Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 5302–5304, September 1983
Alternative Viewpoint and Reply

Time-averaged chemical potential of proteins and the detailed-balance principle (An Alternative Viewpoint)

(chemical equilibrium/protein reaction)

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In a recent article, Xu and Weber (1) describe a theoretical model for protein dimerization at thermodynamic equilibrium. However, their proposed reaction scheme must fail because it violates the detailed-balance principle.

The main assumptions of Xu and Weber are as follows: (*i*) Each protein has a set of structural substates and the effective chemical potential is a time average over the structural fluctuations. (*ii*) The interaction free energy between subunits in a dimer depends on the particular substate occupied by each subunit. Thereby, proteins that are parts of a dimer will tend to be in a different preferred substate from those that are free monomers. (*iii*) If the structural transitions are much slower than the association–dissociation reactions, the proteins will not have time to relax to their respective preferred substate during the short life times of the monomers and dimers. Instead, they will be trapped in some intermediate state. In this way, it is proposed that by "time averaging" over the association–dissociation reactions, both monomers and dimers will have chemical potentials that depend on the time scales of these reactions. Thereby, the chemical potentials will depend on the concentrations in a nontrivial way.

While assumptions *i* and *ii* seem entirely reasonable, *iii* is totally untenable because it violates detailed balance in at least two ways. First, to have any meaning, the proposed time averaging implies that the small internal relaxation (assumed unidirectional and therefore irreversible) during the short lifetime of the monomer is counterbalanced by a corresponding relaxation during the lifetime of the dimer. This creates a net flux along a closed loop in the reaction diagram and an ensuing dissipation that is at variance with the equilibrium assumption.

As a further consequence of the detailed-balance principle, the equilibrium distribution over internal states cannot be changed *solely* by the introduction of new reaction pathways; i.e., the distribution over the internal states of a free monomer must be the same regardless of whether it is alone in the reaction vessel—such that no dimerization can take place—or the degree of dimerization is high.

DETAILED BALANCE

The principle of detailed balance is based on the time-reversal symmetry of the microscopic equations of motion (microscopic reversibility). For macroscopic states—such as the structural substates of a protein—that are averages over a large number of microscopic degrees of freedom, it is also necessary that Liouville's theorem be invoked. (For a recent discussion, see, e.g., ref. 2.) Thus, the detailed-balance principle has a firm basis in the dynamic equations.

At equilibrium, the principle states that the expected numbers of transitions per unit time between any two states mutually balance each other:

$$w_{ij}P_i = w_{ji}P_j, \qquad [1]$$

where P_i is the probability of finding the system in state i and w_{ij} is the transition rate from state i to state j. The relative occupation

$$P_i/P_j = w_{ji}/w_{ij} = \exp(-\Delta G_{ij}/kT) \qquad [2]$$

can serve as a definition of the free-energy difference between states i and j. Obviously, this relative occupation cannot change when the state space is expanded through the introduction of new reactions unless the transition rates w_{ij}/w_{ji}—and thereby the free-energy difference—are explicitly dependent on some parameter associated with such an expansion.

In the particular case of protein dimerization, the equilibrium distribution over the internal states of the monomer cannot change solely because of the introduction of the association–dissociation reactions, however fast they may be compared with the internal relaxations. The fact that this introduction involves a coupling between unimolecular and bimolecular processes is of no particular consequence; the macroscopic state space can be suitably defined to include the necessary spatial distributions.

EQUILIBRIUM BINDING CONSTANT

To be more explicit, let us consider an association–dissociation reaction between two molecular species

$$A + B \rightleftharpoons AB, \qquad [3]$$

each of which has a set of structural substates $\{x_A\}$ and $\{x_B\}$. At equilibrium, each molecule must have a well-defined distribution over these substates (compare assumption *i* above):

$$\rho_A^0(x_A) = Z_A^{-1}\exp[-G_A(x_A)/kT] \qquad [4]$$

for unbound A,

$$\rho_B^0(x_B) = Z_B^{-1}\exp[-G_B(x_B)/kT] \qquad [5]$$

for unbound B, and

$$\rho_{AB}(x_A,x_B) = Z_{AB}^{-1}\exp\{-[G_A(x_A) + G_B(x_B) + \Delta G(x_A,x_B)]/kT\} \qquad [6]$$

for AB complexes. $G_A(x_A)$ and $G_B(x_B)$ are the internal free energies for molecules A and B in substates x_A and x_B, respectively, and $\Delta G(x_A,x_B)$ is the interaction free energy in a complex with subunits in states x_A and x_B. In principle, these free energies can be defined by the distributions 4–6. The respective partition functions Z_A, Z_B, and Z_{AB} are of course the usual integrals over all internal states that normalize the distributions. The effective binding constant can be defined from the partition functions

$$K_{AB} \equiv Z_{AB}/Z_AZ_B \qquad [7]$$

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and one finds that

$$K_{AB} = \int\int dx_A dx_B \rho_A^0(x_A)\rho_B^0(x_B)\exp[-\Delta G(x_A,x_B)/kT]$$
$$= \langle\langle \exp(-\Delta G/kT)\rangle\rangle_0. \quad [8]$$

Thus, the equilibrium constant is an average of $\exp(-\Delta G/kT)$ taken over the *uncomplexed* equilibrium distributions. By assumption *ii* above, the interaction free energy $\Delta G(x_A,x_B)$ between subunits depends only on their respective internal states. By detailed balance, ρ_A^0 and ρ_B^0 are independent of the degree of complex formation.

Equivalently, one could derive the effective binding constant from the chemical potentials. For a free A molecule in substate x_A, the chemical potential is

$$\mu_A(x_A) = G_A(x_A) + kT \ln[c_A\rho_A^0(x_A)], \quad [9]$$

where c_A is the total concentration of free A such that $c_A\rho_A^0(x_A)$ is the concentration of free A in state x_A. Thus, the average chemical potential is

$$\bar{\mu}_A \equiv \int dx_A \rho_A^0(x_A)\mu_A(x_A) = -kT \ln(Z_A/c_A). \quad [10]$$

The effective binding constant [8] follows from this and the corresponding expressions for B and AB.

To get a concentration-dependent binding constant, one can introduce a concentration dependent shift in the free-energy levels of the internal states. Molecularly, such a shift could occur due to the fact that at higher concentrations a protein would be more likely to find itself close to another—perhaps even associated through dispersion forces or such—although not bound in the sense that is registered by the experimental procedure. Thus, there is ample room in a traditional equilibrium description to allow for a binding constant that depends on concentration or on the degree of complex formation.

DISCUSSION

To explain their dissociation data for enolase, Xu and Weber require a concentration-dependent equilibrium binding constant. As discussed above, this could be achieved by introducing nonideal solutions or by considering higher-order aggregates; i.e., one should go beyond the simple two-body interaction scheme. No new principle need be invoked at the expense of fundamental physical theory.

The author has benefited from discussions with Drs. M. Ehrenberg, G. Lindblad, and C. Blomberg. Financial support from the Swedish Natural Science Research Council is gratefully acknowledged.

1. Xu, G.-J. & Weber, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5268–5271.
2. Kreuzer, H. J. (1981) *Nonequilibrium Thermodynamics and Its Statistical Foundations* (Oxford Univ. Press, Oxford), p. 53.

Stability of oligomeric proteins and its bearing on their association equilibria (A Reply)

(enolases/fluorescence/equilibria)

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The experimental observations of Xu and Weber (1) on yeast enolase and similar earlier data of Shore and Chakrabarthy (2) on the dissociation of malate dehydrogenase cannot be explained without assuming that the apparent standard free-energy change in the association of each of these two protein dimers varies with the extent of the association reaction. That much is clear, though opinions may differ as to the origin of the variation. Xu and Weber believe that it follows from actual changes in the chemical potentials of monomer and dimer: At intermediate degrees of association, the conformations of monomer and dimer are assumed to differ from those characterizing these species when either is greatly predominant in the mixture.

Berg (see above) thinks that this point of view contradicts the principle of detailed balance. We can examine the implications of this principle for our case by reference to Fig. 1. This figure depicts the free-energy relations between the protein forms present at extreme degrees of association ($\alpha = 0$) and dissociation ($\alpha = 1$). A gross violation of detailed balance will be incurred if every dissociation is followed by the full change in conformation $M(\alpha = 0) \rightarrow M(\alpha = 1)$ and, in every association, the change in conformation of the monomer within the dimer is of equal magnitude. In this case, circulation along the path ABCD occurs at equilibrium. However, if, at intermediate degrees of dissociation α', the protein forms present are not those characterized by the free energies of $M(\alpha = 1)$ and $D(\alpha = 0)$ but instead have intermediate chemical potentials, the circulation will be confined to the course A'B'C'D' in the figure. The directional free-energy change δG occurring within the lifetime of a monomer or dimer corresponds in that case to the projection of B'C' or D'A' on the free-energy axis. We expect δG to be a fraction of $\Delta G(0) - \Delta G(1)$ of the order of: time of one binding-association cycle divided by time for attainment of equilibrium after dilution. In yeast enolase, equilibration after dilution takes many minutes (1) while a cycle of binding and dissociation may take only a small fraction of a second, if one is to judge by the typical times for such cycles in the binding of small ligands by proteins. The experimental figure for $\Delta G(0) - \Delta G(1)$ is 1.5 kcal/mol (1 cal = 4.18 J) so that in absolute value δG will be much smaller than the thermal energy kT. I do not believe that it has much meaning—except as an intellectual exercise unrelated to experimental reality—to debate about the application of detailed balance to the interconversion of molecular forms that differ by free energies significantly smaller than the thermal energy (3).

One has to recognize that the analysis presented above provides no substitute for the detailed knowledge of the microscopic states of the protein particles at intermediate degrees of dissociation, a knowledge indispensable to decide on the validity of our hypothesis: the variation of the chemical potential with the extent of reaction. Lacking this knowledge, we must for the present remain content with considering possible models that are intuitively satisfying and that suggest significant experiments to test the properties of oligomeric proteins. The proposal of Xu and Weber should be viewed in this light: the variation of the chemical potential with degree of association explains not only the specifically observed effects but also the difficulty—often amounting to unfeasibility—of finding a con-