

Water-protein interactions

In a recent paper, Madhusudhan and Vijayan¹ reported that it was possible to delineate the flexible and rigid regions of the lysozyme molecule as a function of hydration. This rather interesting study, consistent with earlier reports of a similar kind²⁻⁵, brings to fore the need of some generalizations to explain the underlying mechanisms. The need becomes more acute since we (Sitaramam and Mathai⁶) have reported recently a similar situation with membrane proteins on the basis of altogether different kinds of experiments. The common problem is the variable contact between the solvent and the proteins (water in the case of lysozyme and the lipid in the case of membrane proteins) and structural perturbations associated with the proteins.

The experimental situations are apparently different. In the case of lysozyme the water of hydration of the crystal in a capillary was controlled by altering the salt content of a salt solution, which was only in 'vapour-phase equilibrium' with the crystal and not in direct contact, such that hydration alone was changed. In the case of membrane proteins, we could show that the lipid domain 'moves' on (i.e. interpenetrates into) the polypeptide chains, exposing and burying charged groups, which was detected through binding of the fluorescent probe 8-anilino-1-naphthalene-sulfonic acid (ANS). In the case of lipid, the motive force was definitely identified as an exergonic reaction such as respiration, in that the variable lipid/protein contact was observed only during respiration and was abolished when respiration ceased in submitochondrial particles⁶.

These two experimental situations may be quite comparable and not as diverse as they may appear to be. Since conformation of a polymer pertains to a state of equilibrium, what is the motive force for structural perturbations in the lysozyme molecule? Since water of hydration alone is controlled experimentally, a simple way to look at the problem is to assume that the dielectric profile intrinsic to the molecule, which exhibits spatial heterogeneity, would be hydration to structural perturbations. Since water is associated largely with the charged groups of the protein, the perturbations are mostly on the surface, a

solution for the relevant molecular dynamics would relate charge density and the geometry (e.g. radius) *vis-a-vis* the dielectric.

If we consider the simplest case of the electrostatic energy of a homogeneous charged surface of a sphere, $E = Q^2/2Dr$, where Q are the total charges on the surface, D the dielectric, and r the radius of the sphere, the dielectric and the charge density would contribute to defining the free energy of the system and hence the configuration (geometry). What was important to recognize in our membrane studies was the fact that the effective charge density (of the exposed/unscreened charges) needs to be defined not only in terms of the ionic strength but also in terms of the local pH milieu. Since water ionizes very little, small changes in pH are energetically equivalent to relatively large shifts in ionic strength. Thus ionic strength as well as local pH become important extrinsic variables, which influence the intrinsic variables, viz. the dielectric profile and the charged groups, to define the energy profile, i.e. the configuration of the proteins and fluctuations thereof. Variation in local pH at the membrane surface, particularly in relation to migration of surface protons, has attracted considerable interest in recent years from an energetics point of view.

How justified are we in extrapolating water-protein interactions to lipid-protein interactions on the pretext that these represent primarily solvent-polymer interactions? The homology, if any, is not restricted to this class of observations alone. Catalysis by proteins is generally considered to be associated with structural fluctuations to account for enthalpy-entropy compensation as expected of processes at equilibrium⁷. However, determined efforts have yielded < 5% (notional) changes in volume/activity as an effect of hydrostatic pressure on globular proteins⁸. On the other hand, we could demonstrate that membrane proteins exhibit 200-300% changes in activity with (osmotic) stretch/compression of the bilayer, instantiating the appropriateness of membrane proteins for such structure-activity relationships⁹. Solvent-polymer interactions will be of major importance in understanding the molecular dynamics associated with catalysis¹⁰. The full impact of the crystallographic detail will also become more evident if these

studies increasingly focus on the mechanistic basis of structural fluctuations.

1. Madhusudhan and Vijayan, M., *Curr. Sci.*, 1991, **60**, 165.
2. Huxley, H. E. and Kendrew, J. C., *Acta Crystallogr.*, 1953, **6**, 76.
3. Perutz, M. F., *Proc. R. Soc. Lond.*, 1954, **A225**, 264.
4. Kodandapani, R., Suresh, C. G. and Vijayan, M., *J. Biol. Chem.*, 1990, **265**, 16126.
5. Salunke, D. M., Veerapandian, B. and Vijayan, M., *Curr. Sci.*, 1984, **53**, 231.
6. Sitaramam, V. and Mathai, J. C., *Biochim. Biophys. Acta*, 1990, **1018**, 21.
7. Kemenny, G., *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 2655.
8. Gekko, K. and Hasagawa, Y., *Biochemistry*, 1986, **25**, 6563.
9. Sitaramam, V., in *Membrane Structure and Function—State of the Art* (eds. Gaber, B. P. and Easwaran, K. R. K.), Adenine Press, New York (1991, in press).
10. Somogyi, B., Welch, G. R. and Damjanovich, S., *Biochim. Biophys. Acta*, 1984, **768**, 81.

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Fullerenes from flames

Research on fullerenes has become the rage of the day after the discovery of a method of preparing them in macroscopic quantities by the contact-arc vapourization of graphite. More recently, Howard *et al.* (*Nature*, 1991, **352**, 139) have found C₆₀ and C₇₀ in varying proportions in laminar flames of benzene and oxygen. These authors stabilized the flame in a partial helium atmosphere employing a specially designed low-pressure chamber and found the fullerenes preferentially in non-sooty flames. We have been examining the nature of condensable compounds and soot in benzene-air flames for some time and have found fullerenes C₆₀ and C₇₀ in sooty flames from a simple laboratory spirit lamp using benzene as the fuel. We consider this to be of some general interest.

The technique employed by us involved quenching part of the sooting end