Tracking Water Molecules on Protein Surfaces

Helmut Durchschlag[1], Inst. of Biophysics and Phys. Biochem., Univ. Regensburg, Germany
Peter Zipper, Inst. of Chemistry, Univ. Graz, Austria

Water is recognized to have many important roles in influencing protein structure, folding and function. Understanding protein hydration requires the elucidation of the effects of both the solvent and the protein. While a variety of physical techniques (e.g., X-ray crystallography, small-angle X-ray scattering (SAXS), electron microscopy, NMR spectroscopy, computer simulations) are available for the characterization of protein structures in the solid state and in solution, the description of the behavior of water molecules around proteins is much more problematic, owing to the peculiarities of preferentially bound waters (short residence times, higher average density than bulk water, etc.). Localizing water molecules on protein surfaces or in crevices requires the determination of anhydrous and hydrated protein volumes and surfaces, on the one hand, and elucidation of exact topographies of protein envelopes and possible (hydrophilic) water binding sites, on the other. The latter aspect requires knowledge of the 3D structure of proteins and of the amino acid (AA) building blocks.

1 Determination of Protein Volumes, Surfaces, and Hydration

Among the approaches to extract structural information of dissolved proteins, the X-ray techniques are biophysically significant ones. SAXS allows the characterization of the protein size and shape in terms of various molecular parameters, including hydrated particle volume, V_2, and surface area, S_2, by exploiting either the scattering intensity, $I(h)$, or the pair-distance distribution function, $p(r)$. Comparing the hydrated SAXS volume, V_2, with the anhydrous volume, $V_{2,dry}$ (calculated from the molar mass, M_2, of the protein and its partial specific volume, $\bar{v}_2$), allows the amount of hydration, δ_1, to be estimated:

$$\delta_1 = \left(\frac{V_2 N_A}{10^{24} M_2 \bar{v}_2} - 1\right) \bar{v}_2 \rho_1 \qquad (1)$$

where N_A is Avogadro's number and δ_1 symbolizes the solvent density. In accord with other methods, an overall hydration of 0.35 g of water per g of protein has been found for many proteins [1].

2 Modeling of Proteins and Water Molecules

Low-resolution shapes of hydrated proteins can be obtained from SAXS-based conventional or *ab initio* modeling approaches [2], whereas high-resolution 3D information preferably is retrieved from crystal data. The latter approach, however, usually leads to anhydrous protein models, although the available data bases (e.g. the Protein Data Bank, PDB) frequently contain some water coordinates. AA information is usually stored in separate data bases such as SWISS-PROT.

[1] E-mail: helmut.durchschlag@biologie.uni-regensburg.de

Based on the AA and 3D information of proteins, modern calculation programs allow the exact surface topography of proteins ('molecular dot surface') to be calculated analytically, applying some kind of rolling-ball strategy (cf. [3]). The normal vectors of a huge variety of obtained dot surface points may be exploited for creating hypothetical positions of water molecules on the protein surface. Out of this pool of points, special hydration algorithms (program HYDCRYST for atomic coordinates and program HYDMODEL for AA coordinates) have been applied for selecting appropriate positions of water molecules [1, 3–9]. The number of waters assigned to each accessible AA residue is derived from previous NMR or thermodynamic data. A fine-tuning of several input parameters, together with the comparison with reference data (e.g. the waters fixed in crystallographic work), allows scrutinizing the obtained hydration values. The obtained results allow evaluating the results from various X-ray and hydrodynamic techniques more accurately. In addition, from the biophysical point of view, our specific hydration approach is much more realistic than the usually applied assumptions for hydration contributions. In the context with the application and the behavior of nano-compounds, the prediction of their behavior in aqueous solvents is of high biological and technological interest, since water molecules are the lubricant that allows motion of the building blocks.

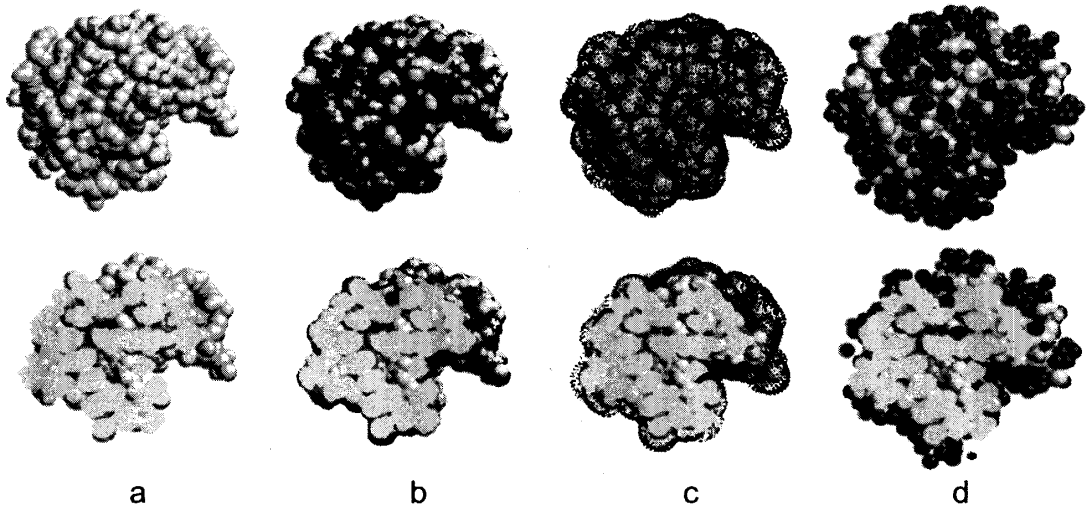

Figure 1: Space-filling models for anhydrous and hydrated lysozyme, together with central slabs: (a) Model for the anhydrous protein; the basic protein atoms are given in light gray. (b) The anhydrous model and dot surface points created for the anhydrous contour. (c) The anhydrous model and surface points created by HYDCRYST for the contour of potential water points located at a certain distance from the initial surface points. (d) Model for the hydrated protein as obtained by HYDCRYST; bound waters are displayed in dark gray.

References

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