Raising Water to New Heights

R. P. Rand

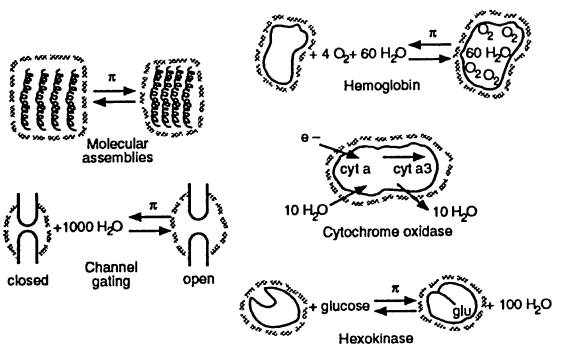
The contribution of hydration to the energetics of molecular assembly and protein catalysis has been recognized for a long time (*1*). Now as described on page 655 of this issue, Colombo, Rau, and Parsegian (*2*) have provided an elegant demonstration that as four oxygens bind to hemoglobin (Hb), so do 60 additional water molecules. Sixty is a surprisingly high number. It suggests that hydration/dehydration reactions contribute far more to the energetics of conformational changes than usually thought. The demonstration is a strong warning not to ignore water as a ligand in allosterism. And it shows how to measure water's contribution in practically any system.

These findings stem from the recent history of using osmotic stress (OS) to measure water's role in molecular assembly (*3*). The strategy, shown schematically in the figure, is remarkably simple, generally applicable, and capable of determining whether even extremely weakly perturbed water molecules are energetically significant. Forces measured between phospholipid layers (*4*) and between macromolecules (*5*) show that surface-perturbed water, as little different from bulk water as a fraction of a calorie/mole, can dominate interaction energies when many such water molecules are involved. Further, the cost of removing all the perturbed water, or the benefit of fully hydrating a newly exposed surface, is universally high at 1.5 to 15 kcal/mol per 100 Å^2 (adenosine triphosphate hydrolysis yields 7.3 kcal/mol).

The OS strategy has been applied to individually functioning molecules that gate ion channels in membranes. Channels open (hydrate) with increased difficulty the face of decreased water activity in their vicinity (*6*). A measure of that difficulty shows that additional water molecules become associated with the open channels, usually to an extent related to their conductance, as if most of the newly associated water is hydrating a newly created aqueous cavity.

More recently, studies of three proteins working under osmotic stress have shown three qualitatively different solvation reactions on substrate binding: one hydrates, one shows a hydration-dehydration cycle, and one dehydrates. The numbers of water molecules involved in the conformational changes are large and their possible roles intriguing.

The 60 additional waters associated with oxyhemoglobin are related to its quaternary structure since O_2 binding to myoglobin seems to show no such effect (*2*). Is the hydration of 600 Å^2 of newly exposed surface of the oxy conformation energetically significant? If that surface is like others, its full hydration would yield more than 8 kcal/mol Hb, or 2 kcal/mol per heme group. Thinking of water as a ligand, their multiplicity and energy may help to better understand intramolecular stresses and the long-range heme-heme interactions behind the cooperativity of oxygen binding.



Osmotic stress strategy. Any aqueous compartment that is inaccessible to a "neutral" solute, indicated here by the space enclosed by the broken line, has its water activity controlled by that solute's osmotic pressure, π. In the case of single proteins, the solutes that work are those excluded from the protein's "hydration shell," as described by Timasheff and his colleagues (*13*). Under such osmotic stress, π, the components of molecular assemblies get pushed together. Isolated proteins undergoing reversible transitions shift their equilibrium toward the dehydrated conformation. The sensitivity of that shift to π gives the difference in the number of water molecules associated with each conformation (*2*).

Kornblatt and Hoa (*7*) have shown that ten additional water molecules associate with cytochrome oxidase as cytochrome a binds an electron. These waters are then shed as the electron is passed internally to cytochrome a_3. Far from being "futile," the water cycle appears necessary for the sequential conformational changes and may be part of the coupled process of moving protons through an associated membrane.

Hexokinase releases about 100 water molecules in the process of binding glucose (*8*). The expulsion of so much water, a mass ten times that of the substrate itself, presumably reflects the dehydration required to confine the phosphorylation to glucose and prevent the phosphorylation of water (*8*).

That water activity alone can affect protein function raises a fundamental experimental problem in studying the direct effects of ligands on proteins. The obligatory coupling of solute and water activities means that any measured effect of a ligand cannot be easily apportioned between ligand and solvent, unless the activity of one of them is kept constant. A dramatic example of this comes again from Hb. The classical measure of 1.6 chloride ions that bind to Hb on deoxygenation becomes an integral 1.0 ion when the effect of sodium chloride on water activity itself is taken into account (*9*).

The nature of the surface perturbation of water remains an enigma (*10*). The most weakly perturbed waters are unlikely to be seen either in the crystal structures of proteins (*11*) or by nuclear magnetic resonance (NMR) (*12*), since their residence time is so short. Yet their removal modifies function. So the OS strategy is an invitation also to crystallographers and NMR spectroscopists to study the structural consequences of removing these weakly "bound" waters. Because while their number can be large, it is now a challenge to determine where they are and to assess their contribution to the overall energetics of any allosteric change. In any case, from the molecule's perspective, water is looking like pretty sticky stuff.

The author is in the Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1, Canada.

REFERENCES

1. See, for example, *Biophysics of Water*, F. Franks and S. Mathias, Eds. (Wiley, New York, 1982).
2. M. F. Colombo, D. C. Rau, V. A. Parsegian, *Science* **256**, 655 (1992).
3. V. A. Parsegian, R. P. Rand, N. L. Fuller, D. C. Rau, *Methods Enzymol.* **127**, 400 (1986).
4. R. P. Rand and V. A. Parsegian, *Biochim. Biophys. Acta* **988**, 351 (1989).
5. D. C. Rau and V. A. Parsegian, *Science* **249**, 1278 (1990).
6. J. Zimmerberg and V. A. Parsegian, *Nature* **323**, 36 (1986); M. D. Rayner, J. G. Starkus, P. C. Ruben, D. A. Alicata, *Biophys. J.* **61**, 96 (1992).
7. J. A. Kornblatt and G. Hui Bon Hoa, *Biochemistry* **29**, 9370 (1990).
8. R. P. Rand and N. L. Fuller, *Biophys. J.* **61**, A345 (1992); T. A. Steitz, M. Shoham, W. S. Bennett, *Philos. Trans R. Soc. London B* **293**, 43 (1981).
9. M. F. Colombo, D. C. Rau, V. A. Parsegian, *Biophys. J.* **61**, A56 (1992).
10. S. Marcelja and N. Radic, *Chem. Phys. Lett.* **42**, 129 (1976); A. A. Kornyshev and S. Leikin, *Phys. Rev. A* **40**, 6431 (1989).
11. H. Savage, *Biophys. J.* **50**, 967 (1986); R. P. Rand, N. L. Fuller, V. A. Parsegian, D. C. Rau, *Biochemistry* **27**, 7711 (1988).
12. G. Otting, E. Liepinsh, K. Wüthrich, *Science* **254**, 974 (1991).
13. T. Arakawa and S. N. Timasheff, *Biochemistry* **24**, 6536, 6545 (1985).