Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Solvation and Conformational Effects in Aqueous Solutions of Biopolymer Analogues [and Discussion]

F. Franks and M. C. R. Symons

Phil. Trans. R. Soc. Lond. B 1977 **278**, 33-57
doi: 10.1098/rstb.1977.0030

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1977 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B. **278**, 33–57 (1977) [33]
Printed in Great Britain

Solvation and conformational effects in aqueous solutions of biopolymer analogues

By F. Franks
Biosciences Division, Unilever Research Laboratory Colworth/Welwyn, Colworth House, Sharnbrook, Bedford

Biopolymer conformational transitions play a fundamental rôle in life processes. These transitions are triggered and controlled by subtle changes in the 'solvent medium' and are therefore likely to depend on the hydration states of the biopolymer and the soluble small molecule species involved. Three distinct types of hydration behaviour common to biopolymer systems are discussed:

(1) Ionic hydration is of an electrostatic nature, i.e. long range and radial but specific effects exist in the manner in which water molecules are oriented about different ions and this may well be the origin of the various lyotropic series of ion specific effects which are so common in colloid, polymer and biochemistry.

(2) The term 'specific hydration' is used to describe the direct interaction by hydrogen bonding between water and polar sites on organic molecules capable of acting as proton donors or acceptors. Since the hydrogen bond potential is very orientation specific, it follows that 'specific hydration' effects depend sensitively on the detailed stereochemistry of the molecular hydration sites, i.e. their distances of separation and mutual orientations. Thus in cases where molecules can exist in several anomeric or diastereoisomeric forms, or where different conformational states can arise by rotation about carbon–carbon bonds, specific hydration interactions may significantly affect the conformational energy minimum such that solvent dependent conformational states may exist. Similarly the solvent will influence the positions of the equilibria between various isomeric states, e.g. of sugars.

(3) Hydrophobic hydration probably arises from the reorientation of water molecules in the vicinity of an apolar molecule or residue, such that the OH vectors are not directed towards the apolar moiety. This is an entropically unfavourable process which can be partly reversed by the association of two (or more) such hydrophobically hydrated residues. In this way some of the perturbed water can relax back to its normal bulk state in which more molecular orientations are possible. A reassessment of the hydrophobic interaction shows, however, that contrary to currently held views, the potential well due to two alkyl groups in aqueous solution is shallower than it is for two similar groups *in vacuo*, and also that the entropy gain from the pair interaction is not as large as has been believed. These findings necessitate a reappraisal of the molecular details of the hydrophobic interaction.

Introduction

The interrelationship of biological processes and the properties of the aqueous substrate can be developed within the framework of the following sequence of statements:

(1) Life on this planet developed in water, and it is therefore hardly surprising that the universal substrate for *in vivo* processes is water, and more specifically $^{1}H_2O$.†

(2) Biologically significant conformations, i.e. native states, of natural macromolecules

† Although certain lower forms of life can adapt to $^{2}H_2O$, this appears to be impossible for higher plants and all forms of animal life.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

occur spontaneously only in aqueous media, and then only within narrow ranges of temperature, pressure and medium composition.

(3) The mechanisms whereby biopolymers promote or participate in life processes commonly include at least one step in which such a macromolecule undergoes a conformational rearrangement. Rearrangements may be of a major or minor nature, but they always involve rotations about covalent links along the polymer main chain.

(4) Conformational transitions of natural macromolecules, and hence biological changes, are triggered by chemically and physically very minor perturbations of the solvent medium, e.g. the Na^+/K^+ or Mg^{2+}/Ca^{2+} ratios, release of low concentrations of a specific organic molecule, such as acetylcholine or histamine, or changes in pH.†

(5) These 'minor' changes are known to influence in a fundamental manner the phenomenon often referred to as 'water structure' – the unique spatial and orientational order characteristic of liquid water.

TABLE 1

model compounds	features studied
amides	hydrogen bonding and hydration of CO..HN group, solute–solute interactions
amino acids	hydration and interactions with ions
di- and oligopeptides	conformational equilibria, medium effects
polyamino acids	order–disorder transitions, effects of pH
synthetic homo and block copolypeptides	hydrophobic aggregation, ion binding, order–disorder transitions
synthetic polymers, e.g. polyvinyl pyrrolidone, polyethylene oxide	hydrophobic effects and rudimentary tertiary structure in solution
solvent perturbants	
ionic	
reacting ions	H^+, OH^-
neutral cations	alkali metals, Mg^{2+}, Ca^{2+}, lanthanides
neutral anions	SO_4^{2-}, PO_4^{3-}, F^-—insolubilizing Cl^-, Br^-—neutral I^-, ClO_4^-, CNS^-—solubilizing
'hydrophobic' ions	R_4N^+, where R = Me, Et, Pr, Bu RSO_4^- where R = dodecyl
non-electrolytes	
chaotropic agents	urea, guanidinium salts
hydrophobic structure	alkanols, ketones, ethers
promoters	
polyfunctional molecules	glycerol, erythritol, sorbitol, sugars

The above five statements, taken together, strongly suggest that the complex conformational rearrangements, so fundamental to biological processes, are closely coupled and very sensitive to the type and degree of intermolecular hydrogen bonding which exists in the solvent medium. In addition, direct interactions between soluble low molecular mass species and specific sites on the polymer must also be considered. In order to develop an understanding of the various interacting factors which promote conformational changes in natural polymers, it is necessary to examine the system in its entirety, i.e. the polymer, the solvent or dispersion medium, and the

† We are of course dealing with a chicken and egg situation, because these 'minor' changes are themselves triggered by another conformational transition in the system. The *primary* stimulus giving rise to a series of reactions must be of an external nature, e.g. a change in temperature, pressure, humidity, or an optical or electrical impulse.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

distribution of other soluble or bound species. Furthermore, since we are dealing with conformational transitions, both the equilibrium and kinetic aspects of the systems need to be considered. In view of the complexity of biopolymer systems in their native environment it is hardly surprising that the state of our knowledge is at best rudimentary.

Over recent years various simplyfying measures have been adopted to reduce the overall problems to manageable proportions. They range from polymer conformational calculations (Poland & Scheraga 1970) based on vacuum potential functions (i.e. ignoring the solvent contribution) to more rigorous efforts of studying solvation interactions in solutions of biopolymer analogues. In the middle ground there exists a large body of experimental information based on *in vitro* studies of isolated biopolymer systems, and this has led to a number of useful empirical treatments of conformational stability and the solvent influence on conformational equilibria (von Hippel & Schleich 1969).

It is our aim in this contribution to the Symposium to discuss the various descriptions of systems composed of molecules commonly used as model for biopolymers, water, and other water soluble species which affect biopolymer conformational equilibria. As an example table 1 lists the various model compounds commonly employed to study the effects of solvent perturbations on the conformational properties of proteins.

Similar simplifying approaches have been adopted to study solvent effects on the conformational equilibria of polynucleotides and polysaccharides, although the latter class of polymers has not yet received the attention it deserves (Suggett 1975).

The molecular description of hydration interactions

We start from the premise that an isolated solute molecule or ion, when introduced into water, will perturb the predominantly tetrahedral arrangement of water molecules, usually referred to as 'water structure'. Such time averaged structure is formally described by a molecular pair correlation function $g(r, \Omega)$ where r is the distance between the centres of mass of the two molecules and Ω expresses the orientational contributions. Clearly $g(r, \Omega)$ depends on the nature of $U(r, \Omega)$ the pair potential function. The knowledge of $g(r, \Omega)$ is important as it permits the calculation of the thermodynamic properties of the liquid. A common approximation is to treat the water molecules as hard spheres, because this removes the orientation dependence from the pair correlation function. In practice this means that the position of the oxygen atoms alone is required for an evaluation of $g(r)$. Figure 1 shows $g(r)$ for liquid water and liquid argon as a function of r^*, the reduced hard sphere radius. The comparison shows the essential difference between the two liquids: the peaks in the argon curve fall at $r^* = 1, 2, 3$, indicating close packing, whereas in water the peaks correspond to $r^* = 1, 1.5$ and 2.5, characteristic of tetrahedral packing. Also the areas under the peaks are proportional to the coordination numbers, 11 in the case of neon and 4.4 for water.

The perturbation due to a single solute (s) particle must be examined in terms of changes in $g_{ww}(r)$, where w refers to a water molecule and also in terms of $g_{sw}(r, \Omega)$, the solute–water pair correlation function which describes the geometry of the primary hydration sphere. It is also possible that r_{ww} remains largely unchanged and that the hydration perturbation affects mainly the orientation dependent part of the pair correlation function (Hertz 1964).

Finally, with increasing solute concentration, solute–solute effects need to be considered and these cannot take place without simultaneous changes in the surrounding hydration spheres,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

leading to increasing overlap as r_{ss} diminishes. In practice the work required to bring two solvated molecules together is called the potential of average force, $W(r)$ (neglecting orientational effects).† This is made up of two terms: (1) the 'vacuum' potential $U(r)$ which includes short range attractions and repulsions, hydrogen bonding and long-range electrostatic effects if present, and (2) the solvation free energy, $A(r)$ which is intimately related to $g_{sw}(r)$ and which modifies $U(r)$. $W(r)$ for a solution can be compared to $U(r)$ for the molecules in a gas. Thus the osmotic pressure of a dilute solution is given by

$$P^* = \frac{RTn}{V}\left(1+\left(\frac{n}{V}\right)b+\ldots..\right), \tag{1}$$

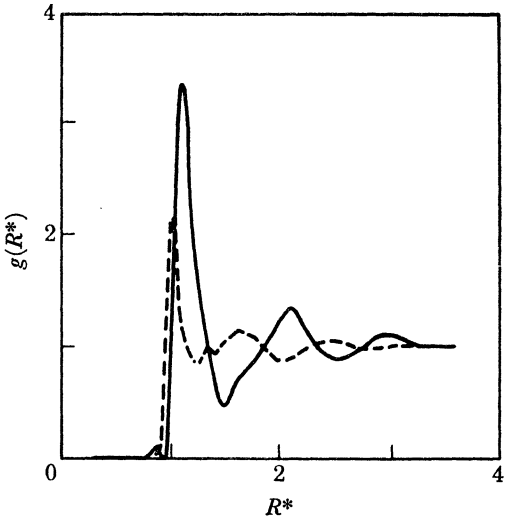


FIGURE 1. The molecular pair correlation function (radial distribution function) $g(R^*)$ for liquid argon at 84.25 K (——) and liquid water at 277.2 K (– – – –); R^* is the reduced distance r/σ, where σ is the hard sphere molecular radius (0.282 nm for water and 0.34 nm for argon). (From Ben-Naim 1972.)

where n/V is the number density in molecules of solute per litre, and b is the second virial coefficient. This quantity is related to $W(r)$ by

$$b = -\tfrac{1}{2}K\int_0^\infty (e^{-W(r)/kT}-1)\,4\pi r^2\,dr, \tag{2}$$

where K is a constant. Equation (2) applies to spherically symmetric solute molecules and is therefore of very limited usefulness for molecules of biological interest where orientational and stereochemical specificity are very important in determining the distance of separation r. One further problem in the effective utilization of $W(r)$ lies in its conversion to $g(r)$ which is of more immediate practical value for the interpretation of experimental data. Thus the 'excess' osmotic pressure – that due to solute–solute effects (the second term on the right hand side of equation (1)) – is given by

$$P^{*E} = -\frac{1}{6}\left(\frac{n}{V}\right)^2\int_0^\infty r\frac{\partial W(r)}{\partial r}g(r)\,4\pi r^2\,dr \tag{3}$$

† Water being a highly asymmetric molecule, the orientation dependent part of the pair correlation function is of great importance in determining the properties of aqueous solutions. Unfortunately it is not readily accessible to experimental determination and therefore in the following discussion it is omitted. There are various procedures which allow oriental averaging to be performed, so that the resulting $g(r)$ would then refer to an *effective* distance of separation.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

from which expressions for excess internal energies and partial molar volumes are readily obtainable. The adequate conversion of $W(r)$ into $g(r)$ presents difficulties even with monatomic fluids, and for binary mixtures various semi-empirical devices or computer simulation methods have to be used; in fact the whole subject of statistical mechanics of aqueous mixtures is still in its infancy (Ben-Naim 1974).

However, even with the rather rudimentary tools at our disposal, there is a wealth of experimental data which could be processed to yield valuable information about specific and non-specific hydration effects and the influence of hydration 'structures' on mutual solute orientations or on specific conformational properties of molecules such as peptides or carbohydrates.

Classification of hydration interactions

In the absence of rigorous theoretical methods for the formulation of $A(r, \Omega)$ or adequate experimental methods for the determination of $g_{sw}(r, \Omega)$, we are forced to adopt a classification of hydration interactions based on the different chemical types of molecular and ionic hydration sites.

(*a*) *Ionic hydration*

This is a subject which has received adequate attention over many years (Friedman & Krishnan 1973). Most of the rigorous studies have been performed on monatomic ions, in particular the alkali metal and halide ions. Interest centres on the differences between water on the one hand, and other polar media (alcohols, propylene carbonate (PC), dimethylsulphoxide (DMSO)) as solvating media on the other, and in recent years the specific features of mixed aqueous solvents have received attention (Bennetto & Feakins 1968; Kay 1973). The experimental characterization of ion solvation has been based mainly on isopiestic, e.m.f. and heat measurement studies, and it has become clear that the Born electrostatic model, which treats the standard free energy of solvation $\Delta G_s^{\ominus}$ in terms of ion charge and size and the dielectric permittivity of the solvent medium, hardly provides an adequate description even in non-aqueous solvents. This becomes quite evident when the temperature and pressure derivatives, (i.e. the enthalpy, entropy and volume of solvation) are considered. The same is true for other electrostatic models which treat the solvent as a dielectric continuum (Friedman & Krishnan 1973).

Useful information about differences in solvation behaviour is derived from thermodynamic properties associated with the ionic transfer from one solvent to another, e.g. DMSO or PC to H_2O, or D_2O to H_2O.

Figure 2 graphically illustrates the complex behaviour encountered. Ionic solvation enthalpies in a series of solvents are compared with those in a given reference solvent – in this case PC. It is seen that the most extensive deviations from the simple ionic radius dependence occur in aqueous solution, but that qualitatively similar trends exist in methanol.

A new insight into the interactions between ions and discrete solvent molecules has been provided by mass spectroscopic studies of ion solvation in the gas phase. From a comparison of pairs of isoelectronic ions of the alkali halide series, e.g. K^+, Cl^- and Rb^+, Br^-, it was found, as expected, that the cation exhibits a larger hydration energy (ΔH_h) for low hydration numbers, n_h (Arshadi, Yamdagni & Kebarle 1970). However, as n_h increases, i.e. with increasing cluster size, the interactions with anions become more favourable. This is explained in terms of the greater ease of packing of water molecules about the anion. The results show that irrespective

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

of the nature of the ion, $-\Delta H_h$ reaches a limiting value for $8 < n_h < 12$, i.e. beyond this extent of hydration $-\Delta H_h$ is the same for cations and anions. One further interesting fact arises from these studies: there appears to be no particular value for n_h at which the hydration complex exhibits particular stability. The opposite is true for $H^+ \cdot nH_2O$ where a maximum in the stability is observed for $n = 4$ (Grimsrud & Kebarle 1973) with another, less pronounced maximum at $n = 6$.

So far the gas phase results have not yet been exploited in studies of solution behaviour, but it is conceivable that the experimental gas phase hydration energies could be combined with spectroscopic data to provide a more detailed description of the primary and secondary hydration spheres. Without such a development it will probably not be possible to gain an understanding of the so-called Hofmeister or lyotropic series of ion specific effects (Hofmeister 1888) which is so ubiquitous in colloid and biochemistry and will be referred to several times.

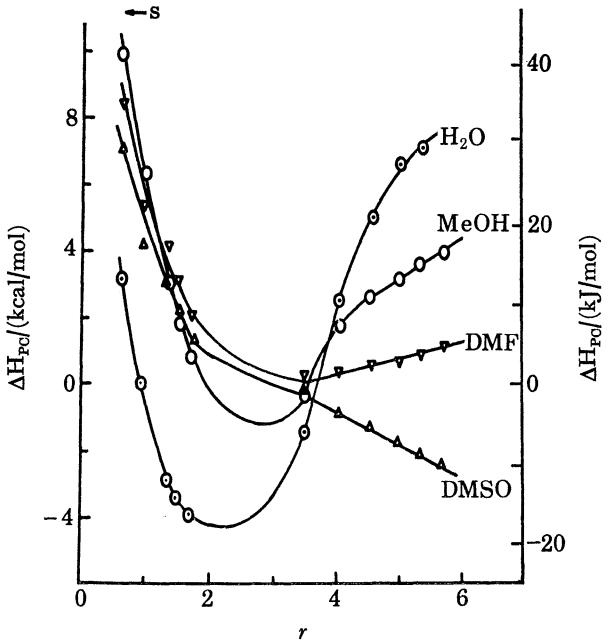


FIGURE 2. Limiting ionic enthalpies of transfer from propylene carbonate from a series of solvents, as a function of ionic radius. (From Friedman & Krishnan 1973.)

The extreme sensitivity of ions to the aqueous solvent environment is well demonstrated by the dramatic effects produced by low concentration of alcohols on ionic transport properties, such as self-diffusion, conductance, transport number and viscosity. It has been shown conclusively that the observed complex aqueous solution behaviour cannot be accounted for in terms of simple preferential solvation by one or the other solvent species, but probably derives from an alteration of the water–water interactions (see below) (Kay 1973).

In principle, infrared and Raman spectra should be of value in providing details about the nature of the ionic hydration sphere. In solution of monatomic ions these techniques are of course limited to observations of the water spectra and the main problems here are the still unresolved questions about the correct assignment of some spectral peaks, especially in the overtone region, but also in the low frequency intermolecular (hydrogen bond stretching and bending) region (Luck 1973). Comparative studies in H_2O–D_2O mixtures are of value here because

Downloaded from rstb.royalsocietypublishing.org

the effects of vibrational coupling can be eliminated, since the peaks due to O–H and O–D stretching vibrations are well separated.

Investigations of hydration induced spectral changes in the solute are limited to polyatomic ions and this introduces orientational complications, resulting from the detailed geometry of the ion hydration sites.

Indirect structural information about the orientations of H_2O molecules in the primary hydration sphere of an ion can be derived from the nuclear magnetic relaxation rates of ions which relax wholly or partly by magnetic dipole–dipole interactions. Unfortunately the alkali metal ions (with the exception of ^7Li) do not fall into this class and neither do the halide ions, except for $^{19}F^-$.

Thus by considering the effect of ^1H and ^{17}O on the relaxation rate of the ^{19}F, Hertz & Rädle (1973) have been able to show that the H_2O molecules are asymmetrically oriented in the fluoride ion hydration sphere and that the F^-–H–O distance cannot deviate much from 0.275 nm.

For ions which relax by quadrupolar mechanisms (this includes the other alkali metal and halide ions) structural information can be obtained by a more roundabout route, given a knowledge of the ionic and solvent self-diffusion coefficients.

Finally, it is appropriate to mention theoretical and computer simulation methods for studying ionic hydration and ion–ion interactions. Apart from rigorous *ab initio* approaches, the technique which currently shows promise is the molecular dynamics simulation in which a computer is used to solve the equations of motion for a system containing a given number of ions and solvent molecules in which the various pair potentials have been carefully specified. The method has been particularly successful in providing a picture of the equilibrium and dynamic properties of water over a large part of the P-V-T surface. Two recent studies have extended the method to aqueous solutions of LiCl (Heinzinger & Vogel 1974) and CsCl (Vogel & Heinzinger 1975). One of the advantages of the technique is that all atomic pair correlations can be obtained, even those which are presently inaccessible to experimental investigation. Thus for the electrolyte M^+X^- in aqueous solution, the relative densities of H and O atoms about M^+ and X^- and about the oxygen atoms of water molecules can be found and this provides information about the orientations of water molecules with respect to the ions. For CsCl the results show that the ions reach equilibrium at a separation of 0.8 nm, confirming the absence of ion-pairing. The nature of $g_{Cs^+-O}(r)$ shows that the primary hydration shell of Cs^+ is not well developed and that the concept of a hydration number is of no great value (Vogel & Heinzinger 1975). The hydration shell about the Cl^- ion is better developed, suggesting that $n_h = 8 \pm 1$ in CsCl and 6 ± 1 in LiCl. The water–water correlation function shows a distinct similarity to that observed for water at high temperature and/or pressure, supporting the concept of the 'structural temperature', first advanced by Bernal & Fowler (1933). The results for the average orientations of water molecules with respect to the ions tend to support the available experimental evidence, but a similar comparison between the calculated and experimental self-diffusion coefficient of water in CsCl does not show the same good agreement.

(*b*) *Specific molecular hydration*

This term is used to describe the interactions of water with proton donor or acceptor sites on neutral molecules, e.g. –OH, –NH, –O–C>=O. Since the hydrogen bonding potential is very orientation sensitive, it follows that this type of hydration interaction is likely to depend

Downloaded from rstb.royalsocietypublishing.org

on the detailed stereochemistry of the solute molecule, i.e. the relative distance between, and mutual orientations of the hydration sites. As mentioned above, the hydration interaction competes with the hydrogen bonding between water molecules, and therefore solute molecules which are able to interact with the solvent without major perturbations in the solvent structure are likely to interact preferentially. This concept which we have termed solvent compatibility can help to explain the observed differences in the solution behaviour of a series of chemically and physically similar molecules, which differ mainly in the locations of their hydration sites. The hexose sugars in their pyranose forms constitute such a group of molecules. They differ only in the distribution of axial and equatorial OH groups on carbon atoms 1–4. Thus β-glucose has the maximum of four equatorial substitutents. Until recently it was generally assumed that sugars were almost ideal solutes in aqueous solution and that their solution properties could be adequately described by a series of hydration equilibria (Stokes & Robinson 1966). However, more detailed studies now suggest that, especially at low temperatures, different sugars interact with the solvent in specific ways, although at higher temperatures, this distinctive behaviour is lost, so that at 80 °C glucose, galactose and ribose all appear to exist as a monohydrate in solution (Tait *et al.* 1972).

TABLE 2. ESTIMATED HYDRATION NUMBERS (n_h) FOR MONO- AND DISACCHARIDES AT 5 °C

	n_h (mol water/mol sugar)	
sugar	dielectric relaxation	glass composition
glucose	3.7 ± 0.2	3.7
mannose	3.9 ± 0.4	—
ribose	2.5 ± 0.4	2.9
sucrose	6.6 ± 0.6	6.3
maltose	5.0 ± 0.4	—

Apart from a thermodynamic characterization of the solution properties of sugars (Franks, Ravenhill & Reid 1972), a combination of n.m.r. and dielectric relaxation techniques has been of particular value in the probing of solute and water motions (Tait *et al.* 1972; Franks, Reid & Suggett 1973; Suggett 1976). Thus n.m.r. methods allow the study of a particular component (nucleus) but suffer from uncertainties associated with fast exchange of water between different environments, so that relative populations of these environments cannot be unambiguously specified. On the other hand, dielectric relaxation measurements are non-discriminating in that every polar species contributes to the observed spectrum, but they do not suffer from the limitations imposed by exchange processes. Thus, *if* the dielectric spectrum can be resolved into several components, each with a characteristic correlation time τ_1 and amplitude A_1, originating from, say, bulk water, solute, and perturbed water, located in the hydration sphere, then the corresponding n.m.r. spin lattice relaxation times can be calculated and compared with the experimental values. This procedure will in turn lend credibility to the dielectric model employed in the resolution of τ_1 and A_1 values.

By applying the above methodology to a number of mono and disaccharides, Suggett & Clark (1976) have demonstrated that the dielectric relaxation spectra of some sugar solutions are best interpreted in terms of two Debye relaxations of which one (τ_1 = 22 ps at 5 °C) corresponds to the major solvent reorientation process, and the other ($\tau_2 \sim 100$ ps) to the coupled

Downloaded from rstb.royalsocietypublishing.org

motions of the sugar molecule and its associated hydration sphere (Suggett & Clark 1976), i.e. the exchange of water molecules between the bulk and the hydration sphere is slow compared to τ_2.

From the amplitudes A_1 and A_2, hydration numbers have been calculated and these are summarized in table 2, together with data derived from non-equilibrium freezing experiments and an analysis of sugar–water glasses, i.e. 'unfrozen water' (D. S. Reid, unpublished results).

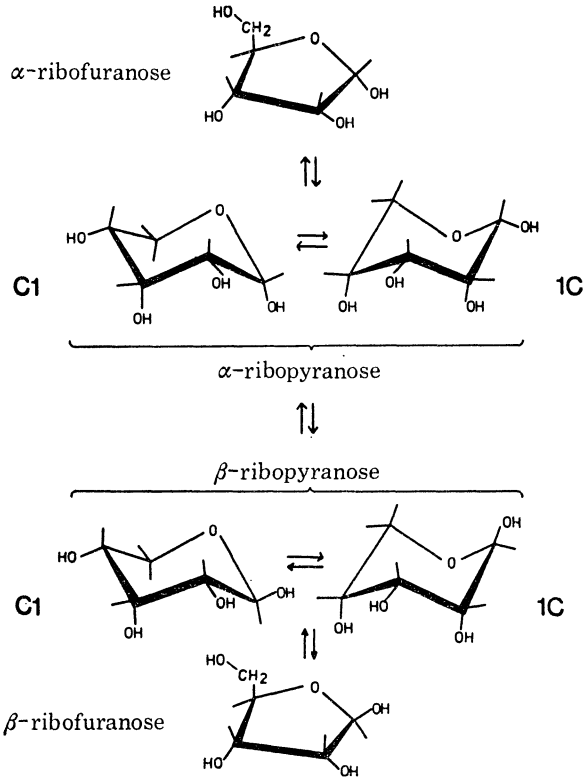


FIGURE 3. Molecular species which coexist in solutions of D-ribose.

From a combination of time domain dielectric relaxation experiments with measurements of ^{1}H, ^{2}H, ^{17}H and ^{13}C n.m.r. relaxation rates on solutes and water, as appropriate, it has been possible to chart the molecular motions and interactions in such systems in a fair degree of detail and to demonstrate effects which are quite specific to given sugars and given conformations and are therefore likely to depend critically on their detailed stereochemistry (Franks *et al.* 1973; Suggett 1976).

A general result of these studies is that a 'water compatible' stereochemistry favours hydration interactions (Tait *et al.* 1972). Thus, for pyranose sugars, the distances between equatorial –OH groups on pairs of alternate carbon atoms (i.e. 1, 3 and 2, 4) – see figure 3 – are identical with the distance of the second peak in the water pair correlation function (see figure 1) where $r = 0.485$ nm, implying that equatorially substituted sugars can interact with water with a minimum of perturbation (Kabayama, Patterson & Piche 1958), as shown schematically in figure 4. In this way any perturbation beyond the primary hydration sphere should be very limited, and this may well be the reason why sugars form 'pseudo-ideal' aqueous solutions

Downloaded from rstb.royalsocietypublishing.org

(Stokes & Robinson 1966); solute–solute interactions are effectively screened by the hydration sphere which closely resembles pure water (Franks *et al.* 1972).

An important consequence of the specific hydration model concerns the effects of solvation interactions on conformational and anomeric equilibria. We can distinguish between three types of equilibria:

(1) Simple anomerization – simple only in the sense that the process follows first order kinetics.

(2) Other conformational equilibria exhibited by monosaccharides, e.g. Cl pyranose $\rightleftharpoons$ 1C pyranose, pyranose $\rightleftharpoons$ furanose.

(3) Rotation about the glycosidic linkage in the case of di- or oligosaccharide.

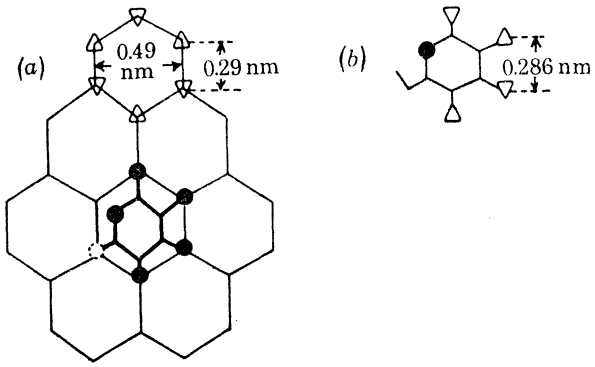


FIGURE 4. Simple model for monosaccharide hydration (*a*) in this case β-D-glucose (*b*) – assuming a hexagonal water lattice at 25 °C; the orientation of the triangles ($\triangledown$ or $\triangle$) indicate whether the equatorial –OH groups lie above or below the plane of the sugar ring. (From Tait, Ablett & Franks 1972.)

Some of these equilibria are illustrated (for ribose) in figure 3. Primarily they are governed by the intrinsic free energies of the various anomers and conformers, and estimates of these are available (Angyal 1969). (It must, however, be pointed out that these estimates are based on experimental measurements on *aqueous* solutions and they cannot therefore be regarded as true vacuum potentials.) However, for any equilibrium in solution, a solvation free energy must be included and this may enhance or reduce the stability of a given conformer and may vary with the solvent.

Some recent experimental results will now be discussed to illustrate the solvation effects on sugar conformation.

Solvent effects on aldohexopyranose conformations have been investigated by Thom (1973) who used measurements of compensated molecular rotation $[M_c]$ as an index of conformational changes:

$$[M_c] = \left([M]_S^T \frac{(n_{H_2O}^{25})^2+2}{(n_S^T)^2+2} \frac{d_S^{25}}{d_S^T}\right)_S^T \left(\frac{(n_{H_2O}^{25})^2+2}{(n_S^{25})^2+2}\right) \quad (4)$$

where $[M]_S^T$ is the molecular rotation at T °C in a solvent S of refractive index n_S^T and density d_S^T. Equation (4) compensates for changes in refractive index. Small changes in $[M_c]_S$ were observed when the temperature was raised from 25 to 80 °C; when the solvent was changed under isothermal conditions, these changes became more pronounced but were still much smaller than would be expected for a major conformational change. Table 3 summarizes some $\Delta[M_c]_S$ values of several methyl pyranosides for three pairs of solvents. Significant solvent effects are observed and two possibilities for their origin have been considered: (1) they might

Downloaded from rstb.royalsocietypublishing.org

arise from conformational changes involving rotations about the C5–C6 bond and/or the C1–OCH_3 bond and these can be calculated by semi-empirical methods; (2) the solvent might affect the chromophores and hence influence the magnitude of the rotational parameters in the empirical equations used to calculate $[M]_S$, e.g. changes in the wavelengths of the far ultraviolet o.r.d. peaks or in the intensity of their Cotton effects. Neither alternative can adequately account for the observed $\Delta[M_c]_S$ values, especially for those involving water as one of the solvent pairs. Thom concludes that both alternatives are likely to contribute to the observed effects and that in addition, water may promote small changes in the ring chair conformation, e.g. ring flattening. He also makes the point that for those sugars which are of importance in biological processes there appear to be solvent specific changes in the rotational equilibria of the $-CH_2OH$ group about the C5–C6 bond. It is perhaps premature to speculate whether and how the highly developed intermolecular order of water is responsible for the sugar specific effects observed in aqueous solution, but the experimental evidence strongly points to a close relation.

TABLE 3. DIFFERENCES IN COMPENSATED MOLECULAR ROTATIONS, $[M_c]$ (SEE EQUATION (4)) FOR METHYL PYRANOSIDES IN PAIRS OF SOLVENTS

(For the figures in parentheses allowance has been made for possible conformational changes about the C5–C6 bond. This still leaves large differences between, say methyl α-D-glucoside and methyl α-D-galactoside, showing that the observed $\Delta[M_c]_s$ values cannot be accounted for completely by rotation about the C5–C6 and the C1–OMe bonds (see text) (Rees & Smith 1975).)

	$[M_c]_s$/deg		
methyl ester	dioxan–DMSO	dioxan–H_2O	DMSO–H_2O
α-D-glucose	+8 (+26)	+3 (+18)	−5 (−8)
α-D-xylose	+26	+18	−8
α-D-mannose	+72 (+90)	+63 (+78)	−9 (−9)
α-D-galactose	−32 (+3)	−69 (−56)	−38 (−59)
β-L-arabinose	+3 (−19)	−56 (−7)	−59 (+11)
β-D-glucose	−4	−5	−1
β-D-xylose	−19	−7	+11
β-D-galactose	−25 (+10)	−47 (−34)	−22 (−43)

D. Thom, Ph.D. thesis, University of Edinburgh 1973.

The effects of different solvents on anomeric equilibria and kinetics are currently receiving attention. For glucose the specific hydration model in figure 4 predicts that in aqueous solution at low temperature (where the intermolecular 'order' in liquid water is greatest) solvation will favour the β-anomer (with four equatorial OH groups) over the α-anomer and this is indeed found to be the case. To assess the degree of preferential stability, we can compare the less specific (?) solvent effect of DMSO and such a comparison is shown in the form of a van't Hoff plot in figure 5 (F. Franks, P. J. Lillford & G. Robinson, unpublished results). Glucose conformations other than the α- and β-pyranose (Cl) have such high free energies that they do not contribute to the equilibrium mixture. The changes in relative conformational free energies which can be brought about by solvation are well illustrated by the fact that in *N*,*N*-dimethylformamide the glucose equilibrium mixture contains 4% furanose (Reine, Hveding, Kjolberg & Westbye 1974).

Downloaded from rstb.royalsocietypublishing.org

The situation is rather more complex with D-ribose which exists as an equilibrium mixture of six different modifications in DMSO as well as in water (see figure 3). We have studied the effect of temperature on the equilibrium composition (F. Franks & G. Robinson, unpublished results). In both solvents at low temperature the predominant modification is β-ribopyranose (C1) which also happens to be the most 'water compatible' conformer. Thus in DMSO at 0 °C it amounts to 33 %, but in water to 46 % of the equilibrium mixture. The difference might be ascribed to the particularly favourable hydration interactions.

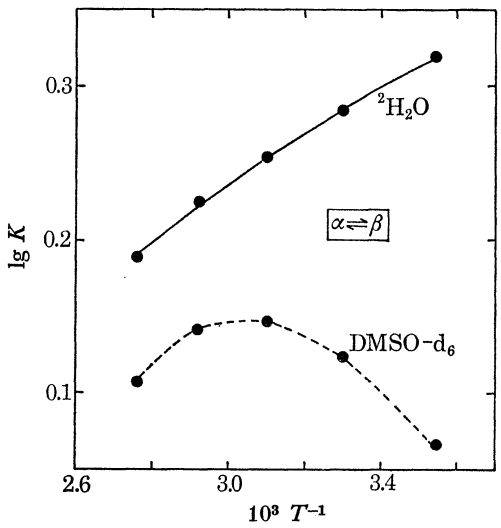


FIGURE 5. van't Hoff plots characterizing the anomeric equilibria of D-glucose in water and in dimethyl sulphoxide. (Results are based on n.m.r. measurements and therefore refer to deuterated solvents.)

The kinetics of mutarotation also exhibit complex solvent effects. The reaction rates in non-aqueous solvents are so low that little reliable information about the solvent catalysed reaction can be obtained; but in some mixed aqueous solvents, particularly in water-*tert.*-butanol and water–tetrahydrofuran (THF), remarkable and complex changes can take place in the activation parameters with increasing cosolvent concentration. Figure 6 shows the solvent composition dependence of $\Delta H^{\ddagger}$ associated with the mutarotation of glucose (Livingstone 1974; Livingstone & Frank 1977) and, for comparative purposes, the hydrolysis of *p*-nitrophenyldichloroacetate (Engbersen & Engberts 1975). Both these reactions involve water-catalysed nucleophilic attack by water. Upon the initial addition of cosolvent the decrease in the rate is governed by the change in $\Delta S^{\ddagger}$ (not shown in figure 6), but as the cosolvent concentration is increased the further decrease in the rate constant is governed by $\Delta H^{\ddagger}$. This type of solvent composition dependence cannot be accounted for either by changes in solvent polarity or by a reduction in the concentration of water. The commonly observed large negative $\Delta S^{\ddagger}$ values in themselves suggest that, relative to the ground state, the transition state is highly and specifically hydrated.

Finally, let us discuss briefly the effect of solvent on the conformations of molecules where the possibility of rotation about covalent bonds exists. In the case of a disaccharide the conformation of the two sugar units relative to one another is defined in terms of the two dihedral angles ϕ and ψ. For a disaccharide consisting of a reducing (R) and a non-reducing (N) monosaccharide residue, Rees has shown that the observed molecular optical rotation $[M_{NR}]$ can

Downloaded from rstb.royalsocietypublishing.org

yield a parameter $[\Lambda]$, the 'linkage rotation', i.e. the optical activity contributed by the actual values of ϕ and ψ (Rees 1970). Thus

$$[\Lambda] = [M_{NR}] - \{[M_{MeN}] + [M_R]\}, \quad (5)$$

where MeN is the methyl ester of the non-reducing sugar, having the same anomeric configuration as the disaccharide NR. From a knowledge of the crystal structure of NR and the optical rotations of N and R it is thus possible to predict $[M_{MR}]$. This treatment has recently been applied to investigations of solvent and temperature effects on a range of disaccharides (Thom 1973). For cellobiose the $[\Lambda]$ values in water and DMSO were almost identical and agreed with that calculated from the crystal structure. This lends support to the suggestion that in solution (as also in the crystal) the disaccharide is stabilized by intramolecular hydrogen bonding.

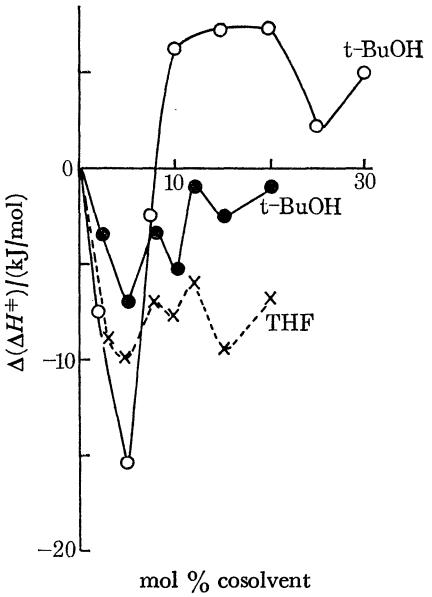


FIGURE 6. The dependence on solvent composition (*tert.*-BuOH and tetrahydrofuran) of the enthalpy of activation associated with the mutarotation of D-glucose (●, ×) and the hydrolysis of *p*-nitrophenyldichloroacetate (○).

TABLE 4. OBSERVED AND CALCULATED LINKAGE ROTATIONS FOR β-MALTOSIDE

	observed†			calculated		
	in DMSO	in dioxane	in H_2O	from crystal conformation	van der Waals minimum (I)	van der Waals minimum (II)
$[\Lambda]_D$/deg	−19	−23	+46	−109	−24	+85

† D. Thom, Ph.D. thesis, University of Edinburgh (1973).

Striking solvent effects were found for β-methyl maltoside and the experimental $[\Lambda]$ values are compared with calculated values in table 4. In the first place it is apparent that the disaccharide in solution does not adopt the crystal configuration. Conformational energy calculations (vacuum potential functions) suggest $[\Lambda] = -24°$, in reasonable agreement with the observed value in non-aqueous solvents. However, allowing for the possibility of hydrophobic

Downloaded from rstb.royalsocietypublishing.org

interactions between the sugar residues another minimum on the ϕ, ψ conformational energy map corresponds to a folded configuration for which $[\Lambda] = +85°$. The aqueous solution value must then be accounted for in terms of yet another, different conformation, or in terms of an equilibrium mixture of the two van der Waals (vacuum) conformations. Although the latter suggestion has been favoured (Rees & Smith 1975), structurally more direct studies of solvent effects on the conformations of small molecules (e.g. adenosine monophosphate) indicate that water can indeed promote a conformation which is different from that favoured by DMSO and that the experimental results are not necessarily compatible with an equilibrium between radically different configurations (Barry *et al.* 1971).

The linkage optical rotation approach to conformation has also been extended to oligosaccharides and it is being increasingly realized that the nature of the solvent and particularly that of an aqueous solvent can profoundly influence ϕ and ψ and hence the shapes of polysaccharide molecules in solution (Suggett 1975; Thom 1973).

Attempts have also been made to elucidate by molecular orbital methods specific hydration interactions and the way in which these interactions influence the conformations of polar molecules. This approach is based on the identification of probable (i.e. most favourable) hydration sites and the adequate specification in terms of positions and orientations of the solute and water molecules; the system is thus treated as a supermolecule and the hydration stabilization energies can be calculated. Having ascertained the geometries and energetics of the solute–water associations, the effects of these interactions on the internal conformation of the solute molecule can be investigated (Pullman & Pullman 1975). This approach has been extensively applied to molecules of pharmacological interest in efforts to eludicate biologically 'active' conformations which in turn might provide information about the 'shapes' of drug receptor sites. Histamine is a good example of such a compound: two states have been investigated (Pullman & Port 1974). I, the monocation which is the major species at physiological pH and II, the dication which should be stable at low pH. Table 5 summarizes the conformational states of I and II *in vacuo* and in their hydrated forms, it being assumed that the quaternary NH_3^+ group has three hydration sites (118 kJ/mol each) and the ring N_1 atom interacts, albeit more weakly, with one water molecule. The ring NH group is not considered as a favourable hydration site.

The results in table 5 shows the calculated changes in the conformations of the histamine ions upon hydration. The intramolecular hydrogen bond between the quaternary nitrogen atom and the imidazole ring which is a feature of the isolated ion disappears in the hydrated state. The theoretical predictions are consistent with the known crystal structure of II and also with ionic species related to I on the one hand, and with n.m.r. coupling constant data of histamines in solution on the other.

In principle, such methods would provide information about $A(r, \Omega)$ and hence $g_{sw}(r, \Omega)$. However, the credibility of such calculations is still open to question, since hydration is treated in terms of isolated nearest neighbour water molecules only, without regard to any spatial and orientational mismatch with more distant water molecules. Thus the results obtained on this basis might be better compared with the gas phase hydration of organic molecules; they may not necessarily provide reliable details of solution conformations.

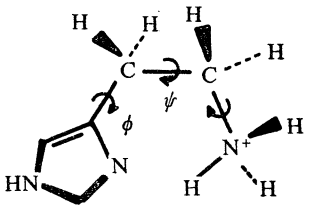
The rôle of the solvent in determining the conformation of peptides has not yet been thoroughly investigated, although significant hydration sites have been identified by the application of *ab initio* techniques (Pullman & Pullman 1975).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Amino acid hydration numbers have been estimated from the intensity of the residual liquid component (unfreezable water) in the ^1H n.m.r. spectrum of amino acid solutions cooled to $-35\,^{\circ}C$ (Kuntz, Brassfield, Low & Purcell 1969). Hydration numbers lie in the range of 1 mol water/mol amino acid for apolar amino acids to 7.5 for the ionized form of glutamic acid and tyrosine. Here again, however, the particular method employed is likely to monitor only those water molecules directly hydrogen bonded to polar sites, but is unable to probe structure or dynamics beyond this primary hydration sphere. Nevertheless the extension of measurements of 'unfreezable water' to proteins has produced good agreement with estimates based on the sum of individual amino acid hydration values.

TABLE 5



I

II
histamine

	structure I	structure II
isolated		
ϕ	30°	120°
ψ	60°	180°
conformation	gauche	trans
ΔE (trans→gauche)	−46	+34
hydrated		
ϕ	60°, *60°*	180°
ψ	60°, *180°*	180°
preferred conformation(s)	gauche⇄trans	trans predominates
ΔE (trans→gauche)	—	-2 kJ mol^{-1}

Recent studies of the interactions between amides and ions in aqueous solution clearly point to the specificity of ion (especially anion) binding to amides (Hamabata & von Hippel 1973). Not surprisingly, the free energy of binding follows the lyotropic anion series, but only when the amide residues carry alkyl substituents (see below for a more detailed discussion). This is an important extension of previous knowledge regarding the influence of ions on macromolecule conformational stability (von Hippel & Schleich 1969).

The ion binding data indicate that the site of binding is the amide dipole which in its unperturbed state is known to take up the trans-planar configuration. It would be of interest to know whether, and to what extent, this configuration can be perturbed by the ion. Also the origin of the ubiquitous lyotropic series which, so Hofmeister speculated (Hofmeister 1888), 'probably arises from differences in the affinities of different ions for water', is still unknown.

That the molecular conformations of simple peptides are indeed very sensitive to the nature of the aqueous solvent medium has been elegantly demonstrated by the use of paramagnetic n.m.r. chemical shift probes (Levine & Williams 1975).

It appears that dipeptides in aqueous solution take up specific conformations which among other factors depend on the nature of the shift probe and on the amino acid side chain. Thus, peptides carrying bulky side chains, e.g. leucine or tyrosine, take up a conformation such that the side chains fold back towards the probe, in this case Tm_{III}. Pr_{III} complexes adopt a some-

Downloaded from rstb.royalsocietypublishing.org

what different conformation, a fact which might suggest that the particular conformation is at least partly determined by the nature of the probe.

The degree of conformational folding of Tmiii-dipeptide complexes (but not that of the Priii complexes) is influenced by those ions (and urea) which also affect the conformational stability of proteins – once again it is found that the order of effectiveness of different ions follows the lyotropic series.

In summary, the conformational changes are specific to the nature of the probe, the nature of the amino acid side chains (apolar residues promote such changes) and the nature of the aqueous medium.

What emerges therefore both from the ion binding studies and the lanthanide induced p.m.r. shifts is that the conformation of a peptide is determined by the complex interaction between the hydrated ion and the hydrated amide dipole, but that this interaction is in some way modified by the vicinity of a hydrated alkyl (or aryl) group. This last observation implicates the phenomenon of hydrophobic hydration which constitutes the final class of hydration interactions to be discussed.

(*c*) *Hydrophobic hydration*

The peculiar thermodynamic properties of aqueous solutions of chemically inert species (rare gases, hydrocarbons) was first treated in a quantitative manner by Frank & Evans (1945). They showed that the low solubility (large positive free energy of solution ΔG_s) of such species did not necessarily originate from an unfavourable interaction energy but from a very large unfavourable entropy term in ΔG_s. Frank and Evans further concluded that in molecular terms this effect arose from spatial and orientational constraints imposed on the hydrogen bonded array of water molecules in the vicinity of an 'inert' molecule. The term 'iceberg' was used in this connection, although it was never implied that the perturbed water resembled ice in its configurational properties. It was later shown that whatever were the 'structures' favoured by the apolar solutes, they were thermally very labile and could be easily destroyed by polar molecules such as urea or polyhydroxy compounds. Different theoretical and experimental methods have been employed to develop more or less adequate molecular and energetic descriptions of such solutions of inert species, occupying cavities within the perturbed hydrogen bonded water matrix; these have recently been reviewed (Ben-Naim 1974; Franks 1975).

Of great help was the realization that entropy driven solution processes were not confined to hydrocarbon/water mixtures, but were characteristic of aqueous solutions of monofunctional alkyl and aryl derivatives generally. This realization led to an intensive study of such solutions by a wide variety of experimental techniques which could not be applied to solutions of hydrocarbons because of their very low solubilities.

Thus, it is now well established that in the presence of low concentrations of such alkyl derivatives the intramolecular vibrations of water, as well as its rotational diffusion, are affected in a way which makes the liquid appear more viscous and more extensively hydrogen bonded. A useful structural model for water in this state is provided by the crystalline clathrates which are formed by the same molecule species which affect water in the manner just described. In terms of the orientations of water molecules with respect to one another and with respect to the solute, the convex water cage, typical of the clathrate structure, demands that none of the OH vectors be directed towards the centre of the hydration cage. In its unperturbed state the favoured configuration of pairs of water molecules resembles that which exists in ice, as shown in figure 7*a*. The corresponding clathrate configuration is achieved by rotating the molecules

Downloaded from rstb.royalsocietypublishing.org

about the hydrogen bond joining them, as shown in figure 7*b*. Since in the clathrate configuration hydrogen atoms are in eclipsed positions, this is likely to be a higher energy state. Also the clathrate configuration is not compatible with the ice-like configuration and therefore the hydration region of a hydrophobic residue must extend beyond one layer of water molecules. That the primary hydration environment of an alkyl group is indeed consistent with a clathrate cage geometry has been demonstrated by Hertz & Rädle (1973), and Stillinger has investigated the statistical mechanics of convex water cages surrounding apolar particles (Stillinger 1973).

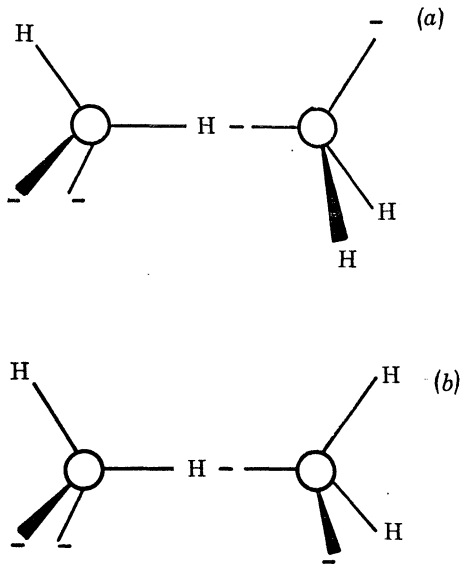


FIGURE 7. Mutual preferred orientations of two water molecules in (*a*) ice, and probably bulk water, and (*b*) a clathrate hydrate, and probably in a hydrophobic hydration sphere.

Of more immediate practical interest than the molecular configuration of the hydrophobic hydration shell is the consequence of this type of hydration – the hydrophobic interaction between alkyl residues in aqueous solution. This is commonly regarded as a partial reversal of the entropically unfavourable process of water cage formation (Tanford 1973): it is postulated that two or more alkyl groups can approach one another, share a common hydration sphere and some of the perturbed water is thus allowed to relax to its 'normal' state.

Quantitatively the interaction between two hydrated solutes is expressed in terms of $W(r, \Omega)$, or by suitably averaging or, more frequently, neglecting orientational effects, in terms of $W(r)$, as already described. The available theoretical treatments of the hydrophobic interaction are based on the assumption, explicitly or implicitly, that the attractive forces between two alkyl groups in water are stronger than they are in the vapour phase. Various estimates are available for the free energy of the hydrophobic interaction, ranging from -3 to -8.5 kJ per mol pair CH_2 groups. The point has been made that in conformational energy calculations of biopolymers the hydrophobic contribution to the free energy has probably always been underestimated, because the vacuum potential $U(r)$, rather than $W(r)$ has been used (Dashevsky & Sarkisov 1974).

We have recently re-examined the various available theoretical descriptions of the hydrophobic interaction and extended the calculations of Friedman & Krishnan (1973*a*). Using the lower alkanols as model hydrophobic species, these authors formulated $W(r)$ in terms of an

Downloaded from rstb.royalsocietypublishing.org

exponential core potential, repulsive at all values of r, and a hydration term $A(r)$ which arose from the destructive overlap of the two hydrophobic hydration spheres. Using the osmotic pressure second virial coefficient b (see equation (2)), they were able to evaluate $W(r)$ and hence $A(r)$. In our calculations we employed a $U(r)$ based on the sum of atom–atom potentials, averaged over 500 orientations. The results yielded gas phase second virial coefficients in good agreement with experimental values. Using the same simple model hydration sphere as that adopted by Friedman & Krishnan, we have been able to calculate $A(r)$ and thus to estimate A_{xx}, the hydrophobic free energy per mole of water excluded (Clark, Franks, Pedley & Reid 1977). The results are compared with the results of Friedman & Krishnan in table 6. The surprising feature is that A_{xx} is positive for all alcohols, indicating that the depth of the $W(r)$ potential well in aqueous solution is shallower than it is in the vapour phase. This is illus-

TABLE 6. GURNEY FREE ENERGY PARAMETERS, A_{xx} (J mol^{-1} WATER), CALCULATED BY FITTING EXPERIMENTAL OSMOTIC PRESSURE DATA

(T = 298 K)

	Friedman & Krishnan	Clark *et al.*
CH_3OH	−357	+141
C_2H_5OH	−428	+172
n-C_3H_7OH	−462	+228
n-C_4H_9OH	−500	+271
t-C_4H_9OH	−437	+292

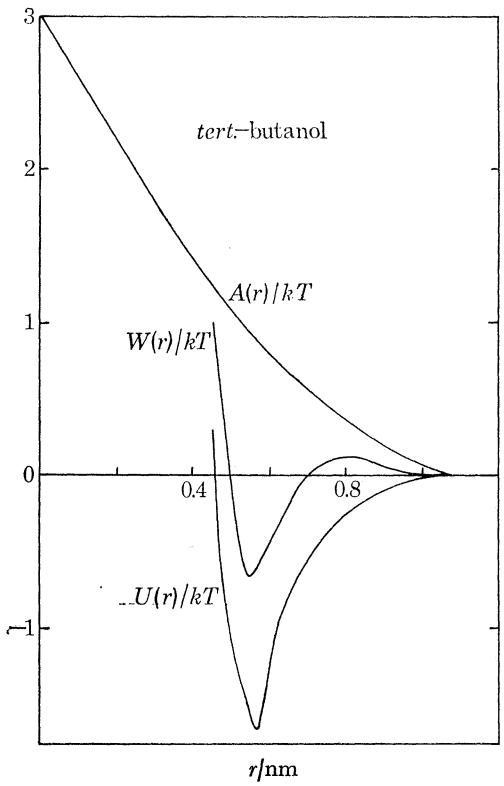


FIGURE 8. The vacuum potential $U(r)$, the hydration potential, $A(r)$ and the potential of average force, $W(r)$, describing the mutual interactions of two *tert.*-BuOH molecules in aqueous solution.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

trated for *tert.*-butanol in figure 8. The small peak near 0.8 nm is of particular interest because it suggests the intriguing possibility of a further potential well beyond $r = 0.8$ nm. Since the calculations were based on a one molecule thick (0.275 nm) spherical hydration shell, this possibility could not be further investigated. Such a long range interaction, if it could be established, would be able to account much better for the observed thermodynamic and dynamic properties of solutions of alkyl derivatives than does the conventional model of the hydrophobic contact interaction (Franks 1975). It would also be compatible with the observation that some polyamino acids form α-helices in aqueous solution, the stability of which depends on the number of CH_2 groups in the amino acid side chains. In such α-helical structures contact interactions by side chain groups are ruled out on steric grounds.

A recent computer simulation study also lends support to the concept of a longer range, water separated hydrophobic interaction (Rahman 1974). The experiment consisted of a molecular dynamic simulation of a system of 214 water molecules and two neon atoms. (The water molecules used in the simulation are in fact neon atoms with four peripheral point charges placed at the vertices of a regular tetrahedron.)

It was found that at equilibrium the two inert particles assumed a separation of $1.5\,\sigma$ ($= 0.42$ nm), where σ is the diameter of the particle, that the water molecules in the immediate vicinity of the inert particles were more strongly hydrogen-bonded to each other than they are in bulk water, and that they adopted a clathrate-like configuration in which two closed pentagonal loops could be identified.

In summary therefore, it seems that both the energetics and the spatial and orientational properties of hydrophobic hydration structures are due for a reappraisal before the concept of hydrophobic effects can be reliably incorporated into complex calculations such as those pertaining to protein stability (Franks & Eagland 1975).

The complex effects of hydrophobic association at higher concentrations have already been touched upon, see figure 6. The available evidence is consistent with the picture that at low concentrations (< 15 mol per cent) the alkyl groups act mainly by modifying the water–water interactions in a way that appears to increase the degree and strength of hydrogen bonding, probably by shifting the spatial and orientational distribution of water molecules. Certainly the net effect is a clustering of solute molecules which is detectable by small angle X-ray scattering. The data suggest a radius of gyration of 0.8 nm for the regions of molecular inhomogeneity, increasing with rising temperature (D. Atkinson, F. Franks & A. H. Clark, unpublished results). This type of behaviour suggests the onset of lower critical demixing which is indeed commonly observed in solutions of alkyl derivatives. The existence of thermolabile association structures is also consistent with the very large partial molal heat capacities of such solutions (Leduc & Desnoyers 1973). (It is important not to confuse these structures with micelles which possess dramatically lower heat capacities (Leduc & Desnoyers 1973).)

It is suggested therefore, that the chief effect of low concentrations of alkyl groups is the transformation of water into a different solvent, more highly organized on a molecular scale. At higher concentrations these unique properties disappear abruptly and the solvent mixture then behaves like a typical mixture of polar molecules. Such a picture can explain the observed solvent effects on the kinetics of reactions in which peculiar transition state solvation effects seem to play a crucial rôle. It is also compatible with the observed effects of mixed aqueous solvents on micellization of surfactants and on the conformational stability of proteins and some synthetic polymers (Brandts & Hunt 1967).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Complex hydration interactions – the effect of urea on aqueous solution behaviour

In most systems of practical interest the observed solvent effect depends on the particular experimental technique employed but is usually the resultant of a number of different individual processes such as those described in the previous sections. Thus, the sensitivity of most native biopolymer conformations to minor perturbations in the solvent medium shows how delicate must be the balance between the various solvation effects in which the different functional groups participate. However, it does not require the complexity of a protein, polysaccharide or polynucleotide to illustrate the existence of constructive or destructive solvation effects in aqueous solution.

Urea is known to promote remarkable changes in the properties of many aqueous solutions and for this reason it is commonly used as chaotropic agent. What is truly surprising, however, is the great diversity of views held on the mechanisms by which urea modifies hydrocarbon solubility, reaction rates, micelle formation, protein stability, etc. (Franks & Eagland 1975). It is therefore appropriate that this discussion of multicomponent hydration phenomena should be based on the remarkable effects produced by urea.

According to statistical thermodynamic principles, the properties of ternary systems are expressed in terms of virial expansions containing pair, triplet and higher order terms relating to interactions between like and unlike molecules. Thus, for example, the excess enthalpy, H^E of such a ternary system (water, solute x and solute y) can be expressed as

$$\begin{aligned} H^E(m_x, m_y) &= H(m_x, m_y) - H_w^0 - H_x^0 m_x - H_y^0 m_y \\ &= h_{xx} m_x^2 + 2h_{xy} m_x m_y + h_{yy} m_y^2 + h_{xxx} m_x^3 + 3h_{xxy} m_x^2 m_y + \ldots, \end{aligned} \tag{6}$$

where $H(m_x, m_y)$ is the enthalpy of that quantity of solution which contains 1 kg water, H_x^0 and H_y^0 are the limiting partial molal enthalpies, and m is the concentration in mol kg^{-1}.

The pair and triplet interaction coefficients h_{ij} and h_{ijj} can be obtained from a series of calorimetric determinations of the individual enthalpies of dilution of the two solutes and their enthalpy of mixing. A similar expression can be written for other thermodynamic quantities, e.g. ΔG^E, although this particular one is hard to determine experimentally for a ternary system, especially if both solutes are volatile. Unfortunately, equation (6) or others of a similar type provide no information about the hydration interactions of the two solute species. Furthermore, any changes in the hydration interactions which might occur when pairs of solute molecules interact will be included in the experimentally obtained value of h_{ij}. Equation (6) is therefore related to $W(r)$ (see equations (2) and (3)) rather than to $A(r)$.

Before examining the effect of urea on the aqueous solutions of other solutes, it is well to summarize the properties of binary water–urea mixtures. Both molecular species possess multiple hydrogen bonding sites, but whereas the hydrogen bonding between water molecules is essentially of a tetrahedral type, urea is a planar molecule and the way in which it can participate in hydrogen bonding with the solvent is not compatible with the hydrogen bonding pattern in bulk water. This suggests two possibilities – either urea forms preferential hydrogen bonds to other urea molecules (i.e. urea aggregates) or urea destroys the three-dimensional long range order characteristic of water. Both of these alternatives have in the past been advanced to account for the thermodynamic properties of aqueous urea solutions (Stokes 1967; Frank & Franks 1968). Using the free energy analogue of equation (6), g_{xx} and g_{xxx} can be

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

obtained from the osmotic pressure equation (1); the results, together with the recently determined enthalpy and entropy parameters are shown in table 7 (F. Franks, M. Pedley & D. S. Reid, unpublished results).

Table 7. Thermodynamic pair and triplet interaction parameters for urea in aqueous solution at 25 °C

	pair J mol^{-1} (mol/kg)$^{-1}$	triplet J mol^{-1} (mol/kg)$^{-2}$
gg_{xx}, g_{xxx}	−81	+8
h_{xx}, h_{xxx}	−351	+21
Ts_{xx}, Ts_{xxx}	−270	+13

It is apparent that molecular pairing is a favourable process because of the negative enthalpy, but that triplet formation is not. This is in direct contrast to the corresponding parameters for solutes which are subject to hydrophobic interactions, where the triplet term dominates, but this is due to a large positive entropy terms (Franks, Pedley & Reid 1976). It is therefore unlikely that aggregates of urea exist in aqueous solution. This conclusion is supported by the dynamic properties of urea and water molecules in such mixtures. The rotational diffusion rate of urea is quite incompatible with the existence of linear (or cyclic) aggregates. On the other hand urea enhances the rotational diffusion of water (Finer, Franks & Tait 1972). These and other results allow the conclusion to be drawn that urea destroys the particular intermolecular order characteristic of water, without at the same time promoting some other long range order. On the other hand urea molecules are extensively hydrogen bonded to other urea molecules and to water. The fact that h_{xx} is small suggests that the various possible types of hydrogen bonds have very similar energies so that they are statistically distributed. Urea has hence been termed a 'statistical water structure breaker' (Frank & Franks 1968).

Given that this is a realistic description of the urea–water system then it can be deduced that urea would interfere with those interactions which are critically dependent on the long range order in water. Of these the most important one is the hydrophobic interaction, and the above deduction is in agreement with experimental findings. Thus urea inhibits the aggregation of dyestuffs and the micellization of surfactants. It also solubilizes hydrocarbons and destabilizes most biopolymer tertiary structures. This latter phenomenon (at least in the case of proteins) has frequently been treated in terms of urea binding, e.g. to peptide bonds; but of course the fact that some type of observed behaviour can be fitted by a binding isotherm does not mean that such binding (with reasonable life times) actually takes place or that it necessarily leads to the destabilization of a given conformation (Franks & Eagland 1975).

As part of a study concerning the nature of the hydrophobic interaction we have determined h_{ij} and h_{ijj} in various ternary mixtures containing urea (species x). Components y were chosen such that their only hydrogen bonding sites were lone electron pairs – i.e. acetone, methylethylketone (MEK) and tetrahydrofuran (THF). It has already been pointed out that any change produced by x or y on the molecular nature of the solvent medium cannot be explicitly evaluated and will automatically be included in the solute interaction parameters. However, ever with this limitation we found h_{xy} (J mol^{-1} (mol/kg)$^{-1}$) values of +50, +121 and +295 for acetone, MEK and THF respectively, compared to $h_{xx} = -351$ (see table 7). The corresponding h_{yy} values (characteristic of hydrophobic interactions) were +770, +1188 and +1182. This shows that urea does not interact preferentially with the other solute and therefore

Downloaded from rstb.royalsocietypublishing.org

may well exert its action by altering the nature of the solvent medium. Unfortunately calorimetric measurements alone provide only half the answer; ideally g_{xy} should also be determined, but this presents severe experimental problems. In the meantime it is encouraging that a similar conclusion has been reached from measurements of the free energy of transfer of urea from water to water–THF mixtures (Treiner & Tzias 1975).

The picture is, of course, far from complete; for obvious reasons there exist few free energy data on ternary systems, and the claim that urea binds to peptide bonds needs to be substantiated by thermodynamic measurements which allow the application of equation (6). Another approach to the problem would be the dynamic one, r.g. by n.m.r. intermolecular relaxation rates of hydrophobic probes in the presence of (deuterated) urea.

More evidence for interference between different types of hydration structures has been obtained from a calorimetric study similar to that described above, by using pairs of molecules with identical numbers of carbon atoms, of which one is hydrophobic and the other hydrophilic, e.g. $C_2H_5OH/C_2H_4(OH)_2$, $C_3H_7OH/C_3H_6(OH)_3$, etc. In each case it was found that $|h_{xy}| < |h_{xx}|, |h_{yy}|$, suggesting an unfavourable hydration sphere overlap. Surprisingly, for the polyols h_{yy} is positive and increases with molecular size (F. Franks, M. Pedley & D. S. Reid, unpublished results).

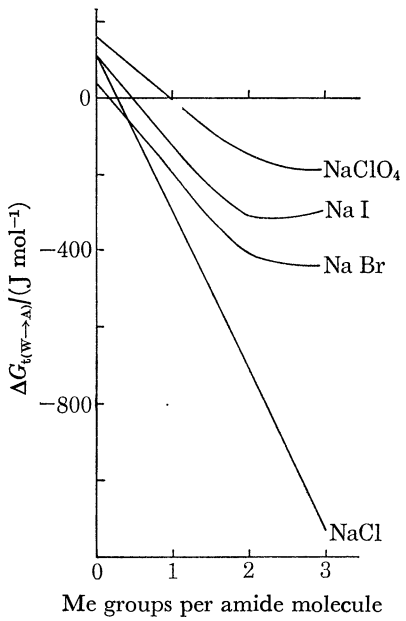


FIGURE 9. Free energies of transfer (water to aqueous solutions of amides) of sodium salts as function of the degree of methyl substitution. The origin refers to formamide.

Reference has already been made to the specific ion binding to amides, the degree of specificity depending on the degree of alkyl substitution in the molecule $R.CONR_2$, where R = H or Me (Hamabata & von Hippel 1973). This is illustrated in figure 9 for NaCl, NaBr and Nal and $NaClO_4$. The system water–ion–substituted amide provide a good example of the complex and subtle interactions between the three different types of hydration spheres described in detail above: the ion hydration sphere, the orientation specific hydrogen bonding between water and hydrogen bonding sites on the amide group, and the clathrate-like cavity induced by the methyl group. It is not known how this latter hydration sphere can give rise to specific inter-

Downloaded from rstb.royalsocietypublishing.org

actions between ions and the amide dipole and it is likely that this problem will not be solved until the pecularities of the various types of hydration interactions and their influence on solute conformation are somewhat better understood than is the case at present.

I wish to acknowledge a debt of gratitude to my colleagues M. D. Barratt, A. H. Clark, P. J. Lillford, D. S. Reid, A. Suggett, M. J. Tait and D. Thom for many helpful discussions and for their major contributions to any improvement in the understanding of hydration phenomena that may have been achieved as a result of our studies.

References (Franks)

Angyal, S. J. 1969 The composition and conformation of sugars in solution. *Angew. Chem. Int. Ed.* **8**, 157–166.

Arshadi, M., Tamdagni, R. & Kebarle, P. 1970 Hydration of the halide negative ions in the gas phase. II. *J. phys. Chem.* **74**, 1475–1482.

Barry, C. D., North, A. C. T., Glasel, J. A., Williams, R. J. P. & Xavier, A. V. 1971 Quantitative determination of mononucleotide conformations in solution using lanthanide ion shift and broadening n.m.r. probes. *Nature, Lond.* **232**, 236–245.

Ben-Naim, A. 1972 *Water – a comprehensive treatise* (ed. F. Franks), vol. 1, p. 417. New York: Plenum Press.

Ben-Naim, A. 1974 *Water and aqueous solutions – introduction to a molecular theory*. New York: Plenum Press.

Bennetto, H. P. & Feakins, D. 1968 *Hydrogen bonded solvent systems* (eds A. K. Covington & P. Jones), pp. 235–242. London: Taylor & Francis Ltd.

Bernal, J. D. & Fowler, R. H. 1933 A theory of water and ionic solution, with particular reference to hydrogen and hydroxyl ions. *J. chem. phys.* **1**, 515–548.

Brandts, J. F. & Hunt, L. 1967 The thermodynamics of protein denaturation. III. The denaturation of ribonuclease in water and in aqueous urea and aqueous ethanol mixtures. *J. Am. chem. Soc.* **89**, 4826–4838.

Clark, A. H., Franks, F., Pedley, M. D. & Reid, D. S. 1977 Solute interactions in dilute solutions. 2. A statistical mechanical study of the hydrophobic interaction *J. Chem. Soc. Faraday I* **73**, in press.

Dashevsky, V. G. & Sarkisov, G. N. 1974 The solvation and hydrophobic interaction of non-polar molecules in water in the approximation of interatomic potentials: The Monte Carlo method. *Molec. Phys.* **27**, 1271–1290.

Engbersen, J. F. J. & Engberts, J. B. F. N. 1975 Water structure and its kinetic effects on the neutral hydrolysis of two acyl activated esters. *J. Am. chem. Soc.* **97**, 1653–1668.

Finer, E. G., Franks, F. & Tait, M. J. 1972 Nuclear magnetic resonance studies of aqueous urea solution. *J. Am. chem. Soc.* **94**, 4424–4429.

Frank, H. S. & Evans, M. W. 1945 Free volume and entropy in condensed systems. III. Entropy in binary liquid mixtures: partial molal entropy in dilute solutions; structure and thermodynamics in aqueous electrolytes. *J. chem. phys.* **13**, 507–532.

Frank, H. S. & Franks, F. 1968 Structural approach to the solvent power of water for hydrocarbons; urea as a structure breaker. *J. chem. Phys.* **48**, 4746–4757.

Franks, F. 1975 *Water – a comprehensive treatise* (ed. F. Franks), vol. 4, ch. 1, pp. 1–94. New York: Plenum Press.

Franks, F. & Eagland, D. 1975 The role of solvent interactions in protein conformation. *Crit. Rev. Biochem.* **3**, 165–217.

Franks, F., Pedley, M. & Reid, D. S. 1976 Solute interactions in dilute aqueous solutions. 1. A microcalorimetric study of the hydrophobic interaction. *J. Chem. Soc. Faraday I* **72**, 359–367.

Franks, F., Ravenhill, J. R. & Reid, D. S. 1972 Thermodynamic studies of dilute aqueous solutions of cyclic ethers and simple carbohydrates. *J. Solution Chem.* **1**, 3–16.

Franks, F., Reid, D. S. & Suggett, A. 1973 Conformation and hydration of sugars and related compounds in dilute aqueous solution. *J. Solution Chem.* **2**, 99–118.

Friedman, H. L. & Krishnan, C. V. 1973 *Water – a comprehensive treatise* (ed. F. Franks), vol. 3, chap. 1, pp. 1–118. New York: Plenum Press.

Friedman, H. L. & Krishnan, C. V. 1973*a* Studies of hydrophobic bonding in aqueous alcohols: enthalpy measurements and model calculations. *J. Solution Chem.* **2**, 119–138.

Friedman, H. L. & Krishnan, C. V. 1973*b* *Water – a comprehensive treatise* (ed. F. Franks), vol. 3, p. 1. New York: Plenum Press.

Grimsrud, E. P. & Kebarle, P. 1973 Gas phase ion equilibria studies of the solvation of the hydrogen ion by methanol, dimethyl ether and water. Effect of hydrogen bonding. *J. Am. chem. Soc.* **95**, 7939–7943.

Hamabata, A. & von Hippel, P. H. 1973 Model studies on the effects of neutral salts on the conformational stability of biological macromolecules. II. Effects of vicinal hydrophobic groups on the specificity of binding of ions to amide groups. *Biochemistry* **12**, 1264–1271.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

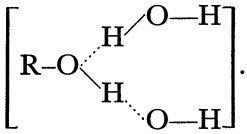
Downloaded from rstb.royalsocietypublishing.org

Heinzinger, K. & Vogel, P. C. 1974 A molecular dynamics study of aqueous solutions. 1. *Z. Naturf.* **29**a, 1164–1171.
Hertz, H. G. 1964 The structure effect of nonpolar particles in aqueous solution. *Ber. Bunsenges. Phys. Chem.* **68**, 907–931.
Hertz, H. G. & Rädle, C. 1973 The orientation of the water molecules in the hydration sphere of F^- and in the hydrophobic hydration sphere. *Ber. Bunsenges. Phys. Chem.* **77**, 521–531.
Hofmeister, F. 1888 Zur Lehre von der Wirkung der Salze. *Arch. Exp. Pathol. Pharmakol.* **24**, 247.
Kabayama, M. A., Patterson, D. & Piche, L. 1958 The thermodynamics of mutarotation of some sugars. 1. Measurement of the heat of mutarotation by microcalorimetry. *Can. J. Chem.* **36**, 557–562.
Kay, R. L. 1973 *Water – a comprehensive treatise* (ed. F. Franks), vol. 3, ch. 4, pp. 173–209. New York: Plenum Press.
Kuntz, I. D., Brassfield, T. S., Law, G. & Purcell, G. 1969 Hydration of macromolecules. *Science, N.Y.* **163**, 1329–1331.
Leduc, P. A. & Desnoyers, J. E. 1973 Apparent molal heat capacities and volumes of tetrabutylammonium carboxylates and related solutes in water at 25 °C. *Can. J. Chem.* **51**, 2993–2998.
Levine, B. A. & Williams, R. J. P. 1975 The determination of the conformations of small molecules in solution by means of paramagnetic shift and relaxation perturbations of n.m.r. spectra. *Proc. R. Soc. Lond.* A **345**, 5–22.
Livingstone, G. 1974 Ph.D. thesis, University of Bradford.
Livingstone, G. & Franks, F. 1977 The effects of aqueous solvent structure on the mutarotation kinetics of glucose. *J. Soln. Chem.* **6**, in press.
Luck, W. A. P. 1973 *Water – a comprehensive treatise* (ed. F. Franks), vol. 2, ch. 4, pp. 235–321. New York: Plenum Press.
Poland, D. & Scheraga, H. A. 1970 *Theory of helix-coil transitions in biopolymers*. New York: Academic Press.
Pullman, A. & Pullman, B. 1975 New paths in the molecular orbital approach to solvation of biological molecules. *Q. Rev. Biophys.* **7**, 505–566.
Pullman, B. & Port, G. N. J. 1974 Molecular orbital study of the conformation of histamine; the isolated molecule and solvent effect. *Molec. Pharmacol.* **10**, 360–372.
Rahman, A. 1974 *Report on workshop on ionic liquids*, Centre Européen de calcul atomique et moléculaire, p. 263.
Rees, D. A. 1970 Conformational analysis of polysaccharides. Part V. The characterization of linkage conformations (chain conformations) by optical rotation at a single wavelength. Evidence of distortion of cyclohexaamylose in aqueous solutions. Optical rotation and the amylose conformation. *J. chem. Soc.* B 877–884.
Rees, D. A. & Smith, P. J. C. 1975 Polysaccharide conformation. IX. Monte Carlo calculations of conformational energies for disaccharides and comparison with experiment. *J. chem. Soc. Perkin II*, 836–840.
Reine, A., Hveding, J. A., Kjølberg, O. & Westbye, O. 1974 The mutarotation of D-glucose and its dependence on solvent. III. The existence of α and β-D-glucofuranose in *N,N*-dimethylformamide. *Acta Chem. Scand.* **B28**, 690–691.
Stillinger, F. H. 1973 Structure in aqueous solutions of nonpolar solvents from the standpoint of scalded-particle theory. *J. Solution Chem.* **2**, 141–158.
Stokes, R. H. 1967 Thermodynamics of aqueous urea solutions. *Aust. J. Chem.* **20**, 2087–2100.
Stokes, R. H. & Robinson, R. A. 1966 Interactions in aqueous nonelectrolyte solutions. 1. Solute–solvent equilibria. *J. phys. Chem.* **70**, 2126–2131.
Suggett, A. 1975 *Water – a comprehensive treatise* (ed. F. Franks), vol. 4, ch. 6, pp. 519–567. New York: Plenum Press.
Suggett, A. 1976 Molecular motions and interactions in aqueous carbohydrate solutions. 3. A combined n.m.r. and dielectric relaxation strategy. *J. Solution Chem.* **5**, 33–46.
Suggett, A. & Clark, A. H. 1976 Molecular motions and interactions in aqueous carbohydrate solutions. 1. Dielectric relaxation studies. *J. Solution Chem.* **5**, 1–15.
Tait, M. J., Ablett, S. & Franks, F. 1972 *Water structure at the water–polymer interface* (ed. H. H. G. Jellinek), p. 29. New York: Plenum Press.
Tait, M. J., Suggett, A., Franks, F., Ablett, S. & Quickenden, P. A. 1972 Hydration of monosaccharide: a study by dielectric and nuclear magnetic relaxation. *J. Solution Chem.* **1**, 131–151.
Tanford, C. 1973 *The hydrophobic interaction*. New York: John Wiley & Sons.
Thom, D. 1973 Ph.D. thesis, University of Edinburgh.
Treiner, C. & Tzias, P. 1975 Thermodynamic transfer functions for urea and thiourea from water to water–tetrahydrofuran mixtures from precise vapour pressure measurements. *J. Solution Chem.* **4**, 471–483.
Vogel, P. C. & Heinzinger, K. 1975 A molecular dynamics study of aqueous solutions III. *Z. Naturf.* **30**a, 789–796.
von Hippel, P. H. & Schleich, T. 1969 Ion effects on the solution structure of biological macromolecules. *Acc. Chem. Res.* **2**, 257–265.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Discussion

M. C. R. Symons. (*Department of Chemistry, Leicester University*). My colleagues, Drs R. Naftalin, J. Harvey & I have found that if aqueous sugar solutions are cooled below *ca.* 0 °C separate proton resonances from the OH protons are resolved on the low-field side of the water resonance. Above this temperature exchange with water protons is fast and the resonance is a single averaged line. The low-field shift implies strong hydrogen-bonding and conclusively requires the presence of at least two water molecules per O–H group  . Hence the 'solvation number' for, say, glucose must be $\geqslant 10$. The water resonance is shifted to low-fields relation to pure water, indicating strong hydrogen bonding to the sugar molecules. In reconciling these results with those reported by Professor Franks I imagine one must particularly differentiate between 'static' and 'dynamic' definitions of solvation numbers.

F. Franks. In reply to Professor Symons' comment I agree that there are indications that apart from the relatively long-lived primary hydration shells, the sugars also affect other water molecules. This manifests itself for instance in the slightly lengthened correlation times of what we refer to as 'bulk water'. This effect has been discussed in terms of an exchange-averaged value for the bulk water and a secondary hydration layer, but it is well possible that an explanation based on two types of hydration water could be a plausible alternative (Suggett 1976).

However, the evaluation of hydration numbers from chemical shift measurements appears to me not quite as well founded as the procedure based on rotational correlation times. One further point which relates particularly to experiments at low temperatures, is that in 'frozen' solutions at $\leqslant -20$ °C, the limiting sugar:water ratio, i.e. where no more ice separates from the mixture, agrees well with the hydration numbers obtained from n.m.r. and dielectric relaxation measurements at $+5$ °C as described in my lecture.

Finally the type of hydration proposed by Professor Symons would not be compatible with the Kabayama & Patterson configurational model of equatorial group hydration. On the other hand we have found this model to be particularly useful in the interpretation of differences between the hydration behaviour of different sugars.

Reference

Suggett, A. 1976 *J. Solution Chem.* **5**, 33.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES