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Detailed results, including correlations with nature of the molecular surface that is withdrawn from contact with solvent (19), will be presented.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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The energy of interaction between a unique conformation of the protein and a single water molecule was found to be a valuable description of solvent space, when presented as a contour map in three dimensions. The zero-energy contour surface separates protein and solvent space and may be considered to define an effective protein surface. Solvent space within the zero-energy contour consists of a single network of channels and spaces of various sizes and shapes plus one isolated low-energy volume that contains one water molecule. All but one of the 47 crystallographically-located water molecules are within the zero-energy contour, and many are in volumes of quite low energy.

A Monte Carlo simulation was performed to sample the equilibrium ensemble of protein-solvent configurations. Translation/rotation of solvent and internal rotation of side chains provided motion. Provision was made for small motions to obtain rapid local equilibration, and for large motions to obtain proper distribution of solvent. Simulated solvent structure is found to be highly ordered: all water molecules in a first, and part of those in a second layer at the protein surface maintain a unique hydrogen-bonded network, that may be considered "anchored" to the protein at low-energy positions of solvent space. The network contains twice as many ordered water molecules as have been located by x-ray crystallography in the crystal of trypsin inhibitor. Extent and order of simulated water are more similar to solvent structure determined by x-ray crystallographic refinement of another small protein, rubredoxin (2). Possible reasons for the differences include high molarity of salt in the crystals, incomplete freedom of motion in the simulated crystal, insufficient size of the simulated statistical sample, use of a model for water-water interactions (3) that exaggerates local order, and incomplete crystallographic refinement of solvent structure.

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WATER-PROTEIN INTERACTIONS

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The interaction of macromolecules with solvent water is an important determinant of their properties, but this relationship has not yet been described satisfactorily. The following experiments focus on the process of protein hydration—the addition of water to dry protein to obtain the solution state. A detailed description of the sequence of hydration events is