Biophysics of Water

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weight is known. Papain was chosen because its molecular structure is known and it is known not to change in alcohol or dimethylsulphoxide solutions.[3] Parameters for this protein are $M_r = 23\,400$, Σb_i (in D_2O buffer) = 880×10^{-12} cm assuming 80% deuteration of labile hydrogen.

The radius of gyration is around 16 Å in D_2O and drops to around 14 Å for the highest probe concentration used. Half of this drop can be accounted for from model calculations using the molecular structure and the rest is undoubtedly caused by the bound water found on the surface. The results are shown in Figure 2. The least squares line is $1050–32\,000\ \rho_s$. From the least squares line we get $\Sigma b_i = 1050$, i.e. the scattering of bound water is 170×10^{-12} cm, corresponding to approximately 90 water molecules or a volume of 3500 Å^3. The volume of the molecule can be estimated using standard density for proteins and is around 28 500 Å. In this estimate, there is an implicit assumption that, on average, the density of bound water around the protein is the same as bulk. We find this reasonable since the protein surface is heterogeneous and contains charged groups which will electrostrict water as well as hydrophobic groups which will repel it. Subtracting the molecular volume from the total volume we get an impenetrable water volume of 3500 Å^3. The error is estimated to be around 1000 Å^3. One layer of water would correspond to a volume of between 10 000 and 15 000 Å^3 bound to the surface. The present measurements show, therefore, that a large part of the surface is directly accessible to the probe molecules at least when these are in relatively low concentrations.

References

1. B. Jacrot, *Rep. Prog. Phys.*, **39**, 911 (1976).
2. G. Zaccaï, in Proc. of the NATO Adv. Study Institute on Scattering Techniques Applied to Supramagnetic and Non-Equilibrium Systems, 1981.
3. J. Drenth, J. N. Jansonius, R. Koekoek and B. G. Wolthers, *Adv. Protein Chem.*, **25**, 78 (1971).

On the role of interfacial water in protonmotive systems

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Summary

The nature and role of protonmotive systems in bioelectrochemical information transfer is briefly reviewed. It is likely that 'energized' coupling protons are relayed from their sources to their sinks along the surfaces of the coupling membrane. The possible role of interfacial water molecules and other factors in effecting this transfer is outlined. Attention is drawn to those areas to which water biophysicists can contribute an improvement in our understanding of protonmotive systems.

Introduction

It is now widely accepted that an important means of bioelectrochemical energy transduction and information transfer is effected by a current of 'energized' protons, which are pumped across 'coupling' membranes that themselves serve to separate two aqueous compartments. Such systems are known as protonmotive systems. Current controversy is focused upon the extent to which such energized coupling protons are osmotically active. In the chemiosmotic formulation[1,2] the coupling protons are described energetically as forming a protonmotive force between the two bulk aqueous phases that the membrane serves to separate, given as a sum of electrical and chemical terms:

$$\Delta p = \Delta\psi - 2.3RT\Delta\mathrm{pH}/F \qquad (1)$$

where Δp is the protonmotive force, $\Delta\psi$ is the electrical potential across the membrane, ΔpH is the pH differential across the membrane, and R, T and F have their usual thermodynamic meanings. In this view the protonmotive force between one membrane surface and the other is equal to that between the two bulk phases in the steady state. In an alternative view, generalized by

Williams[3] and developed in more specific terms by others,[4,5] the coupling protons do not become osmotically active and are retained on the membrane surfaces. It is at present unknown whether such a retention of energized coupling protons is engendered by additional proteins, by biophysical forces including hydrogen-bonded chains of interfacial water molecules, or by both. It is, however, important to realize that this type of protonmotive system behaves as a molecular machine, as defined by McClare,[6,7] and care must be exercised in its thermodynamic description.

Space does not permit an extensive analysis of the evidence favouring the view that the 'energized' coupling protons of processes such as electron transport phosphorylation, active solute transport and flagellar rotation are indeed membrane-bound, and such evidence has been reviewed at some length elsewhere;[4,5] we confine our present enquiries to the relative importance of interfacial water molecules and 'proton-transferring proteins' in effecting such directed vectorial proton transfer.

Proton transfer along membrane surfaces; what is the mechanism?

The existence of a layer of 'structured' water within the inner Helmholtz plane adjacent to biological membrane surfaces could, in principle, give rise to a vectorial proton transfer pathway parallel to a membrane surface.[3] There is at present little evidence available to indicate whether or not this actually occurs *in vivo*. However, it is obvious that for those working in the field of 'vicinal water'[8,9,10] the possibility that such water molecules may be involved in bioelectrochemical proton transfer seems well worth exploring. In this regard it is worth noting Freund's proposal[11] that proton transfer in bioenergetic systems occurs not via the mechanism believed to occur in ice[12] but via a dual 'proton band' mechanism, as found for certain inorganic hydroxides.[13]

The alternative to an involvement of 'structured' water in effecting preferential H^+ transfer parallel (as opposed to perpendicular) to a coupling membrane surface is that there exists in such coupling membranes proteins whose normal function is specifically to channel 'energized' protons between their membrane-located sources and sinks.[4,5] Such proteins are taken to interact cooperatively, and we have referred to the proton-transferring network that they constitute as a 'protoneural' network.[5] In this type of model the energy of the 'energized' protons is conserved in the form of field-induced strained protein conformational states, which relax as they pass energized protons to the next element of the network, finally delivering them to a proton sink, such as an ATP synthase enzyme, which will perform useful biological work.

Thus, at our present state of knowledge, it is of the greatest importance to find or to develop methods which will allow us to distinguish between proton transfer along chains of adsorbed water molecules and proton transfer along

chains of H-bonding amino acid residues, both along the surfaces of coupling membranes[4,5] and through channels within the proton pumps themselves.[14–16]

The existence, likely nature and properties of strongly-adsorbed water molecules at the surfaces of membranes and proteins have been excellently summarized by the other contributors to this volume. Our task is to draw the attention of water biophysicists to the possible involvement of such water molecules in vectorial proton transfer as a means of bioelectrochemical information transfer. Schwartz[17,18] has outlined the thermodynamics of membrane-located proteins containing large (hundreds of Debye units) permanent dipoles, and the significant conformational changes that even single bond charges can exert upon them as a transmembrane field is set up. We therefore hope that the extremely barren outline of protonmotive systems that we have given here may stimulate workers in the field of water biophysics to join the interdisciplinary effort that will be needed to further our understanding of protonmotive bioenergetic systems.

References

1. P. Mitchell, *FEBS Lett.*, **78**, 1 (1977).
2. P. Mitchell, *Science*, **206**, 1148 (1979).
3. R. J. P. Williams, *FEBS Lett.*, **85**, 9 (1978).
4. D. B. Kell, *Biochim. Biophys. Acta*, **549**, 55 (1979).
5. D. B. Kell, D. J. Clarke and J. G. Morris, *FEMS Microbiol. Lett.*, **11**, 1 (1981).
6. C. W. F. McClare, *J. Theoret. Biol.*, **30**, 1 (1971).
7. C. W. F. McClare, *Ann. N.Y. Acad. Sci.*, **224**, 74 (1974).
8. W. Drost-Hansen, in *The Chemistry of the Cell Interface, Part B* (ed. H. D. Brown), pp. 1–184, Academic Press, New York (1974).
9. H. H. G. Jellinek (ed.), *Water structure at the water-polymer interface*, Plenum Press, New York (1972).
10. R. Cooke and I. D. Kuntz, *Ann. Rev. Biophys. Bioeng.*, **2**, 95 (1974).
11. F. Freund, *Trends Biochem. Sci.*, **6**, 142 (1981).
12. N. Riehl, B. Bullmer and H. Engelhardt (eds), *Physics of Ice*, Plenum Press, New York (1969).
13. F. Freund and H. Wengeler, *Ber. Bunsenges. Phys. Chem.*, **84**, 866 (1980).
14. M. K. F. Wikström and K. Krab, *Biochim. Biophys. Acta*, **549**, 177 (1979).
15. S. R. Caplan and M. Ginzburg (eds), *Energetics and Structure of Halophilic Microorganisms*, Elsevier/North Holland, Amsterdam (1978).
16. A. K. Dunker and D. A. Marvin, *J. Theoret. Biol.*, **72**, 9 (1978).
17. G. Schwartz, *J. Membr. Biol.*, **43**, 127 (1978).
18. G. Schwartz, *J. Membr. Biol.*, **43**, 149 (1978).