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)

GREGORIO WEBER

Roger Adams Laboratory, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801

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-

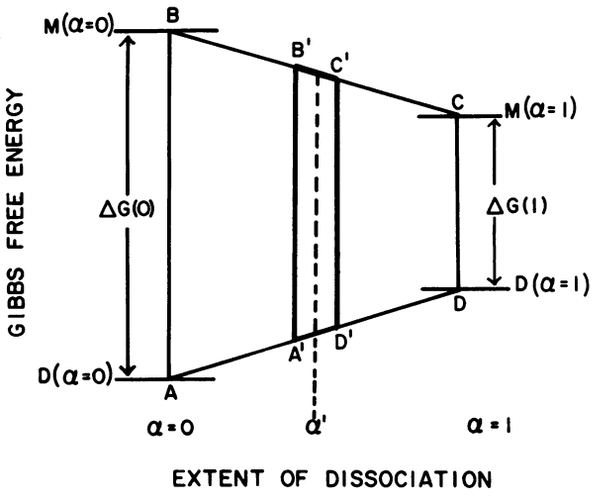

FIG. 1. Schematic of Gibbs energy levels of monomer M and dimer D at various degrees of dissociation α. $\Delta G(0)$, $\Delta G(1)$ are the free energy changes on dimerization at $\alpha \rightarrow 0$ and $\alpha \rightarrow 1$.

centration range in which an easily displaceable equilibrium exists between a protein aggregate and its constitutive oligomers. It indicates why intermediate situations between these cases and those of invariant chemical potentials are to be found in the simplest aggregates (dimers). It also predicts that dissociating perturbations that are rapidly reversible [e.g., pressure (4, 5)] can result in authentic initial aggregates with altered conformational properties.

Berg offers alternative explanations for the experimental findings: protein activity coefficients that differ from unity and higher states of aggregation beyond those considered in the formulation of the equilibrium by Xu and Weber. Both of these suggestions can be dismissed: the first by noticing that at the experimental concentrations (0.1–1 μM) used, the average distance between neighbor protein centers is 650–1,400 Å; the second because one of the methods employed (fluorescence polarization of labeled enolase or malate dehydrogenase) determines the degree of reaction through measurement of the average volume of the protein particles.

1. Xu, G.-J. & Weber, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5268–5271.
2. Shore, J. D. & Chakrabarthy, S. K. (1976) *Biochemistry* **15**, 875–879.
3. Brillouin, L. (1960) *Science and Information Theory* (Academic, New York), pp. 163–190.
4. Paladini, A. A. & Weber, G. (1982) *Biochemistry* **20**, 2587–2593.
5. Mueller K., Luedemann, H.-D. & Jaenicke, R. (1982) *Biochemistry* **20**, 5411–5416.