Water and globular proteins

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Dynamic, thermodynamic and structural studies of the hydration of globular proteins indicate how the macromolecule–water interface can influence folding, enzymatic activity and other biological properties.

Cellular organization and the overall biochemistry of an organism depend on water, if only because on a molecular level water determines the reaction rates and thermodynamics of biological processes. Apparently simple questions about hydration have not been adequately answered: How much water is perturbed by macromolecules of the cell? How does the water about proteins and other biopolymers differ from bulk water and from the water about small molecules? How does the water about these polymers enter into or modulate biochemical processes, particularly enzyme catalysis in different environments: aqueous solutions; regions of low water content but high water activity, such as membranes; and anhydrobiotic systems of low water activity?

Measurement of hydration

The techniques used to study the hydration of biopolymers[1] differ in the ways in which they focus on time average or dynamic properties; structure; water or protein properties; solution or solid samples.

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Table I lists the principal approaches and indicates the nature of the measurement and results.

Hydration is the incremental addition of water to dry macromolecules until a dilute solution is obtained, At some level of hydration there is sufficient water to completely hydrate the molecule, the additional water only dilutes the system. Some measurements that can be made at all system compositions, such as IR spectroscopy, EPR relaxation measurements, amide hydrogen exchange and heat capacity, define clearly an endpoint of the hydration process and give a unified picture of hydration that encompasses both the partially hydrated solid state and the solution state.

The endpoint determined by the change in heat capacity during hydration is particularly meaningful, because the heat capacity senses changes in enthalpy and equilibria for the interaction of water with all types of surface elements, including the nonpolar, which should hydrate last. Thus, the heat capacity endpoint corresponds most closely to full hydration. Other time-average measurements give a similar or lower endpoint. Dynamic properties can also be treated within this framework.

The fully hydrated molecule

The following picture[2] (Table II) of a fully hydrated macromolecule is based principally upon measurements of heat capacity[3] and IR spectroscopy[4]. The results are mostly for lysozyme and appear to be typical for other globular proteins[1]. The amount of water required for full hydration, determined by the point at which the heat capacity reaches the dilute solution value, is 0.38 g of water/g of protein (300 moles of water/mole of lysozyme). At this hydration the protein sample is solid and has only half as much water as protein crystals, barely enough for monolayer coverage. The hydration water is in rapid motion. NMR relaxation measurements[5,6] indicate that it is slightly more viscous than bulk water. Dielectric relaxation measurements[7] show two water relaxation times, one of 2×10^{-11}s, close to that of bulk water, and the other of 10^{-9}s. Little hydration water, probably less than 10%, is bound like substrate to an enzyme, with microsecond or longer residence time and greatly restricted rotational motion. EPR measurements[8] show that a spin probe noncovalently bound at the surface is only about 10 times less mobile than the bulk water. The rates of exchange of buried peptide hydrogens are identical to those for the dilute solution state, indicating full protein motion at the hydration endpoint (J. Schinkel, unpublished results); oxygen quenching of protein fluorescence leads to the same conclusion (E. Gratton, unpublished results). The average thermodynamic properties of the hydration water, like the dynamic properties, differ only slightly from those of the bulk solvent (Table II). Enthalpy and entropy differences compensate to give a smaller difference in free energy. The volume is about 10% smaller and the heat capacity about 15% larger than for the pure solvent. The thermodynamic differences

TABLE I. Measurements of protein hydration

Measurement	State of Sample		Type of Information			Information about	
	Solution	Powder/Film	Time-average	Dynamic	Structural	Water	Macromolecule
Diffraction:							
X-ray, neutron	+	(+)	+	(+)	+	+	+
Spectroscopy:							
IR, UV, CD, fluorescence, NMR, EPR	+	(+)	+		(+)	(+)	+
Solution thermodynamics:							
Cp, V, preferential binding	+		+			+	+
Sorption thermodynamics:							
G, H, Cp, V		+	+			+	+
Relaxation:							
NMR, EPR, dielectric	+	+		+		+	(+)
Hydrodynamics:							
sedimentation, viscosity, etc.	+		(+)	(+)		+	+
Computer simulation:							
molecular dynamics and Monte Carlo	+		+	+	+	+	
surface and packing							
Special rate properties:							
enzyme activity, hydrogen exchange	+	+		+	(+)		+
Special time-average properties:							
nonfreezing water	+		+			+	

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agree qualitatively with expectation, and in the case of the heat capacity, studies of model compounds predict a value for the solvated molecule that is within 5% of the experimental observation. The above description emphasizes the average properties of the hydration water and their general similarity to those of bulk water. In fact, the hydration water is heterogeneous (see below).

The protein surface imposes a structure on the hydration water, as a water molecule structures its neighbors in liquid water: only certain arrangements are permitted by the geometry of the protein–water interactions; ordering is local; there is rapid fluctuation between arrangements; and measurements that sense structure reflect average structural properties. The small amount of water at full hydration (0.38 g/g) requires that each water molecule cover, on the average, 20 Å^2 of protein surface, which is twice the effective area of a molecule of bulk water. This paradox can be resolved by noting, first, that water interacts primarily with charged and polar groups[4], second, that the average spacing of these groups on the surface gives 20 Å^2 polar-charged patches, and third, that 20 Å^2/molecule is possible for water with a volume close to that of bulk water. Apparently, the protein surface selects water arrangements that can give high surface coverage per water molecule. The selected set of arrangements must be a subset of those allowed in bulk water, because protein covered with a monolayer fits without perturbation into the bulk solvent. This implies that there is no significant amount of 'multilayer' water and that the hydration water can be structured by the protein surface and yet be similar to bulk water. The mobility of the protein matrix and surface water in the fully hydrated state deserves emphasis, because of its relationship to enzymatic and other functions of proteins (see below).

Stepwise hydration process

Hydration consists of three well-defined stages which are reflected in various properties of the system. Fig. 1 correlates the change with hydration in the IR spectrum, heat capacity, enzyme activity, motion of an EPR probe and rate of peptide hydrogen exchange. Molecular events can be associated with each stage[2] (Table II). Between 0 and 0.07 g of water/g of protein, the hydration process is dominated by interaction with charged groups and includes normalization of the pK of the ionizable side chains. At 0.07 g/g there is a transition in the IR spectrum and the heat capacity, reflecting a change in surface water

TABLE II. The protein hydration process and the properties of a fully hydrated molecule[a]

Hydration level	Thermodynamics	Structure	Motion
0–0.07 (0–60)	Large differences for transfer of water from bulk to hydration layer: average values, $\Delta\bar{G}_t = -1.5$, $\Delta\bar{H}_t = -17$ kcal/mol Heat capacity between that of ice liquid water: $\Delta\bar{C}p_t = -0.2$ cal/K-g Normalization of pK at 0.05 g/g. 'Knee' in adsorption isotherm. Native state very stable.	Structure of protein at low hydration not detectably different from solution. Water bound principally to charged groups (ca 1 water/charged atom site). At 0.07 g/g: transition in surface water, from disordered to ordered and/or from dispersed to clustered state; seen in IR and EPR spectroscopic and thermodynamic properties; associated with completion of charged group hydration.	Water mobility ca 100 × less than for bulk water; increased motion with increased hydration. Bound ligand mobility constant from 0–0.2 g/g: $\tau \sim 4 \times 10^{-9}$s. Enzymatic activity negligible.
0.07–0.25 (60–220)	Differences for transfer of water small and decreasing with hydration: average values, $\Delta\bar{G}_t = -0.2$, $\Delta\bar{H}_t = -0.5$ kcal/mol $\Delta\bar{V}_t = 0$ at ≥ 0.15 g/g Heat capacity greater than for liquid water: $\Delta\bar{C}p_t = 0.4$ cal/K-g Plateau in adsorption isotherm. Stability of native state decreases strongly with increased hydration.	Water bound principally to polar protein surface groups (ca 1 water per polar site). Water clusters centered on charged and polar sites. Clusters fluctuate in size and/or arrangement, as in bulk liquid.	Internal protein motion (H exchange) increases from 1/1000th at 0.04 g/g to full solution rate at 0.2 g/g.
0.25–0.38 (220–300)	Partial molar thermodynamic quantities close to bulk water values. Region of rapid rise in adsorption isotherm. Transition region in heat capacity.	At 0.25 g/g: start of condensation of water onto weakly interacting, unfilled patches of surface; seen in dynamic and thermodynamic properties.	Parallel increase in enzymatic activity and mobility of bound ligand. Fast water motion.
0.38–full hydration (300)	Differences between hydration and bulk water, averaged over all hydration water: $\Delta G = -0.5$ kcal/mol, $\Delta H = -3.0$ kcal/mol $\Delta Cp = 2.5$ cal/K-mol, $\Delta V = -1.7$ ml/mol Thermodynamics of unfolding close to dilute solution behavior.	Monolayer of water covers surface. Interaction with charged and polar surface groups selects locally ordered arrangements of hydration water. Fluctuation between many instantaneous arrangements, as in liquid water. Arrangements selected mesh with bulk solvent and cover large area per water. Hydration water heterogeneous.	Mobility close to bulk water for part and perhaps most of hydration water. Mobile bound ligand: $\tau = 7 \times 10^{-10}$s. Enzymatic activity 1/10th solution value. Full internal motions of protein. Dynamic and thermodynamic coupling between hydration water and protein.

[a] Data and conclusions mostly from experiments on lysozyme, cited in text or in references 2 and 8. Thermodynamic differences at full hydration are excess values per mole of water, with sorption data[23] having been integrated to estimate ΔG and ΔH. Data for ovalbumin[26] were used to estimate ΔV. Stability of the native conformation was determined by scanning calorimetry[27].

arrangements. Between 0.07 and 0.25 g/g clusters of water molecules form and grow until most of the surface is covered. Between 0.25 and 0.38 g/g, the uncovered surface patches become hydrated in a second transition. Apparently water condenses over the less strongly interacting surface elements, presumably non-polar atoms not adjacent to charged or polar atoms and thus not covered by the clusters.

As there are no significant changes in protein conformation during hydration[2,8] one can compare measurements on the nearly dry protein with computations[9] for the desolvated solution structure. There is ample reason to believe that solvation influences protein folding, and it is surprising that removal of water does not significantly alter conformation.

The motion of a noncovalently bound EPR probe (Fig. 1) changes abruptly at the 0.25 g/g level. The environment but not the motional behavior of the probe changes at 0.07 g/g.

The enzymatic activity of lysozyme becomes detectable at 0.2 g/g and changes in close parallel with the motional properties of the spin probe (Fig. 1). The activity also changes with hydration above 0.38 g/g. Clearly, although multi-layer water is not important for time-average behavior, it is for some dynamic properties.

Motional properties of the protein surface and of the protein interior are coupled differently to the surrounding water. The change in mobility of the protein matrix is largely complete at 0.25 g/g (Fig. 1). Tryptophan fluorescence quenching by oxygen, which explores nanosecond motional behavior, (10 orders of magnitude more rapid than hydrogen exchange), is affected similarly by hydration (E. Gratton, unpublished results). Correlation between water and protein motions have been demonstrated by NMR measurements[6]. Neutron diffraction studies[10] have described surface diffusion of water on the 10^{-8}s time scale and suggest that the surface water is clustered at partial hydration.

One should not expect behavior assoated with the partially hydrated molecule to remain unaltered for the fully hydrated state. For example, the thermodynamics of vaporization should be greatly different for a water molecule coordinated about a charged atom of a nearly dry protein and one similarly positioned on a fully hydrated protein, where there would be interactions of the water with neighbors and also greater dispersion of the charge of the protein atom among the several additional waters coordinated about it. Thus classifications of hydration water (Type I, II and III) based on steps in the hydration process are incorrect.

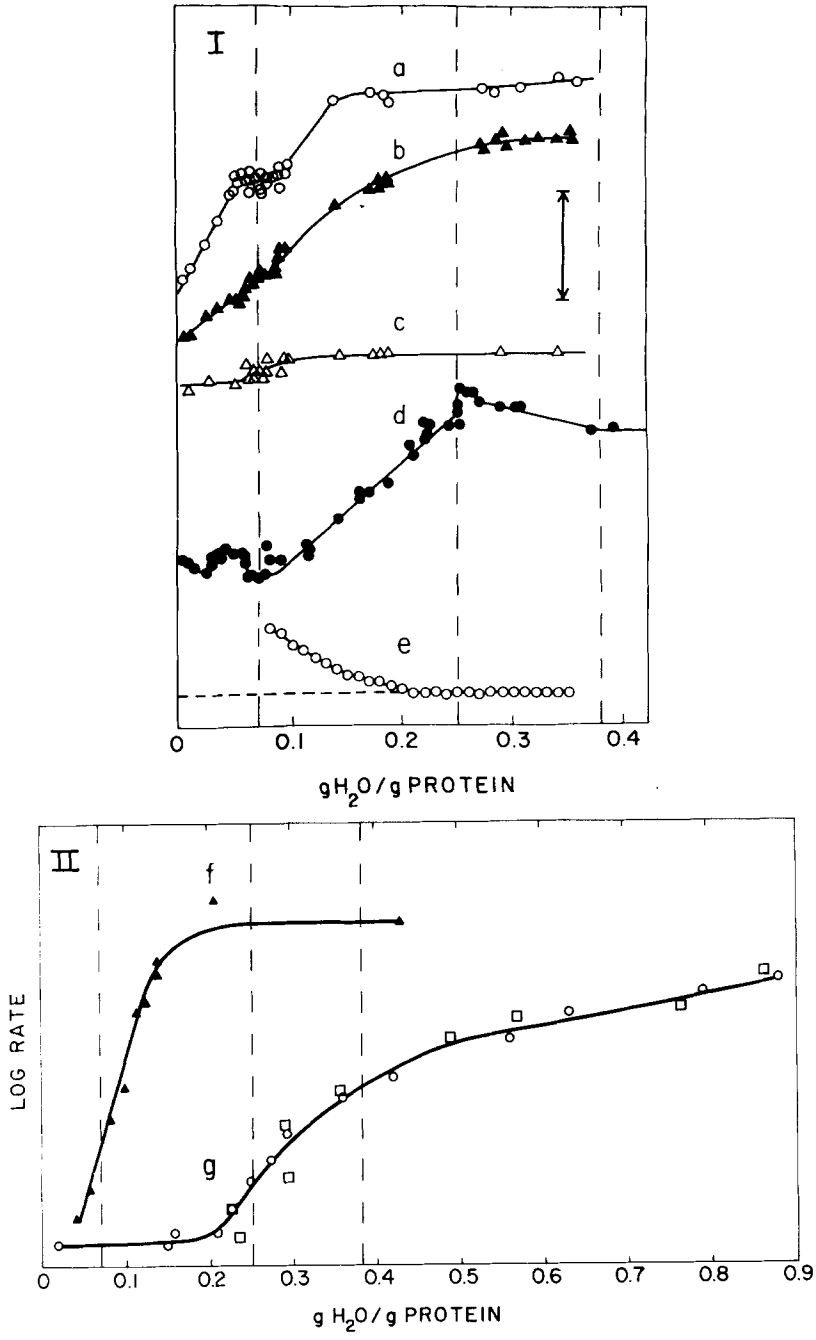

Fig. 1. Effect of hydration on lysozyme. Part I, time-average properties. Part II, dynamic properties. From top to bottom the curves are: (a) carboxylate absorbance (1580 cm^{-1}); (b) amide I shift (ca 1660 cm^{-1}); (c) OD stretching frequency (ca 2570 cm^{-1}); (d) apparent specific heat capacity; (e) diamagnetic susceptibility; (f) log rate of peptide hydrogen exchange; (g) □, enzymatic activity (log v_0), and ○, rotational relaxation time of an ESR probe (log τ^{-1}). Data for curves (a) to (e) from references 9–11. Data for curve (f) from J. Schinkel, unpublished results, and for curve (g) from reference 14.

Physics of hydration

Low hydration (0–0.07 g water/g protein) can be discussed in terms of order–disorder and nucleation theories of embryonic droplets[11]. According to nucleation theory, it is impossible to condense water on an insoluble particle of radius smaller than about 100 Å, unless the pressure is higher than the equilibrium vapor pressure of water. Therefore, under normal laboratory conditions, a dry globular protein molecule could not become hydrated if its surface comprised only amide backbone and other non-ionizable residues. Soluble elements such as ionizable residues allow water vapor to condense on the macromolecule. In accord with this view, the

first detectable event in protein hydration is the ionization of side chains[2]. With increasing hydration, clusters of water about these strong hydration sites grow and the surface backbone and the non-ionizable side chains become involved in a process dominated by surface tension.

It is known that there is order at the surface of hydrogen-bonded liquids[11]. When the surface is of a small sphere, there is an extra positive term in the free energy, corresponding to the work done to destroy the parallel orientation of the surface dipoles. For this reason a very small droplet of water should be a disordered network of dipoles, and only for larger droplets should the ordered surface structure become stable. This disorder-to-order transition takes place at a critical size of about 100 water molecules. The above argument is for a homogeneous nucleation process, but a similar picture should hold for the condensation of water on the heterogeneous protein surface because of the similar hydrogen bonding energies of water–water and water–protein species. Therefore, the transition observed in the IR spectroscopic and heat capacity data at 0.07 g water/g protein (Fig. 1) probably reflects a change from the initial solid-like structure of the water molecules around the ionizable groups to the final liquid-like structure of the water monolayer adsorbed on the macromolecule.

Hill[12] has analyzed the statistical mechanics of localized unimolecular adsorption on a randomly heterogeneous surface for the case of interaction between adsorbed molecules. This model fits the process of protein hydration well. Hill's theory predicts two phase transitions. At some low critical surface water concentration there will be a two-dimensional condensation to form water clusters, stabilized by adsorbate–adsorbate interactions. The 0.07 g/g transition perhaps reflects this event, in addition to the order–disorder transition. Because of strong self-interaction, water clusters may form at even lower hydration and without a sharp transition. The second transition predicted by Hill's theory is at high coverage and corresponds to condensation of water over the weakest interacting portions of the protein surface, as observed at 0.25 g/g (Fig. 1). A condensation event of this kind is an obligatory part of the hydration of a soluble macromolecule.

There are two different time scales for fluctuations of a protein: a 'short' time scale (near 10^{-12}s), where the macromolecular is viewed as a liquid droplet with random and independent motions of small groups of atoms[9]; and a 'long' time scale (near 10^{-8}s), where the macromolecule displays motions of domains. Coupling between the hydration water and the protein on the 'short' time scale is expected because, first, a relaxation time of the hydration water is near 10^{-11}s and, second, binding of water molecules to the backbone increases the polar character of the peptide bond and induces a change in its conformation, owing to increased planarity of the amide group. One can speculate that coupling on the 'long' time scale between hydration water and domain motion correlates with co-operative motion of clusters or networks of water molecules, the existence of which is suggested by the thermodynamics of the hydration process and the geometrical requirements of the special water arrangements at the protein surface. Motions on the 'long' time scale merit special attention because these are crucial for protein function.

Experimental support for coupling between solvent and protein fluctuations is found in the dependence on solvent viscosity of the rates of carbon monoxide and oxygen binding to myoglobin[13].

Water and biological function

Protein hydration studies suggest that the surface of the folded molecule minimally perturbs the surrounding aqueous environment. The least possible amount of water is in contact with the protein because the surface area (20 Å^2) covered per water molecule is maximized, and the protein surface with its hydration monolayer meshes with and does not perturb the bulk water. This observation complements the general understanding that folding minimizes exposure of nonpolar groups and maximizes exposure of polar and, in particular, ionizable groups to solvent.

Free energy and enthalpy changes for many association reactions of proteins with ligands are about the same as those for folding a protein from the random coil state, although the latter process establishes perhaps ten times as many noncovalent interactions[14]. The special arrangements of water and protein surface groups at the folded protein surface may explain this paradox. Presumably replacement of protein groups by ligand, when a complex forms, would alter these arrangements, changing the number of water molecules and interactions of the surface water at the active site. The free energy of hydration of a macromolecule is very large, about 150 kcal/mol of lysozyme (Table II) or 2.5 kcal/100 Å^2 of protein surface. Thus, a small change in the arrangements of surface water or special structure of the surface at the active site will produce a large effect on protein folding, enzyme properties, etc. Richards and Richmond[15] have similarly concluded, from consideration of the surface tension, that there may be special properties for the water associated with an active site.

Large proteins and enzymes usually consist of several massive and nearly rigid domains, the relative position of which may change during catalysis or other function. The recent finding of the global incompressibility[16] of the macromolecule and the evidence for local fluctuations of its structure[17] allow one to conclude that an enzyme works because of domain and active-site mobility. Each domain moves as a Brownian damped oscillator in the field of interdomain weak forces and finds the best orientation of the functional groups by thermal fluctuation. The same holds for the substrate. Thus, catalysis is fast because domain rigidity reduces the number of degrees of freedom of the polypeptide chain, reducing the search time for optimization of the active site environment. In this picture domain co-ordinates are seen as statistical macrovariables, driven by the interdomain fluctuating forces. The importance of water for enzyme dynamics derives from the role of water in determining interdomain forces.

The development of lysozyme activity during the last stage of the hydration and the development of surface mobility accords with studies of the lysozyme mechanism that suggest rearrangement of the enzyme–substrate complex is concerted with bond-breaking in the rate-determining step[18]. It is also in accord with the picture presented in the preceding paragraph. At high hydration (above 0.2 g/g) one expects that inter-domain water bridges are broken at random by thermal energy and then reformed. These random events give rise to fluctuating forces acting between the domains and the substrate, providing the dynamical state of the cleft needed for catalysis.

Whatever the mechanism of an enzyme reaction, within the time of a catalytic event the enzyme-substrate complex must ultimately increase its free energy content at the expense of the surroundings and through interaction with them[19]. The relationship between the correlated fluctuations of enzyme and hydration water can be most simply understood by considering substrate and solvent molecules both as ligands, so one can offer a thermodynamic description of the catalytic events similar to the one used to deal with the multiple equilibria of proteins in binding of several ligands[20]. Co-operative effects allow the protein to add free energy. One obtains a truly kinetic picture when the flexibility of the protein structure is the peculiar property of the macromolecule that handles the multiple

binding. Thus numerous small changes (over nanoseconds) in the location of solvent at the protein surface control the displacement of catalytic residues at the active site. In terms of statistical physics, the space–time fluctuating nature of the solvent–surface interactions affect the course of the catalytic event. This picture of enzyme catalysis based on fluctuations is consistent with the usual thermodynamic description, because the probability of a fluctuation depends exponentially on the free energy change, which is the quantity needed in the thermodynamic description. The existence of a coupling free energy requires a non-vanishing average value of the cross-correlated fluctuations[21]. The kinetic picture can go one step further, because in the catalytic events the time derivatives of the fluctuating variables can be time correlated, i.e., velocities as well as conformation variables can cross-correlate.

Measurements on nucleic acids[22,23] show that the end point of the hydration process, corresponding to full hydration, is about twice the level for proteins. Heat capacity measurements (P.-H. Yang and J. A. Rupley, unpublished results) show that for tRNA the hydration process is stepwise and qualitatively similar to the process for proteins. The difference between protein and nucleic acid hydration appears to be entirely in the last step, i.e., completion of the hydration process after saturation of charged and polar sites. Nucleic acids may require more water because they have multilayer water or a more compact arrangement of surface water within a monolayer, covering, on average, 10 Å²/water molecule.

Concluding remarks

The peculiar character of hydration, in which structure and dynamics are initimately mixed, brings together physicists, biochemists and biologists. We have outlined a unified picture of protein hydration, including a primitive understanding of the relationship between hydration and function, based on domain fluctuations. The exploration of the hydration of membranes and organized elements of the cell is particularly important in view of the controversy concerning the nature of water within cells[24].

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References

1 Kuntz, I. D. and Kauzmann, W. (1974) *Adv. Protein Chem.* 28, 239–345
2 Careri, G., Gratton, E., Yang, P.-H. and Rupley, J. A. (1980) *Nature (London)* 284, 572–573
3 Yang, P.-H. and Rupley, J. A. (1979) *Biochemistry* 18, 2654–2661
4 Careri, G., Giansanti, A. and Gratton E. (1979) *Biopolymers* 18, 1187–1203
5 Hilton, B. D., Hsi, E. and Bryant, R. G. (1977) *J. Chem. Soc.* 99, 8483–8490
6 Bryant, R. G. and Shirley, W. M. (1980) *Water in Polymers – ACS Symposium Series 127* (Rowland, S. P., ed.), pp. 147–156
7 Harvey, S. C. and Hoekstra, P. (1972) *J. Phys. Chem.* 76, 2987–2994
8 Yang, P.-H., Tollin, G. and Rupley, J. A. *Biochemistry* (in press)
9 McGammon, J. A., Gelin, B. R. and Karplus, M. (1977) *Nature (London)* 267, 585–590
10 Middendorf, H. D. and Randall, J. (1980) *Phil. Trans. R. Soc. Lond. Ser. B* 290, 639–655
11 Abraham, F. F. (1974) *Homogeneous Nucleation Theory*, Academic Press, New York
12 Hill, T. L. (1949) *J. Chem. Phys.* 17, 762–771
13 Beese, D., Eisenstein, L., Frauenfelder, H., Good, D., Marden, M. C., Reinisch, L., Reynolds, A. H., Sorensen, L. B. and Yue, K. T. (1980) *Biochemistry* 19, 5147–5157
14 Rupley, J. A., Yang, P.-H. and Tollin, G. (1980) *Water in Polymers – ACS Symposium Series* 127 (Rowland, S. P., ed.), pp. 111–132
15 Richards, F. M. and Richmond, T. (1978) *Ciba Symposium* 60, 23–37
16 Hardy, C., Gavish, B. and Gratton, E. *Biophys. J. Abstracts Amer. Biophys. Soc.* (in press)
17 Careri, G., Fasella, P. and Gratton E. (1979) *Ann. Rev. Biophys. Bioeng.* 8, 69–97
18 Banerjee, S. K., Holler, E., Hess, G. P. and Rupley, J. A. (1975) *J. Biol. Chem.* 250, 4355–4367
19 Careri, G. and Gratton, E. (1977) *BioSystems* 8, 185–186
20 Weber, G. (1975) *Adv. Protein Chem.* 29, 1–83
21 Careri, G. (1974) *Quantum Statistical Mechanics in the Natural Sciences* (Kursunoglu, G. and Mintz, S. L., eds), pp. 15–35, Plenum Press, New York
22 Texter, J. (1978) *Prog. Biophys Mol. Biol.* 33, 83–97
23 Hopfinger, A. J. (1977) *Intermolecular Interactions and Biomolecular Organization*, Wiley-Interscience, New York
24 Drost-Hansen, W. and Clegg, J. (1979) *Cell-Associated Water*, Academic Press, New York
25 Hnojewyj, W. S. and Reyerson, L. H. (1959) *J. Phys. Chem.* 63, 1653–1654
26 Bull, H. B. and Breese, K. (1968) *Arch. Biochem. Biophys.* 128, 497–502
27 Fujita, Y. and Noda, Y. (1978) *Bull. Chem. Soc. Japan* 51, 1567–1568

The Na^+,K^+ pump may mediate the control of nerve cells by nerve growth factor

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The survival of nerve cells, in vitro *and* in vivo, *is controlled by extrinsic agents called neuronotrophic factors. The best known among them, nerve growth factor, has been shown to control the performance of the Na^+,K^+ pump in the membrane of its target ganglionic neurons. In turn, the operation of the pump is essential for the survival of these neurons. Ionic control may be an important mechanism by which cell survival, growth and/or differentiation is regulated by certain extrinsic factors or hormones.*

Extrinsic influences have been invoked to explain the development of the nervous system: the orderly manner in which neuronal cells proliferate, migrate, extend axons to appropriate territories, and connect with the correct target cells (or die if they fail to do so). We believe that similar influences continue to apply in the mature organism, and play important roles in the capability or failure of neurons to perform their functions and to resist, or compensate for, pathological insults. Thus, the problem of identifying mechanisms which control the development and functions of nerve cells is important to both the neuroscientist and the clinical neurologist. To manipulate neuronal maintenance and repair to the best clinical advantage, it will be necessary to identify and understand the extrinsic factors which regulate survival and general growth (neuronotrophic factors), as well as the actions of nerve cells, such as neuritic extension (neurite promoting factors). Such investigations are now under way in a number of laboratories (reviewed in Refs 1,2).

The classical model for neuronotrophic and neurite promoting agents is nerve growth factor (NGF), a protein which specifically addresses sensory neurons in dorsal root ganglia (DRG) and sympathetic neurons in peripheral sympathetic ganglia. Three decades of investigation of NGF have contributed detailed information on

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