VOLUME CHANGES OF GLOBULAR PROTEIN ASSOCIATION

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Water is generally absent from the interior of globular proteins and from intersubunit interfaces (1). The folding and assembly of proteins is, therefore, often represented as a transfer of matter from an aqueous to a less polar milieu. As Kauzmann observed many years ago (2), the volume changes of transfer of small organic molecules lead to the expectation that denaturation, which exposes previously buried side chains to solvent, would occur with a large decrease in volume. Measurements from several laboratories of pressure-induced denaturation reactions have shown that, while the volume changes are negative, they are unexpectedly small (3–6; for a review, see reference 7). As a result, the applicability of model compound transfer data to macromolecular reactions has been questioned, as has the role of hydrophobic properties in stabilizing protein structure (4, 8).

Others, however, have pointed out that the local concentration of side chains in an unfolded polypeptide is sufficiently high that deviations from ideal thermodynamic behavior would be expected to occur (9–11). In support of this, we note that some of the nonideal behavior may arise from the failure of water to gain access to the entire denatured macromolecular surface.

That nonideal behavior is, in fact, implicated is suggested by data on globular protein polymerization. Proteins which polymerize under dilute, relatively ideal thermodynamic conditions yield volume changes comparable to or greater than those of denaturation (12–14). Deoxygenated sickle cell hemoglobin solutions, in contrast, are well known to be nonideal, and their polymerization gives no measurable volume change.[1]

It appears, therefore, that the denaturation experiments give a misleading impression of the volumetric properties of aqueous protein solutions.

To obtain a better understanding of solvent-protein interactions, we are measuring directly the volume changes of association or dissociation of globular subunits. We use proteins whose three-dimensional structures are known. The absence of significant conformational change upon association or dissociation can be proven as well. The volumetric properties can then be correlated with the detailed nature of the molecular surface that is withdrawn from contact with water. The problems that make the denaturation data difficult to interpret thus do not occur, for the solutions are dilute with respect to the relevant reacting species, which are subunits and not side chains, and the nature of the relevant molecular surface is known.

We report here work on the acid-induced dissociation of human methemoglobin (met-Hb) from its normal tetrameric structure to $\alpha\beta$ dimers, as well as measurements on the association of bovine trypsin with bovine pancreatic trypsin inhibitor (PTI).

Volume changes were measured in Carlsberg dilatometers (15, 16) at 20.0 ± 0.001°C. Experiments with trypsin were done in 0.1 M acetate buffer, pH 5.0, to avoid autolysis. For the association of purified β trypsin with PTI the uncorrected volume change is +80 ml/mol. This is comparable to the values cited for chain denaturation (3–6). The measured ΔV, moreover, is a lower limit, for a variety of controls will all contribute to raising the value. These, as well as measurements at higher pH's, are in progress.

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[1]Kahn, P. C., and R. W. Briehl. To be submitted.

Dilatometric titrations of met-Hb were done in the manner of Katz et al. (17) on unbuffered protein in 0.1 M KCl. Where our pH range (pH 7.0–2.0) overlaps that of Katz et al. (17) (pH 6.8–3.5) agreement is excellent. Dissociation to dimers, which occurs between pH 6 and pH 4 (18), yields a measured ΔV of -485 ml/mol of tetramer. Controls for the ΔV of proton binding will make this more negative, and the beginning of denaturation, shown by far ultraviolet, circular dichroism spectra to overlap the end of dissociation, will make it less so. In any case, the dissociation result will still be large and negative.

That the volume changes are large and positive in the direction of subunit assembly is in agreement with expectations based on model compound transfer data. Since the association reaction involves primarily the expulsion of water from the interfacial surfaces into the bulk solvent, an increase of volume upon association requires that the density of hydrating water exceed that of the solvent.

Detailed results, including correlations with nature of the molecular surface that is withdrawn from contact with solvent (19), will be presented.

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MODELING WATER-PROTEIN INTERACTIONS IN A PROTEIN CRYSTAL

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We have used the refined crystal structure of a small protein (trypsin inhibitor (1)) as a system on which to test methods of analysis of solvent structure near protein surfaces in terms of 6–12 and electrostatic potentials.

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