermodynamics (5).

If a bound ligand L—e.g., in the state LE as in Eq. 5—is in equilibrium with free ligand at c_L, then the chemical potential of the bound ligand is equal to μ_L in solution, as given in Eq. 2. However, as part of a steady-state cycle, bound ligand in general will not be in equilibrium with free ligand. It is therefore more satisfactory to employ the *standard chemical potential of a bound ligand*, which is not subject to this complication. One then need not make any approximation nor work only at equilibrium, as Tanford does (3). Standard chemical potentials were used, for this reason, in ref. 2. In any case, in realistic steady-state systems, the difference between the chemical potential and the standard chemical potential of a bound ligand is small (2, 3).

We use the notation $\mu^\circ_L(LE)$ for the standard chemical potential of L bound on E. For brevity, we here define $\mu^\circ_L(LE)$ (1–3, 6) using the conventional relation between a standard free energy change and the corresponding equilibrium constant:

$$\mu^\circ_L(LE) - \mu^\circ_L = -RT \ln K^*_b. \quad [10]$$

The standard state for bound L is E half-occupied by L (1–3, 6). From Eq. 5 we then find

$$\mu^\circ_L(LE) = G_{LE} - G_E \quad [11]$$

$$= -\Delta G' + \mu_L, \quad [12]$$

where $\Delta G'$ is the basic free energy change in the binding process. We see from Eq. 10 that $\mu^\circ_L(LE)$ is simply an alternative way to express K^*_b. Eq. 11 shows how $\mu^\circ_L(LE)$ is related to enzyme state free energies. Note that the interaction between L and E and any perturbation of E, when L is bound, is attributed entirely to L by the thermodynamic quantity $\mu^\circ_L(LE)$. This is a distinctly unsymmetrical and nonmolecular point of view which is basically at the root of the difficulty with Tanford's approach.

SUBDIVISION OF FREE ENERGY OF ENZYME STATES

We now examine the free energy of *enzyme + ligands* in the successive states of the cycle, (*a*) through (*h*) in Table 1. Our objective is to break down each of these state free energies into contributions that can be assigned to: S alone; E and S inextricably; L alone; E and L inextricably; E alone; and E, L, and S inextricably. Tanford would like to be able to assign free energies to S (including P) and L only (the ligands in this model) and thus to detect where in the cycle S hands free energy over to L. However, this is not possible because of the inextricable sharing of free energy between the ligands (S and L) and E (2). In most states, the separate free energies of S and L are not even defined. The closest one can come to Tanford's goal is the classification of the state free energies into the six categories listed above. This, in fact, is a more detailed molecular breakdown than simply into the standard chemical potentials of bound S and L (as Tanford advocates). But even this more detailed breakdown does not answer the essentially meaningless question: Just where in the cycle is free energy transferred from S to L?

We now denote the successive basic free energy changes $\Delta G'_I$, $\Delta G'_{II}$, ... for the steps in Table 1 (see Eqs. 6–8) simply by I, II, ... X is the sum of these; X is usually small and positive and all of I, II, ... are usually small (2). Also, $X = X_S + X_L$, where X_S and $-X_L$ are both large and positive. The free energy of the starting state (*a*) in Table 1 is denoted $G_0 \equiv \mu_S + \mu_{L_i} + G_E$ (for $S + L_i + E$). The free energy levels of the successive states, in terms of basic free energy changes, are then $G_0 - I$, $G_0 - I - II$, etc., as listed in column 8 of Table 1. The final value in column 8 is also equal to $G_0 - X$.

By considering the full kinetic diagram (it is not sufficient to consider only the main cycle), it is possible (2) to break down the total state free energies in column 8 into the separate categories in columns 2 through 7. Three new parameters appear, and are defined by:

$$A = G_{E^*} - G_E \quad [13]$$

$$w_S = G_{LES} + G_E - G_{LE} - G_{ES} \quad [14]$$

$$w_{S^*} = G_{LE^*S} + G_{E^*} - G_{LE^*} - G_{E^*S}. \quad [15]$$

A is presumably large and positive; w_S and w^*_S relate to *any* S,

Table 2. Chemical potential or standard chemical potential of ligands in various states

	State	μ_S	μ_L
I	(*a*) E + S + L_i	μ_S	μ_{L_i}
II	(*b*) LE + S	μ_S	$\mu_{L_i} - I$
III	(*c*) LES	$\mu_S - II$	$\mu_{L_i} - I + w_S$
IV	(*d*) LE*S	$\mu_S - X_S + V + VI + VII + w_S^* - A$	$\mu_{L_i} - X_L + IV$
V	(*e*) E*S + L_o	$\mu_S - X_S + V + VI + VII - A$	$\mu_{L_i} - X_L$
VI	(*f*) E*P + L_o	$\mu_S - X_S + VI + VII - A$	$\mu_{L_i} - X_L$
VII	(*g*) EP + L_o	$\mu_S - X_S + VII$	$\mu_{L_i} - X_L$
	(*h*) E + P + L_o	$\mu_S - X_S$	$\mu_{L_i} - X_L$

L interactive effects, direct or indirect, in the *dual* binding of S and L on E and E*. These latter quantities (w_S and w_S^*) may be small or large.

From the definition of the standard chemical potential of a bound ligand, it is also possible (2) to fill in Table 2 for the state-by-state chemical potential (free ligand) or standard chemical potential (bound ligand) of both S and L.

Table 1 demonstrates that most of the entries are not attributable to S alone or to L alone. Thus, it is not possible in most states to say what the free energy of either S or L is as the system goes through a cycle and consequently it is not possible to follow the transfer of free energy from S to L on a state-by-state basis. We can only say that the transfer is accomplished by a complete cycle, because E has not changed at all in a complete cycle but S and L have.

Table 2 for the chemical potentials of S and L is of even less use in tracing the state-by-state free energy transfer because it is less detailed than Table 1. In fact, the μ_S column in Table 2 is just the sum of the (S), (E,S), and (E,L,S) columns in Table 1 and the μ_L column in Table 2 is the sum of the (L), (E,L), and (E,L,S) columns in Table 1. Note that the (E,L,S) column gets counted twice. This in itself is fatal for Tanford's suggestion as a general procedure. That is, the free energy of the system cannot be cleanly divided into S free energy and L free energy if part of the system free energy belongs to *both* categories.

DISCUSSION

Although the free energy transfer from S to L is well defined and comprehensible only at the complete cycle level, the vital *kinetics* of the system can and must be examined at the individual transition level. In the present model, the kinetic behavior is implicit in the complete set of 14 rate constants in the main cycle plus those additional very small rate constants that isolate the main cycle from the rest of the kinetic diagram for practical kinetic purposes. In principle, each of all these rate constants can be examined as a detailed quantitative, molecular problem. This is basically the approach taken by Jencks and others. Thus, the *kinetics and mechanism* of the hypothetical S, L pump can be understood in full only by a most detailed molecular, even atomic, dissection of the states and transitions of the system. However, in the course of this step-by-step analysis it is *not* possible to follow the *free energy transfer* from S to L because the enzyme E is as intimately involved in the transition energetics as are S and L.

Thus, the chemical potentials of bound ligands cannot be used to follow the details of step-by-step free energy transfer between ligands. Also, being so closely related to equilibrium binding constants (Eq. **10**), they are of little help in understanding the *kinetics* of the system.

Similarly, the step-by-step free energy transfer cannot be followed by use of the basic free energy changes or the gross free energy changes or even the more detailed subdivision of free energies used in Table 1. A non-question has no answer.

Tanford (3) repeatedly states or implies that I have advocated the use of basic free energy changes for the analysis of free energy transduction. This is not the case at all. In my book (5), free energy transduction is covered before basic free energy levels are even defined. In a short review paper (7), the section on free energy transduction makes no mention of basic free energy levels (although they were already introduced). In the long review paper with Eisenberg (2), we stated explicitly on p. 483: "But these equations [expressing the successive basic free energy levels of the states of the main cycle] are of no help in a molecular interpretation of the free energy transduction." In all of these sources the emphasis has been on the free energy transduction as a property of the complete *cycles* of the diagram. This point of view is reinforced in two recent papers (8, 9) in which it is shown that, for steady states near equilibrium, the Onsager coefficients L_{ij} in the linear flux-force equations of a free energy transducing system are related in a very simple way to the one-way *cycle* fluxes of the kinetic diagram, evaluated at equilibrium. No mention is made of basic free energy levels. In a near-equilibrium system, free energy transduction (coupling) is of course governed by the cross-coefficients L_{ij} ($i \neq j$), which are shown in the above-mentioned work (8, 9) to be *cycle* properties.

Basic free energy levels *are* "basic" (analogous to the energy levels of a quantum mechanical system) and are especially useful in kinetic and stochastic analyses (5). Basic levels are *intrinsic* properties of each individual kinetic unit in the ensemble (or collection) of units. They do not depend on the state of the ensemble itself—e.g., whether the ensemble is in a transient or at steady state. In contrast, the gross free energy levels are *ensemble* properties (hence, the name "gross") that depend on the generally time-dependent distribution p_i of units of the ensemble among the possible enzyme states i (5). Gross free energy changes govern the *statistical* direction of spontaneous transitions in the kinetic diagram in accordance with the second law of thermodynamics.

In a current paper (4), Tanford stated that in my book (5) "it is asserted that it is not even possible to arrive at a step-by-step mechanism of the kind that biochemists normally seek." The complete inaccuracy of this statement is apparent from the fact that the entire book is in fact devoted to the *analysis* of such step-by-step mechanisms. That is, specification of the mechanism by way of enumeration of all the states and all the rate constants for possible transitions between these states is a prerequisite for the application of the general results of the book. Far from it not being "possible to arrive at a step-by-step mechanism," my book requires that one *start* with a step-by-step mechanism.

Finally, to return to Tanford's interpretation of chemical potentials of bound ligands: the disagreement on this point is only conceptual. Strength of binding of ligands is unquestionably an important thermodynamic (but not kinetic) component of the mechanism in free energy transducing systems. Considerable attention was paid to strength of binding in the review by Hill and Eisenberg (2). Standard chemical potentials of bound ligands contain equivalent thermodynamic information (2) about the mechanism (Eq. **10**). However, free energies that are actually *separate properties of the ligands themselves* are something else again. They cannot be defined and hence cannot be traced through the transducing cycle. The analogy with the so-called "high-energy phosphate bond," say of ATP, is close. It may be intuitively appealing to think of the high free energy

of hydrolysis of ATP as a separate property of one particular bond in ATP, as is often done, but it is still wrong.

1. Tanford, C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 270–273.
2. Hill, T. L. & Eisenberg, E. (1981) *Q. Rev. Biophys.* **14**, 463–511.
3. Tanford, C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6527–6531.
4. Tanford, C. (1983) *Annu. Rev. Biochem.* **52**, 379–409.
5. Hill, T. L. (1977) *Free Energy Transduction in Biology* (Academic, New York).
6. Hill, T. L. (1960) *Statistical Thermodynamics* (Addison-Wesley, Reading, MA).
7. Hill, T. L. (1977) *Trends Biochem. Sci.* **2**, 204–207.
8. Hill, T. L. (1982) *Nature (London)* **299**, 84–86.
9. Hill, T. L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2589–2590.