BOUND WATER

by

P. Vogelhut and M. Orentlicher

Memorandum ERL-M156

22 April 1966

ELECTRONICS RESEARCH LABORATORY
College of Engineering
University of California, Berkeley
94720

Manuscript submitted: 29 March 1966

This work was supported wholly by the National Institutes of Health under Training Grant No. 1-T1-GM-1418-01.

BOUND WATER

INTRODUCTION

An understanding of the role of bound water is necessary for a complete description of a multitude of physiological processes occurring at the molecular level of cellular activity. Enzyme kinetics, charge transport in lamellar systems in chloroplasts, propagation of action potentials, contraction of muscle fibers, the action of anaesthetic agents, mass transport across membranes--all these topics somewhere deal with the role of water that is bound to the relevant macromolecules. The form of this bound water is obviously not unique since it depends on the adsorbing surface properties. One cannot make general statements about the substance and describe its crystal structure, transport properties, or other physical parameters. One can, however, state methods of measurement and certain areas of physical chemistry where the presence of bound water influences rate processes or equilibria. Another general approach is to treat the bound water surfaces as a solid medium with many imperfections (due to the underlying adsorbing surface); this type of study is concerned with charge transport properties on a macromolecular scale and presupposes an exact knowledge of the atomic configuration of the biomolecule. Since, however, very few

biologically important macromolecules are known in their complete three-dimensional structure, the impact of solid state physics techniques on the bound water problem has been quite marginal.

The term "bound water" is defined as water molecules held by solute molecules in aqueous solution. A water molecule is considered to be held to a solute if the water-solute bond is maintained during solute motion through the solvent. This requires a bond energy which is large relative to the thermal energy and also sets a lower bound of about one kcal for the free energy of binding.

Since it may not be immediately apparent that "bound water" is important to biologists, we shall establish its relevance before proceeding further. Simply stated, the sources of biological sensitivity to the binding of water are:

1. Water is the medium in which biomolecular events occur; therefore, water is always present to interact with biomolecules.
2. The activity of biomolecules (e.g., fiber contraction) is closely related to their existence in marginally stable structures (e.g., α-helix vs random coil). Interaction with water can either stabilize or destabilize a particular structure. This is obviously applicable to the "transition states" of rate theory.

3. The most commonly encountered biological-structure stabilizer is the (intra- or inter- molecular) hydrogen bond. Since water is the hydrogen-bonding substance, it is ideally suited for interaction with the biomolecular H-bond systems.

In the following we deal almost exclusively with biomolecules in aqueous solution. The interaction of water with solid proteins has been studied and has been found to involve some phenomena not often encountered in solution work. This is due to the gradual adsorption of water molecules by solid-protein macromolecules. A short review is given by Haurowitz (1963) and an extensive study was published by Eley and Leslie (1964).

After discussing how and what we know about bound water, we shall proceed to describe its influence on biomolecular equilibria and rate processes.

BOUND WATER

Water is typically bound to biomolecules by hydrogen bonding or by electrostatic bonding to charges on the solute. (For a discussion of the hydrogen bond, see Pauling (1960) or Pimentel and McClellan (1960).) Since the H-bond is the key structural element of water, it may fit into a water structure if it is on an exposed biomolecular site. But buried sites, which surround a bound water molecule with a nonpolar

environment, localize the bound water so that it cannot interact with bulk water. Similarly, charges on the solute molecule may be expected to bind water molecules strongly and destroy their characteristic interaction with the surrounding water (Frank, Wen, 1957).

We have therefore three gross categories of bound water:

1. Water bound on "accessible" H-bond sites,
2. Water bound on "buried" H-bond sites, and
3. Water bound to local charges.

Fortunately, there are experimental means for investigating the bound water of biomolecules. In Protein Structure (Scheraga, 1961), various experimental techniques for studying proteins in aqueous solution are presented, many of which can be used to obtain information about bound water. (The intimate connection between a study of water and a study of biomolecules is indicated by the important theoretical studies of liquid water that Scheraga, a protein chemist, has contributed.)

Some important research tools are hydrodynamic data, titration curves, deuterium-hydrogen exchange, spectroscopy, dielectric measurements, and low-angle X-ray scattering.

1. Hydrodynamic data

If the size and shape of the solute can be estimated as a function of the water bound to it, the hydrodynamic quantities

(diffusivity, viscosity, etc.) will give the water bound to the solute. Since this method must be based on simplified models of biomolecules, it will yield rough values for the total quantity of bound water.

2. Titration Curves

From titration curves, the number of H-bonding sites and charged sites, as a function of pH, can be deduced, given a knowledge of the biomolecule's structure. This method is treated extensively by Scheraga (1961).

3. Deuterium-Hydrogen Exchange

This is a particularly sensitive method for studying proton donor sites on biomolecules. The degree of rapid interchange indicates the number of accessible (proton-donor) H-bond sites. The slow exchange gives insight into the degree to which proton-donor sites are buried or tied up in intra-molecular H-bonding.

4. Spectroscopy

Shifts in the characteristic IR and NMR O-H bond spectra of water can indicate binding of water. Clifford et al. (1965) describe a study of aqueous solutions in which NMR and Raman spectra were both employed. They demonstrated the value of different measurements applied jointly to obtain physically unambiguous results. They present a concise discussion of the interpretation of NMR spectral data: chemical shift, bandwidth, and relaxation time. From these qualities, the extent

of H-bonding and the mobility of water molecules can be estimated. Additional estimates of these properties have been obtained from the IR spectra of aqueous solutions (Scheraga, 1965).

5. Dielectric Measurements

This method is particularly suited to bound water studies. By suitable choice of frequency region, dielectric measurements can give a measure of the water bound "irrotationally," so as to be unable to contribute to the dielectric polarization. Roughly, this corresponds to the amount of water bound to local charges and buried sites. But the water molecules may also be bound into ice-like structures on a biomolecule, and therefore unable to orient with the free water molecules around them. In this case, bound water appears "irrotational" at high microwave frequencies, but contributes to the dielectric constant at low microwave frequencies (Grant, 1965).

6. Low Angle X-ray Scattering

This technique for studying biomolecules in aqueous solution has hardly been exploited, but shows great promise, because the surface-bound water is invisible to X-ray, thus allowing the solute size and shape to be determined (Kratky, 1963). In conjunction with the other methods, an accurate description of the water-solute system can be approached.

With these tools, what have we determined about "bound water?" Both the NMR spectra (Sussman, Chin, 1966) and dielectric measurements

(Grant, 1965) indicate that bound water can exist in extended structures in biological systems. The ordering in these structures appears to be intermediate between the ordering that is characteristic of ice and that of water. But this structuring is not universal. The steric conditions for water-structuring surfaces are well described by Berendsen and Migchelsen (1965) in their original NMR studies of water adsorbed on protein fibres.

From this discussion it should be clear that solutions of the problems of bound water and the structure of biomolecules in aqueous solution will be correlated. It is only by combining these experimental techniques that we may be able to understand either subject.

EFFECTS OF BOUND WATER ON BIOMOLECULES

We divide the effects of bound water on biological phenomena into two categories: equilibrium properties and rate processes.

Equilibrium Properties

A recent publication by Scheraga (1963) on noncovalent bonds in proteins demonstrates the effect of water on the configuration of proteins and on their reactions. He notes that the equilibria either between different configurations or different chemical species should be written as

$$A\ldots H_2O + B\ldots H_2O + (H_2O\ldots H_2O)$$

or

$$A'\ldots H_2O + B'\ldots H_2O + (H_2O\ldots H_2O)'$$

An example of this effect is the stabilization of broken intramolecular H-bonds by H-bonding to H_2O, which is claimed to be the primary determinant of the DNA denaturation temperature (Gordon, Curnutte, Lark, 1965).

The effects of bound water on equilibria is important for other reasons as well as the direct free-energy contribution of the bonds indicated above. For example, the local dielectric constant determining electrostatic interactions will depend on whether the locale is considered nonpolar or aqueous.

Of course, the experimental determination of biomolecule size and shape by the hydrodynamic methods mentioned earlier, depends on proper allowance for bound water (Scheraga, 1961). The same holds true for interpreting dielectric dispersion of protein solutions (Haurowitz, 1963).

Kirkwood and his co-workers relate the acid-base equilibria of polar groups on proteins to charge fluctuations on their surfaces. They were able to account adequately for both the pH dependence

of protein static-dielectric constant increments and for the dielectric relaxation of the protein, as well as protein inter-molecular electrostatic forces (Kirkwood, Shumaker, 1952).

The mechanism of proton transport on protein surfaces will definitely be through H-bonds. Experiments on the conductivity (Eley, Leslie, 1964) and the dielectric constant (Rosen, 1962) of dry and humid protein powders, show that adsorbed water drastically increases the charge mobility of protein powders. Rosen found a sharp increase in dielectric constant at adsorption levels corresponding to a filled monolayer of water on the surface of the molecule. Since proton mobility through the H-bond system of water is very high, this change seems to correspond to collective polarization of the adsorbed layer. It is probable that the mechanism for Kirkwood's "charge fluctuations" also involves proton transport through this H-bond system.

Rate Processes

Discussion of proton transport takes us to the realm of rate processes. Proton transport seems to account for dielectric relaxation and dc conduction in wet proteins. A decisive role for bound water in this transport is suggested by the sharp decrease in activation energy for conduction in hydrated vs nonhydrated organic acid crystals (Pollock, Ubbelohde, 1956). However, as in most kinetic studies, there is disagreement about the mechanism.

Kirkwood (1955) applied his charge-fluctuation theory to the kinetics of enzyme action and demonstrated that the inert-protein portion of the enzyme could contribute to stabilization of enzyme-substrate complexes through advantageous charge distribution. His calculations also predict the proper pH dependence.

Yet another line of speculation concerns the relation of the "ice-like" structure previously described and the efficiency of enzyme action. Since proton-transfer is a key step in many enzymatic processes, water bridges between proton donor and acceptor groups near the active site may be necessary elements of the catalytic structure. Therefore, studies of bound water structure may explain the mode of interaction between the active site and the neighboring groups on an enzyme that bestows catalytic activity to the enzyme.

In the preceding example, water serves passively as a charge-transfer medium in a rate process. However, the Discussion of the Faraday Society containing Kirkwood's paper recalls many enzyme reaction mechanisms involving active participation by bound water. Of course, Kirkwood's theory and the other models for enzyme action are speculative, but it is clear that whatever the actual mechanism may be, water interaction with the enzyme or with the substrate is probable. It is therefore necessary to determine the influence of water before a quantitative kinetic theory can be developed.

We noted previously that competition between protein and water for H-bond sites on the protein is a factor in determining the equilibria between ordered and random protein configurations. This factor, as well as the effect of bound water on the local dielectric constant, carry over into a discussion of the kinetics of denaturation (Scheraga, 1963).

CONCLUSION

We can expect rapid charge transfer and structural H-bonds to enter into most biological reactions. Current research is leading to a relation of these processes to the interaction of biomolecules and their aqueous environment. One important link in this relationship is formed through the studies of bound water.

Some medical application of these concepts is already extant. Examples may be found in the volume of the Annals of the New York Academy of Science entitled "Forms of Water in Biologic Systems." Among them are studies of cell freezing (MacKenzie, 1965; Greaves, Davies, 1965) and the mechanism of anaesthesia (Catchpool, 1965).

For those unfamiliar with this field, the first chapters of the monograph by Scheraga (1961) are recommended as an introduction to the study of biomolecules in aqueous solution. Those with a background in chemistry and physics could profit from the Discussions of the Faraday Society and the Annals of the New York Academy of Science to which we refer.

REFERENCES

1. Berdendsen, H. J. C., Migchelsen, C., 1965, Ann. N. Y. Acad. Sci., 365.
2. Catchpool, J. F., 1965, Ann. N. Y. Acad. Sci., 595.
3. Clifford, J., Pethica, B. A., Senior, W. A., 1965, Ann. N. Y. Acad. Sci., 125, 458.
4. Eley, D. D., Leslie, R. B., 1964, Electronic Aspects of Biochemistry, Pullman, B. (ed), Academic Press, N. Y.
5. Frank, H. S., Wen, W., 1957, Trans. Far. Soc., 24, 133.
6. Gordon, D. E., Curnutte, B., Jr., Lark, K. G., 1965, J. Mol. Biol., 13, 571.
7. Grant, E. H., 1965, Ann. N. Y. Acad. Sci., 125, 418.
8. Greaves, R. I. N., Davies, J. D., 1965, Ann. N. Y. Acad. Sci., 548.
9. Hasted, J. B., 1961, Progress in Dielectrics, 3, Birks, J. (ed), Heywood, London.
10. Haurowitz, F., 1963, The Chemistry and Function of Proteins, Academic Press, New York.
11. Kirkwood, J. G., 1955, Discussions Far. Soc., 20, 78.
12. Kirkwood, J. G., Shumaker, J. B., 1952, Proc. Nat. Acad. Sci., 38, 855.

13. Kratky, O., 1963, Prog. in Biophysics, 13, 105.

14. MacKenzie, A. P., 1965, Ann. N. Y. Acad. Sci., 522.

15. McPollock, A. R., 1956, Ubbelohde Trans. Far. Soc., 52 1112.

16. Pauling, L., 1960, The Nature of the Chemical Bond, New York, Cornell.

17. Pimentel, G. C., McClellan, A. L., The Hydrogen Bond, San Francisco, Freeman.

18. Rosen, D., 1963, Trans. Far. Soc., 59, 2178.

19. Scheraga, H. A., 1961, Protein Structure, Academic Press, New York.

20. Scheraga, H. A., 1963, The Proteins, Neurath, H., (ed) Academic Press, New York.

21. Scheraga, H. A., Thomas, M. R., Schrier, E. E., 1965, J. Chem. Phys., 69, 3722.

22. Sussman, M. V., Chin, L., 1966, Science, 151, 324.