Technical Comment

Understanding Hydrophobic Behavior

The exclusion of nonpolar molecules from water is an important phenomenon in the industrial and environmental processing of chemicals and in the stabilization of biological structure. Unfortunately, semantic ambiguities and thermodynamic subtleties have lent confusion to discussions of the molecular basis for hydrophobic behavior. K. A. Dill (*1*) has cautioned against the loose use of terminology relating to hydrophobicity, and K. P. Murphy *et al.* (*2, 3*) have elucidated thermodynamic aspects of hydrophobicity in proteins. While these authors make useful and valid points, unnecessary semantic and thermodynamic difficulties remain.

This confusion can be clarified by reference to the relations between the changes of state illustrated in Fig. 1, with the use of the conventional notation Δ_{a}^{b} for the change from state a to state b. The distinctive features of water as a solvent, as compared specifically to those of nonpolar organic solvents, are most clearly reflected in the difference between transfer of solute from any solvent independent state (for example, pure gas, g, pure liquid, l, or pure solid, s) to water, w, on the one hand, and a nonpolar organic solvent, o, on the other. Accordingly, the best experimental measurement of the hydrophobic effect is

$$\Delta_{\mathrm{hyd}} = \Delta_{g}^{w} - \Delta_{g}^{o} = \Delta_{l}^{w} - \Delta_{l}^{o} = \Delta_{s}^{w} - \Delta_{s}^{o}$$

Because differences among various nonpolar organic solvents are expected to be relatively small (the most significant being between the aromatic and nonaromatic), it is generally assumed that $\Delta_{o'}^{o} \sim 0$ and therefore, for nonpolar solutes, $\Delta_{l}^{o} \sim 0$. It follows that $\Delta_{\mathrm{hyd}} \sim \Delta_{l}^{w} \sim \Delta_{o}^{w}$. It is also apparent that dissolution of solid nonpolar solute then has an additonal fusion contribution

$$\Delta_{s}^{w} \sim \Delta_{l}^{w} + \Delta_{s}^{l} \sim \Delta_{\mathrm{hyd}} + \Delta_{\mathrm{fus}}$$

and dissolution of a gaseous nonpolar solute has an additional condensation contribution

$$\Delta_{g}^{w} \sim \Delta_{l}^{w} + \Delta_{g}^{l} \sim \Delta_{\mathrm{hyd}} + \Delta_{\mathrm{cond}}$$

In light of these relations, the definition preferred by Dill of the hydrophobic effect as the transfer of nonpolar solute to water with a large ΔC_p (*1*) should be narrowed to specify that the transfer is from the pure liquid or from a nonpolar organic solvent ($\Delta_{l}^{w} \sim \Delta_{o}^{w} \sim \Delta_{\mathrm{hyd}}$), even though $\Delta_{g}^{w}C_p$ is also large and $\Delta_{s}^{w}C_p$ is still positive. Moreover, the data in the paper by Murphy *et al.* (*2*) showing that the unfolding of a protein from the native to the denatured state d(Δ_{n}^{d}) behaves like the dissolution of solid solutes in water ($\Delta_{s}^{w} \sim \Delta_{\mathrm{hyd}} + \Delta_{\mathrm{fus}}$) rather than the dissolution of liquid solutes in water ($\Delta_{l}^{w} \sim \Delta_{\mathrm{hyd}}$), should be analyzed in a fashion that distinguishes the hydrophobic contribution (Δ_{hyd}), which helps to stabilize protein structure, from the fusion contribution (Δ_{fus}), which has nothing to do with hydration or water. This approach would relate the work of Murphy *et al.* more closely to that of R. L. Baldwin (*4*), take into account the distinct difference in temperature dependence that they have documented between $\Delta_{g}^{w}C_p$ and $\Delta_{n}^{d}C_p$ (*5*), and make their results consistent with established views of the contribution of the hydrophobic effect to protein stability.

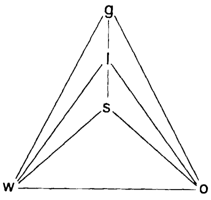

Fig. 1. Transitions between different states of a solute. The letters g, l, and s, correspond to the pure (solvent-free) gas, liquid, and solid states, respectively. The letters w and o correspond to solutions in water and in nonpolar organic solvent, respectively.

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16 November 1990; accepted 28 January 1991