Solvent Structure and Perturbations in Solutions of Chemical and Biological Importance

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1 Background

Many common chemical processes occur in liquid media, and the usual medium is aqueous. Moreover, much of biology also takes place in an aqueous environment. While we may often assume that aqueous solution is a perfectly normal environment for chemical processes, it is surprising perhaps that we often consider water as an inert solvent. Far from being a non-interacting medium, the hydrogen bond through which water molecules interact with each other causes strong interactions with many – or probably most – of the solutes which dissolve to a significant degree in water. Looked at slightly differently, in order to dissolve in a solvent, a solute must interact strongly with the molecules of the solvent.

In biological processes, much work over the past twenty years or so has focused on the possible relevance of the interactions with solvent to fundamental biomolecular interactions.[1] For example, the stability of proteins and nucleic acids relates in some way to the interaction of the chemical constituents of these molecules with their normal aqueous environment, as do enzyme–substrate and drug–DNA interactions. In addition to the specific hydrogen bonding interactions between polar and charged molecular groups on the biomolecule and the polar water molecules of the solvent, much attention has focused also on the less specific so-called 'hydrophobic' interaction that has, since the seminal paper of Kauzmann[2] in 1959, been often invoked as the major driving force to protein folding and stability. Although a complete understanding of the nature of the hydrophobic interaction still eludes us, a simple explanation asserts that, around a non-polar group such as a methyl group, the surrounding water arrangement is somehow 'more ordered' than 'normal bulk' water at the same temperature. There is thus a reduction in entropy of the water as a result of this ordering, whose structure is often envisaged as relating to that of bulk pure water at a lower temperature. If we now bring two non-polar groups in aqueous solution together, some of this 'ordered' or 'restricted' water will be expelled to the bulk, with the system thus gaining entropy from the increase in solvent disorder. It is this entropic contribution that is conventionally argued to be the underlying mechanism of the hydrophobic interaction.

Perhaps largely because of these ideas of water ordering, the solution chemistry literature over several decades has often developed ideas of 'structure making' and 'structure breaking' effects of various solutes, and such ideas have been used to try to rationalize the way in which denaturant or protectant molecules, when added to, *e.g.* a solution of a folded, active enzyme, either break down or enhance the water structure surrounding various groups on the protein's molecular surface, and hence, by modifying the solvent interactions, lead to reduced or enhanced macromolecule stability.

Until recently, these concepts of structure making and structure breaking have been difficult to quantify. For a start, we need to have a reference structure, *i.e.* a knowledge of the structure of normal, bulk water, and our knowledge of this structure is imperfect. Moreover, these concepts have generally arisen in interpreting the results of measurements that are indirect, in that they require a model before they can be interpreted in structural terms. The conclusions therefore depend implicitly on the interpretive model used.

Radiation diffraction techniques have, however, advanced considerably over the past twenty years, to the point where we are now able to perform direct measurements of water structure – and its perturbation by added molecules – close to appropriate (polar, charged, and non-polar) solutes. These advances have been made possible through the development of both neutron sources and instrumentation. In particular, the advent of the spallation neutron source,[3] which produces intense pulses of neutrons by impacting high energy protons from an accelerator onto a heavy metal target, has been particularly important. With appropriate instrumentation it has enabled us to overcome some of the technical problems of working on aqueous solution

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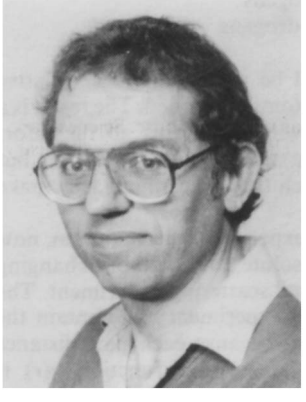
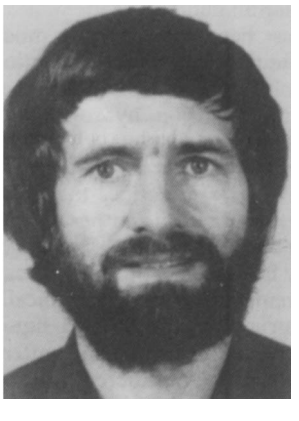


John Finney graduated in Natural Sciences from Jesus College, Cambridge, in 1964. After subsequently gaining a Postgraduate Certificate in Education at Leicester University, he joined J. D. Bernal as a research assistant at Birkbeck College London, where in 1968 he was awarded a Ph.D. in crystallography for his work on liquids. He was subsequently appointed lecturer in crystallography in the Birkbeck department, being promoted to Reader in 1977 and to a personal chair in 1986. In 1988, he moved to the new ISIS pulsed neutron source at the SERC Rutherford Appleton Laboratory as Head of Neutron Science, and subsequently became Chief Scientist. In November 1993, he was appointed to the Quain Chair of Physics at University College London. He is a fellow of both the Royal Society of Chemistry and the Institute of Physics.



systems using conventional fission reactor-based neutron sources Although these techniques are still new, we now have enough experience to be confident of their power in probing for the first time the way in which water – and other hydrogen-containing solvents – is or is not perturbed by solutes of a variety of chemical types We can now begin to see directly how the solvent structure is altered by solutes, and thus test the validity of the conventional ideas of hydrophilic and hydrophobic hydration, and perhaps lay the groundwork for a real understanding of the role of water in chemical and biomolecular processes

In what follows, we outline the techniques that are being developed – mainly at the UK's pulsed spallation neutron source ISIS situated at the Rutherford Appleton Laboratory in Oxfordshire – and give some examples of recent work that is making major advances in our understanding of water itself, and raising problems for the simple conventional ideas of structure making and structure breaking We also take a brief speculative look into the future, to see how the techniques may develop further in helping us understand what actually goes on in the chemistry of aqueous and other solutions

2 Neutron Scattering from Solutions

The neutron is an essential probe for studying the structures of aqueous systems First, unlike *X*-rays, neutrons are scattered strongly by both hydrogen and its deuterium isotope Secondly, the neutron scattering power of an atom is isotope-specific, rather than determined by the chemical species This latter property allows us to perform parallel experiments on systems which, though chemically similar, produce a different response from the neutron scattering probe By making judicious use of this 'isotope substitution' technique first developed in application to electrolyte solutions by Enderby and Neilson,[4] we can obtain much greater detail in liquid state structural studies than is possible by any other technique

Rather than describe the technical details of this technique, which can be found elsewhere,[4–6] we merely indicate here the kind of information that can be obtained Considering first the simplest case of a one-component liquid, we can measure the neutron scattering intensity I as a function of scattering vector Q (relating to scattering angle 2θ and wavelength λ, and defined as $Q = \frac{4\pi}{\lambda} \sin\theta$) After appropriate corrections, Fourier transforming the resulting 'structure factor' results in the *pair correlation function* $g(r)$ which describes statistically the probability of finding an atom at a distance r from any other atom A typical pair correlation function is shown in Figure 1, together with its relation to a model two-dimensional liquid As can be seen, in broad terms the first peak gives information on the distribution of nearest neighbours, ('short-range order'), with the second peak telling us about the average positional arrangements further out (often called the 'intermediate-range order') Peak positions can be related to average distances and angles between molecules, while peak areas tell us the number of neighbours at a particular distance If the first peak is relatively sharp, its area gives us a first neighbour 'coordination number'

The aqueous systems of interest here, however, are much more complex than this idealized liquid Even for just a solution of *e g* methanol in water, our solvent contains two kinds of atoms, while our solute is made up of carbon, hydrogen, and oxygen For a simpler two-component liquid system AB, we can describe the liquid mixture in terms of three *partial* pair correlations, $g_{AA}(r)$, $g_{AB}(r)$, $g_{BB}(r)$ where $g_{AA}(r)$ describes the probability of finding an A atom at a distance r from another A atom and so on Now, if we can change the neutron scattering power of, for example, component A by isotope substitution, we can make neutron scattering measurements on both (chemically similar) liquids Although the structures of the two liquid samples are essentially the same, the neutron scattering pattern is different, and this difference is caused by the different neutron scattering powers in the two cases of the atom which has been

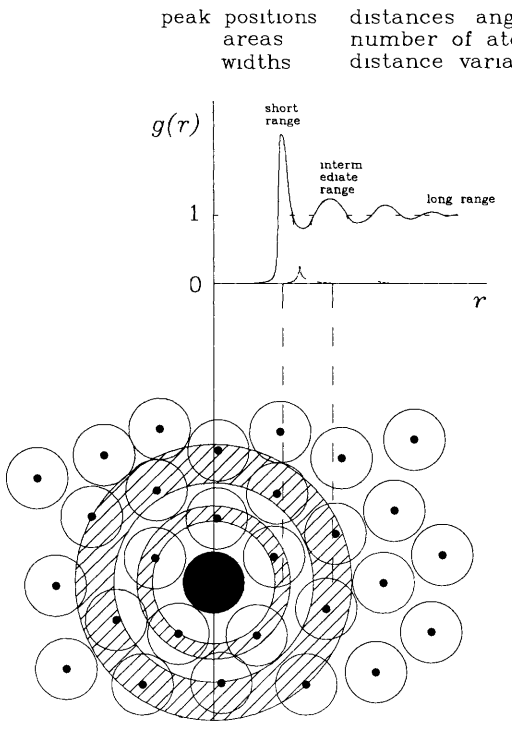


Figure 1 A two-dimensional liquid and its corresponding pair correlation function, showing the relation between the liquid structure and its pair correlation function description

isotope substituted Taking the difference of these scattering patterns and then performing a Fourier transform results in a pair correlation function, *but one which is centred on the A atom In essence, by using isotope substitution on a given atom, we can in effect 'sit on the substituted atom and survey our environment from the vantage point of that atom* By performing a further substitution of the B atom, we can discriminate also the identity of the neighbouring atoms

We illustrate what we are doing in a series of isotope substitution neutron diffraction experiments by referring to Figure 2 Figure 2a is a schematic two-dimensional representation of an aqueous solution, with the solute molecule shaded The relative sizes of the atoms depicted are such as to emphasize the hydrogen of the water, and should not be seen as being physically realistic If we take a single neutron scattering experiment, we obtain the *total* pair correlation function $g(r)$ which is the distribution of all the dashed interatomic pair distances in Figure 2b Thus, our measured total pair correlation function contains all the pair distances between

- solute atoms and water oxygens
- solute atoms and water hydrogens
- solute atoms and other solute atoms
- water oxygens and water oxygens
- water oxygens and water hydrogens
- water hydrogens and water hydrogens

Each of these contributions will be weighted by the relative scattering powers of each of the atoms concerned The result is a total pair correlation function which contains all the information we need to understand the structure of this solution, but unfortunately 'scrambled' so much that we cannot easily make use of it

After performing a scattering experiment on a solution, now let us change the isotope of the solute, and, without changing anything else, perform an identical scattering experiment The data we obtain from this second experiment will contain the same information as before, but, because each pair distance contribution to the total pair correlation function $g(r)$ is weighted by the scattering powers of the atoms, *those pair*

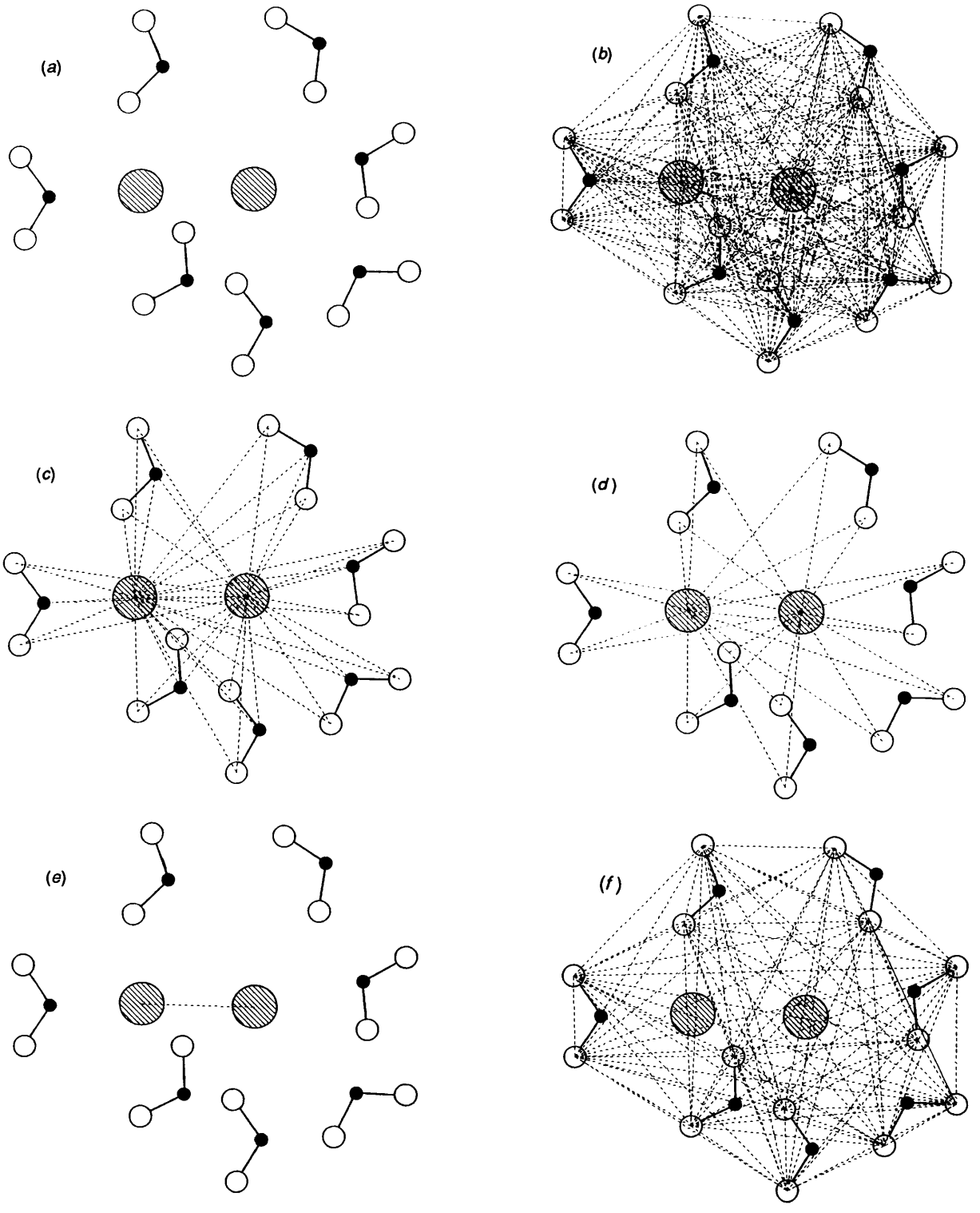


Figure 2(a—f) A schematic two-dimensional aqueous solution (a), showing the pair distances obtained in particular kinds of neutron diffraction experiments. The total pair correlation function is the distribution of *all* pair distances shown in (b), each weighted by the scattering lengths of the atoms involved. A difference experiment in which the solute isotope is changed is equivalent to 'sitting on' the solute atom, giving the pair distances indicated in (c), while changing also the hydrogen isotope on the water molecules means that, sitting on the solute, we see only the solute–water hydrogen distances in (d). A double substitution on the solute gives the solute–solute distances in (e), while a similar experiment changing the hydrogen isotope allows us to access the solvent HH, OH, and OO pair distances, of which the hydrogen–hydrogen distances are shown in (f).

distances involving the solute atom will be weighted differently in the total. Thus, if we now subtract the two sets of data, identical terms (*i.e.* those involving atoms which have *not* been substituted with a different isotope) will cancel out, and we will be left with only those pair distances that involve the solute. This situation is illustrated in Figure 2c; here we essentially 'sit on' the solute atom and look only at the surrounding atoms. We can thus measure directly the hydration of the solute atom or molecule.

We can in fact go further. If we want to separate out the solvent-oxygen from the solvent-hydrogen atoms, we can perform another series of experiments in which the hydrogen

isotope is changed to deuterium (in fact, for technical reasons, we normally use deuterium and then dilute with H_2O to change the scattering power of the hydrogen). The result of this can give us separately the distribution of distances from solute to water-hydrogen and water-oxygen separately – see Figure 2d. Thus we can obtain information on the *orientations* of water molecules in our hydration shell.

We can go even further with isotope substitutions, and obtain two particularly important pieces of structural information. First, we can change the solute isotope *twice* to yield the solute–solute distance distribution alone (Figure 2e). This gives us important information on solute aggregation. Secondly, we can change just the hydrogen isotope twice to obtain the hydrogen–hydrogen distance distributions (*e.g.* see Figure 2f). This gives us information on the water structure *in the presence of the solute*. It is this information which, when compared with the same information from pure water, that tells us how water structure is, or is not, perturbed close to a solute molecule. Hence we can test directly conventional ideas of structure making or breaking. Other combinations of substitutions can, in suitable cases, yield the oxygen–hydrogen and hydrogen–hydrogen distance distributions.

3 The Structure of Bulk Water

As we are interested to probe how water structure is perturbed by adding solutes of various types, it is essential that we establish first the structure of the reference liquid, bulk water. Using H/D substitution, a representative set of data is given in Figure 3. It is worthwhile to spend a little time understanding what these partial pair correlations mean.

Focusing first on the oxygen–oxygen pair correlation function $g_{OO}(r)$ of Figure 3a, the first peak at about 2.85 Å gives us the first neighbour oxygen–oxygen distance, and the area under this peak being about 5 tells us that there are on average five molecules out to 3.5 Å. This is slightly larger than the four that would be expected if each water molecule accepted two hydrogen bonds from neighbours and donated two, as in the simple random tetrahedral network model. Clearly, this coordination number depends critically upon the upper cut-off distance used in performing the integration under the peak and it may well be that some non-hydrogen bonded molecules do approach to within the 3.5 Å distance used here.

Moving now to the second broad peak centred at about 4.5 Å, we see that this corresponds to an O–O–O angle of about 110°. This is close to the tetrahedral angle, implying that the local water molecule geometry is on the average tetrahedral, though the spread in the second neighbour peak shows there is considerable variation around this average.

Figure 4a is a sketch of the local geometry consistent with Figure 3a, and we can use similar sketches (Figure 4b and 4c) to interpret the oxygen–hydrogen and hydrogen–hydrogen pair correlation functions of Figure 3b and 3c. Turning first to the oxygen–hydrogen $g_{OH}(r)$ of Figure 3b, we see a very strong peak at about 1 Å. This corresponds to the intramolecular OH distance, and the fact that it is indeed found to be at the known O–H distance, and the area under it indicates two hydrogen atoms, tells us our experiment is reporting correct results. Thus, we have in effect an internal calibration in the molecular structure: if our results give this structure correctly, then we can be reasonably confident that the data are good. [Alternatively, if the molecular structure of a molecule under particular solvent conditions is not known, we can use these isotope-substitution techniques to determine molecular structure in solution. However, we will not here pursue this particular line of development further.]

The second peak centred on about 1.85 Å gives us the first intermolecular OH distance – the hydrogen-bonded near neighbour distance (see Figure 4b). The third peak centred on about 3.25 Å refers to non-hydrogen-bonded oxygen–hydrogen distances on neighbouring molecules. Again as indicated in Figure 4b, there are several such pair-distances which may not be

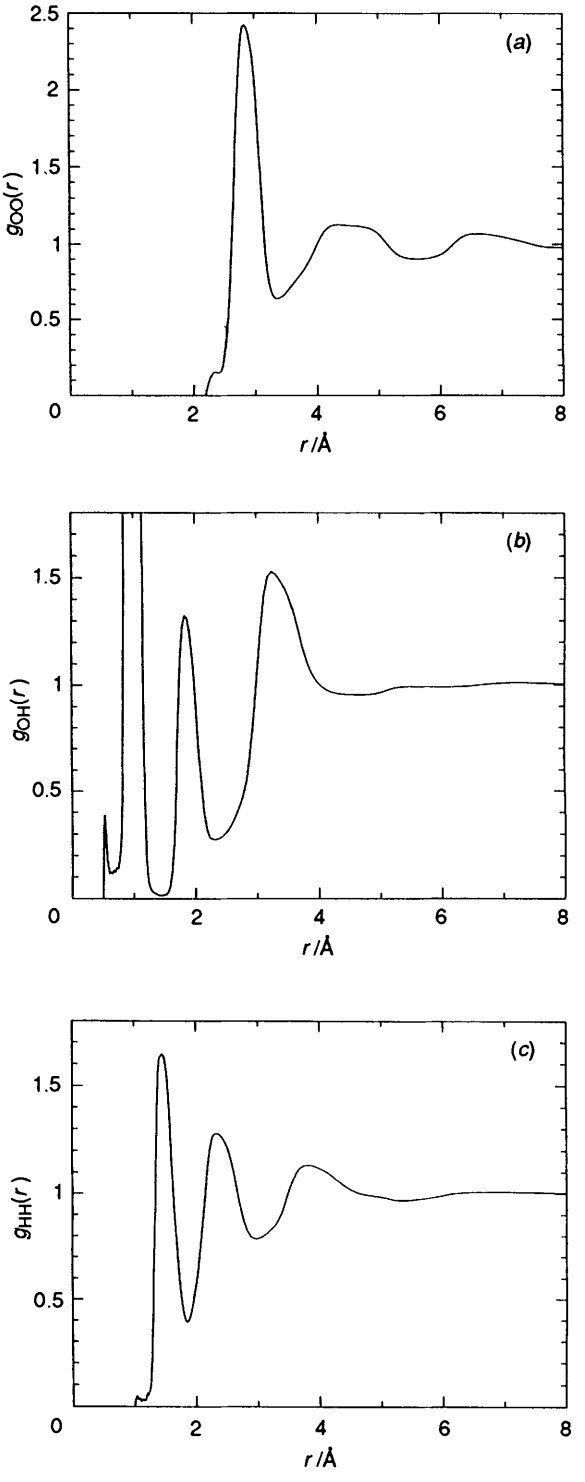


Figure 3 Partial pair correlation functions for water at room temperature; (a) oxygen–oxygen; (b) oxygen–hydrogen; (c) hydrogen–hydrogen. The significance of the various peaks is discussed in the text and illustrated in Figure 4.

equivalent, which may account for the asymmetry of the third peak.

Finally, we turn to the hydrogen–hydrogen $g_{HH}(r)$ pair correlation of Figure 3c. As with the $g_{OH}(r)$, the first peak is at the intramolecular H···H pair-distance of 1.55 Å, and again confirms the molecular geometry of the water molecule. The second peak at just above 2.4 Å refers to the closest HH distances between hydrogen-bonded neighbours, while the third – broad and asymmetric – peak at around 3.7—3.8 Å refers to the more distant HH distances on neighbouring water molecules. These distances are indicated in Figure 4c.

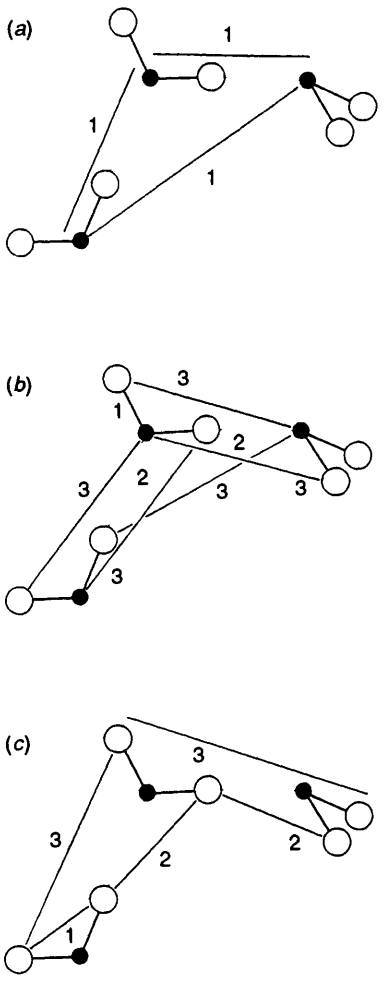


Figure 4 The local geometry in liquid water corresponding to the data of Figure 3 The pair distances relating to the various peaks are indicated, numbered upwards from the lowest r peak

Thus, these partial pair correlation functions give us very detailed information on the local geometry of water molecules in the liquid The $g_{OO}(r)$ of Figure 3a gives what we might call the intermolecular correlation function, as it indicates oxygen–oxygen distances, it can also be obtained from X-ray data,[7] as the hydrogen atom scatters X-rays only weakly The $g_{OH}(r)$ and $g_{HH}(r)$ functions are particularly interesting in that they give us *orientational* information on the water structure This ability will be seen to be particularly important in solution studies, some of which will be described below

Before passing on to these, however, we might comment on what happens to these correlation functions – and hence the water structure – as temperature is raised or lowered Recalling the discussion in the introduction concerning increasing or decreasing structural 'order', we expect that this 'order' will increase as temperature falls, and decrease as temperature rises We might expect this change in 'order' to be reflected in changes in the sharpness of the various peaks in the correlation functions For example, we might expect that reducing the temperature would reduce the spread of the distances and angles in Figure 4, and that this reduction will be reported through a sharpening of the relevant peaks in the appropriate partial pair correlation functions That this is to be expected is supported by a number of computer simulations of water at different temperatures Although neutron experiments as a function of temperature are at an early stage, they do in general terms support this sharpening as temperature is reduced, with broadening as temperature increases [6] There may also be associated small decreases or increases respectively in the peak positions In what follows, we shall look for peak sharpening to indicate enhanced ordering and *vice versa*

4 Aqueous Solutions of Polar Molecules

Some of the earliest isotope-substitution work on polar molecule solutions was performed on urea using nitrogen isotope-substitution to explore the solute–water interactions,[8–10] and subsequently on a series of amides up to and including *N*-methyl acetamide to explore the solvent interactions with the peptide group Figure 5 shows the nitrogen-centred pair correlation function for a 2 molal aqueous urea solution, which reports what is seen from the vantage point of the two (equivalent) nitrogen atoms As in the water case discussed above, the molecular structure is quantitatively reproduced by the peaks at low r, again verifying the validity of the data Further out, the two broad peaks between 2 5 and 4 0 Å relate mainly to nitrogen–water (both hydrogen and oxygen atoms) Further experiments replacing some of the exchangeable deuterium atoms with hydrogen allowed some conclusions to be drawn concerning the relative locations of the oxygen and hydrogen atoms of the water molecules

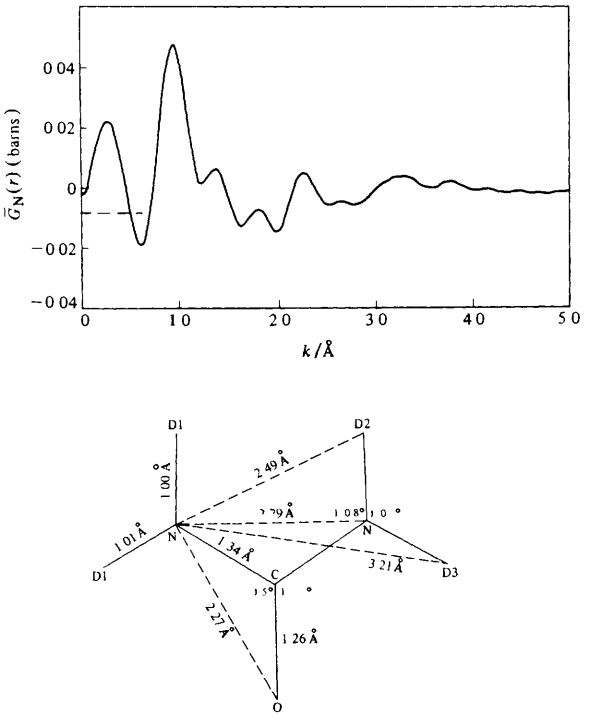


Figure 5 The nitrogen-centred pair distribution function of a 2 molal solution of urea in D_2O The intramolecular pair distances are shown in the lower figure and these can be related in position and area to the peaks close to 1 0 Å, 1 35 Å, and 2 3 Å The molecular geometry is thus reproduced The peaks at around 0 3 and 1 8 Å are known artefacts of the Fourier transform The two broad peaks between 2 5 and 4 0 Å correspond to about 7 water molecules

Unfortunately, as this molecule both donates protons to hydrogen bonds with neighbouring water molecules through the amino groups, and accepts water protons through the carbonyl group, the lack of spherical symmetry around the nitrogen makes interpretation difficult without recourse to modelling or computer simulation Interestingly, if we use these data as a test bed for simulation calculations, none published to date appears capable of reproducing the experimental data [11] This inability to reproduce the structure of water around a molecule containing chemical groups that occur frequently in biological macromolecules perhaps suggests caution is still needed in drawing conclusions from simulations of water around macromolecules such as proteins

Now using H/D substitution to probe the solvent-water structure in the presence of urea, interesting results were obtained for solutions of this molecule which, as mentioned in the introduction, is considered a classical water 'structure breaker' Although the HH correlation function obtained contains contributions from the exchangeable amino hydrogens on the urea molecule, the results at a 10 molal concentration were remarkable for the qualitative resemblance between the HH function for the solution and that for pure water This suggests the conclusion that – far from being the strong water 'structure-breaker' that is usually supposed – urea seems to fit very comfortably into the normal structure of water

Turning now to results on dimethylsulfoxide (DMSO),[12] we see in contrast quite significant changes in the water structure as both concentration and temperature are changed Figure 6 shows the HH pair correlation function for 28 molal DMSO (1 mole DMSO to 2 moles water) at 30 °C (dots) and − 62 °C (line) Comparing the 30 °C data with the equivalent function for pure water (Figure 3c), both the second and third peaks move to slightly larger distance r (by 2—3%) in a manner quite analogous to the changes seen on raising the temperature of water Interestingly, both these peaks move back towards the pure water positions, and sharpen, as the temperature is lowered substantially (see Figure 6) Moreover, the area under the second peak falls significantly from the value of about 5 in pure water to about 2 8, indicating that the water is becoming less hydrogen-bonded This can be explained by the conjecture that DMSO is hydrogen-bonding to water in preference to water itself In a second experiment at the lower concentration of 14 molal (1 mole DMSO to 4 moles water), the peaks at room temperature had moved closer to the pure water positions, indicating that any significant modification to the water structure occurs only at very high concentrations

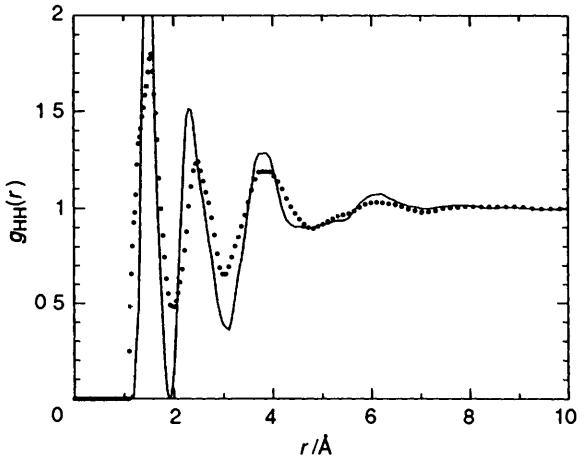


Figure 6 Hydrogen–hydrogen pair correlation function in a solution of 1 mole DMSO in 2 moles water at − 62 °C (line) and + 30 °C (dots) The first intermolecular peak near 2 4 Å moves appreciably with temperature, analogous to water at a higher temperature

The implication of this result is that some solutes at high concentration have an effect on water structure that is analogous to heating the liquid significantly we might comment that a substantial depression of freezing point occurs also in these systems At the same time, we should stress that the local water number density is different from both its pure liquid value and from the average water number density for the solution The extent to which the solute hydrates the water molecules affects the local number density As instrumentation improves further to improve the quantitative accuracy of the measured HH distribution, we will be able to ascertain the trend of local coordination number density with concentration for a number of solutions

In concluding this brief discussion of polar group hydration in aqueous solution, we should perhaps point out the apparent difference in the influence of the urea and DMSO molecules The latter, at high concentration, significantly perturbs the water, in a way which is similar to heating bulk water In contrast, the classical 'structure-breaker' urea does not seem to have the same effect, but rather seems to fit quite comfortably in the bulk water structure Further work is needed to understand precisely what is happening structurally in polar molecule solutions, using where appropriate good modelling and computer simulations to assist the structural interpretation

5 The Tetramethylammonium Ion

The TMA ion is the first member of the series of tetraalkylammonium ions which have been studied extensively by other methods, in particular thermodynamic and nuclear magnetic resonance As the size of the alkyl group increases, thermodynamic measurements imply that the ion behaves in a way which indicates increasing non-polar character They are thus suitable solutes with which to explore not only the hydration of non-polar groups, but also to probe the nature of the hydrophobic interaction itself through looking at the solute–solute interactions

From the point of view of isotopic substitution, these molecules are also extremely promising By substituting the nitrogen, we can study the hydration structure, and the spherical symmetry of the TMA ion eases considerably the interpretation of the results As there are no exchangeable hydrogens on the molecular ion, H/D substitution on the solvent yields results on solvent structure *only* This contrasts with the urea case, and with alcohols, where the exchangeable hydrogens on the molecule contribute to the HH and OH functions, thus potentially complicating the interpretation Furthermore, the large isotope differences achievable through H/D substitution allow us to see solute–solute correlations relatively easily through H/D substitution on the solute molecule Combining H/D substitution of the solute methyl-hydrogens with H/D substitution on the solvent water-hydrogens also enables us to obtain information on the orientational structure of the water hydrating the methyl groups By using various substitutions, we can therefore obtain information on all the three aspects of interest, namely

- solute–solvent correlations
- solute–solute correlations
- solvent–solvent correlations

Moreover, we can begin to probe how each of these might be affected by changing the solution conditions, *e g* by adding ions, or classical 'structure-breaking' or 'structure-making' molecules

A series of such experiments carried out on TMACl[13–15] illustrates the power of the technique, and allow us to draw some interesting conclusions Although this is the lowest member of the series, and therefore the one with least strong 'hydrophobic' behaviour, the results as we shall see do demonstrate that the TMA molecular ion hydrates as a non-polar molecule, and hence we can begin to throw some light on the role of solvent structural perturbations in explaining the hydrophobic effect

We now summarize the results of the following series of experiments performed on several concentrations of aqueous TMACl

Experiment 1: Nitrogen substitution on TMA, with D_2O as the solvent. This allows us to sit on the centre of the TMA ion, and observe the hydration shell from this central vantage point The result is shown in the nitrogen-centred pair correlation function (nitrogen at the origin) which gives the probability of finding any other atom at a distance r from the central nitrogen (Figure 7) In addition to reproducing the TMA structure from the nitrogen's viewpoint (the peak at 1 35 Å corresponds to 4 carbon atoms, while the negative peak at 2 1 Å indicates the 12 methyl hydrogens in the correct position), a broad peak centred between 4 and 5 Å from the nitrogen can

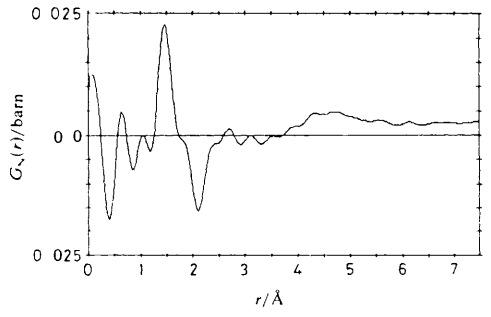


Figure 7 The nitrogen-centred pair distribution function of 1 9 molal TMACl in water The positive and negative peaks between 1 4 and 2 2 Å are consistent with the molecular structure (4 carbons at 1 47 Å and 12 hydrogens at 2 10 Å) Note that the hydrogen peak is negative as the neutron scattering length of hydrogen is negative The broad peak beyond is the hydration region As in Figure 5, the oscillations below 1 3 Å and the ripples above 2 5 Å are known artefacts of the data analysis procedure used

be interpreted as a shell of around 20 water molecules This is about the number we would expect for a classical non-polar 'cage' hydration model (Figure 8) but, without being able to distinguish between hydrogen and deuterium atoms in this region, we cannot yet decide if TMA is hydrating as a cation or a non-polar entity

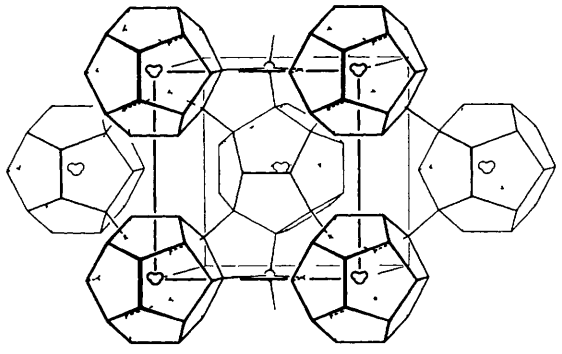


Figure 8 Water cages found in a simple clathrate hydrate crystal structure The oxygen centres occur at the polyhedral vertices, hydrogen atoms will lie mostly along the hydrogen bonds joining them both within the shells and pointing away from each cavity where the shells link with the neighbouring waters through hydrogen bonds Such cage structures have been proposed to occur around non-polar groups in aqueous solution, and the results presented here give evidence for this However we find these structures to be significantly disordered in the solutions that are discussed here

Experiment 2: Nitrogen substitution on TMA, with a 30% H_2O/D_2O solvent. This again yields the nitrogen-centred pair correlation function, but, because neutrons are scattered with a different strength by H and D, then comparing with the result from the first experiment, we can now in principle identify the hydrogens in the hydration region This allows us to assert that indeed TMA is hydrating as a non-polar molecule The results are consistent with a cage structure, perhaps of the clathrate-type it is, however, significantly disordered and should in no way be considered as the well-ordered, almost static cage that is sometimes asserted Thus, we should regard the kind of structure shown in Figure 8 as an idealized model the real structure shows considerable disorder

Experiment 3: H:D substitution on the solvent. This allows us to extract the partial pair correlation functions for *the solvent alone*, of which we focus on $g_{HH}(r)$, the probability of finding an H atom *on a water molecule* at a distance r from any other water molecule hydrogen This clearly depends on both the relative positions *and orientations* of the water molecules Providing the concentration is such that most of the water molecules participate in the hydration shell, we can compare this function with the same function for bulk water This comparison should tell us if the non-polar hydration region of the TMA ion is 'more ordered' structurally, as conventional wisdom would have us believe

Figure 9 shows this comparison The peak at around 1 55 Å denotes the intramolecular H—H distance, and again acts as an internal check of the data this distance should be the same for both pure water and the TMACl system If we now look at the second and third peaks, which relate to H···H distances on neighbouring water molecules (see Figure 4c), within the limitations of the data, there are no differences Thus, within these uncertainties, there is no evidence from these data that the water close to TMA is more ordered than in the bulk Any such 'ordering' we might expect to see as a sharpening of these peaks No sharpening is evident

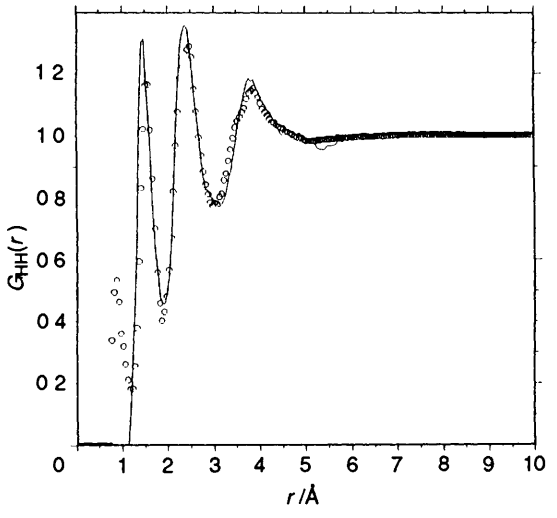


Figure 9 The hydrogen–hydrogen pair correlation function for 1 0 molal TMACl (circles) compared with the results from pure water (line)

Experiment 4: H:D substitution on the solute. This allows us to extract the TMA–TMA pair correlation function which is shown in Figure 10 for a 4 molal solution The broad peak at about 8 2 Å is at the distance we would expect for a uniform liquid-like distribution of TMA ions This result is thus direct evidence against the existence of solvent-enforced ion pairing in this system Thus, the tendency for association of these 'non-polar hydrated' molecules is not strong at this relatively high concentration

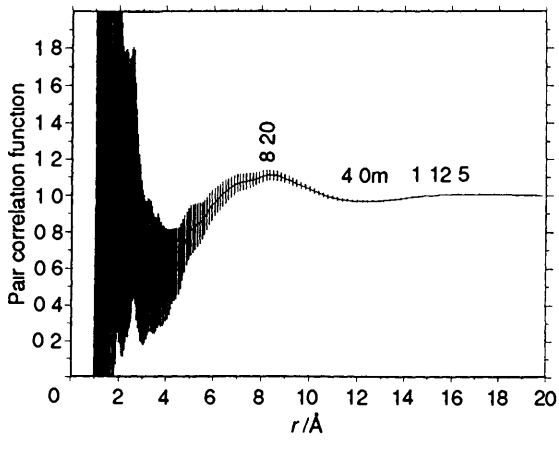


Figure 10 TMA–TMA pair correlation function in a 4 00 molal solution in D_2O The vertical lines represent error estimates

Experiment 5: Repeat experiments 1 to 3, but instead of pure water as solvent, use 2 molal urea. The point of this experiment is to see if the addition of a denaturant such as urea (a classical 'structure-breaker') leads to a significant 'disordering' of the hydration region Again, within the errors of the experiment, there is no significant difference from the nitrogen's viewpoint of the hydration region between water and 2 molal urea for the two concentrations shown in Figure 11 These results are consistent with other work on urea–water solutions discussed above, in which urea, far from 'breaking down' water structure, was seen to fit very comfortably in it

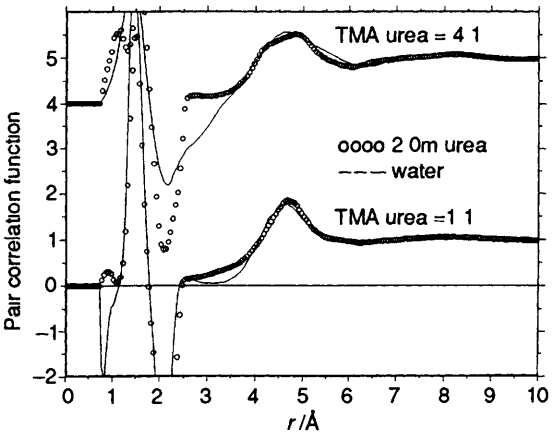


Figure 11 Partial pair correlation functions 'sitting on' the nitrogen in TMA, in 2 0 molal urea solution (circles) compared to solution in pure water (line) at two TMA concentrations

In summary, the neutron results so far on TMACl solutions lead us to conclude that TMA hydrates as an apolar molecule there is evidence for a cage-like average hydration structure, but one which is defective and disordered From the water's viewpoint, its (orientational) structure is not significantly perturbed from the bulk there is *no* evidence for any 'enhanced' structural ordering of the water close to the exposed methyl groups There is also no evidence for any solvent-induced (or 'hydrophobic') association of TMA, which has been proposed on the basis of thermodynamic results,[16] and the addition of the so-called 'structure-breaking' protein denaturant urea seems to have no significant effect on the hydration shell We seem to have a picture in which, although the hydration structure around the TMA molecular ion is what is classically expected of a non-polar molecule, this structure is one into which the water fits easily This is not a surprising conclusion when the nearest neighbour distances and angles of even a relatively well-ordered clathrate cage structure are examined the geometry of the water–water interaction can accomodate these We have no evidence for any structural ordering which can be used to support the conventional explanation of the hydrophobic interaction This is, to say the least, quite an interesting conclusion!

6 Alcohol–Water Systems

As mentioned in the introduction to the previous section, if one looks at the tetraalkylammonium ions in general, there is good thermodynamic evidence from which to argue that 'hydrophobic character' increases as the size of the alkyl group increases[17] and the influence of the charged nitrogen ion is reduced Thus, although the above results showed clear evidence in support of a non-polar hydration structure, we would be wise to perform similar experiments on other systems which are clearly accepted as interacting through a 'hydrophobic interaction' Moreover, although isotope substitution on the chloride ion in the TMACl system has demonstrated that the Cl hydration is normal, the absence of an anion in the system would be preferable

Alcohol–water systems provide a potentially fruitful series on which to perform similar experiments There is a wealth of thermodynamic and dynamic data as functions of both concentration and temperature available on a variety of alcohols,[18] yet little in the way of direct structural information on the hydration of the alkyl groups The hydroxyl group ensures reasonable solubility to make neutron experiments possible, it also complicates the interpretation, though ways can be devised to overcome this problem

Again, the questions we might ask of alcohol–water systems are similar to those tackled above for TMA First, what is the nature of the alkyl group hydration, as seen from the methyl group's viewpoint? Is it a clathrate-like cage structure, and if so, how well-ordered is it? Secondly, from the point of view of the water in the hydration 'shell', how is it perturbed from its bulk organization? Does, as has been suggested, the hydration water have a structure equivalent to that of bulk water at a lower temperature, and is there any evidence for hydrogen-bond strengthening? Both these questions can be tackled with judicious use of H/D substitution

We now summarize the conclusions of very recent work at ISIS We consider first the hydration of the methyl group in methanol from the viewpoint of the methanol molecule Secondly, we look at the solvent structure and its possible perturbation from the bulk, as seen from the water's standpoint, in solutions of ethanol and tertiary butanol Concentrations in all cases are taken close to the respective minima in partial molar volume

6.1 Methyl Group Hydration in Methanol–Water

By H/D substitution on the methyl group hydrogens, combined with H/D substitution on the water, we can extract the pair correlation function between the methyl hydrogen atoms and the hydroxyl hydrogens (the MH function) (As well as reporting on pair distances to water hydrogens, distances to the alcoholic hydrogen are included in this function However, at the concentration used of 1 methanol to 9 waters, this contribution will be at the 5% level, and will be a small perturbation on the results) This function, because of the low symmetry of the methyl hydrogen centred viewpoint, is not easy to interpret Recourse was therefore made to a spherical harmonic expansion procedure developed recently by one of us[19] to construct an orientational pair correlation function which shows how the water molecules tend to orient in the hydration shell of the methyl group

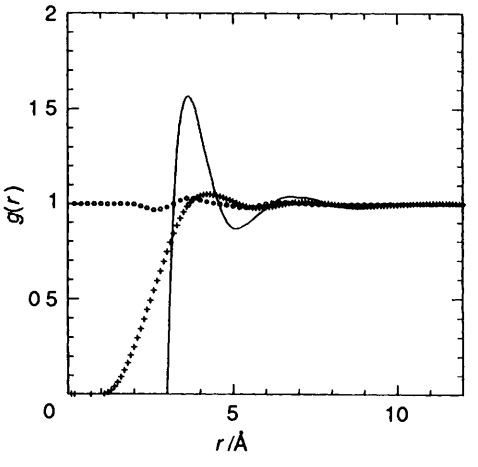


Figure 12 The MH pair correlation in a 1 9 methanol water mixture

Figure 12 shows (solid line) the pair correlation function of the (methanol-centred) molecular centres The peak at 3 6—3 7 Å tells us the first neighbour water molecules are at this distance from the methanol, with the number of waters obtainable by integrating the peak area The crossed line shows the MH function (see above) assuming the water molecules are oriented

isotropically around the methyl group This model does not fit the experimental MH function, and the difference between this isotropic model and the experimental result is shown by the line of circles Two observations are made usefully on this difference plot First, the peak at around 3 6 Å shows 'excess' water hydrogens at this distance, that is, at about the same distance (actually slightly less) from the carbon atom of the methyl group as the water molecules centres Thus, if we imagine drawing a sphere centred on the methyl group with a radius that of the maximum in the molecular centres distribution, there will be a preference for O–H bonds of the water molecules to be approximately tangential to this sphere *This is precisely what we would expect if we indeed had a 'cage-like' hydration structure (see Figure 8)* Furthermore, the dip in the difference curve of Figure 12 at around 2 5—3 0 Å shows there are fewer hydrogens in this region – that is, deficiency of O–H bonds pointing towards the methyl group – than the isotropic model predicts This again is consistent with the idea of a cage-like hydration arrangement to make way for the methyl group, the surrounding water has reoriented itself so that the O–H vectors that might have pointed towards the methyl group have shifted to an orientation approximately tangential to a sphere circumscribing the methyl group and passing through the molecular centres of the surrounding waters

The analysis can be taken further by plotting sections of the methyl–water orientational correlation function which best fits the data In addition to confirming this preferred tangential orientation, these plots quantify the disorder in this 'cage-like' hydration shell the disorder is indeed very significant Thus, we should *not* conclude from these results that the methyl group is hydrated by a clathrate-cage of water molecules that is perfect and long-lived, as has been asserted in traditional explanations of 'hydrophobic hydration' There *is* a preference for O–H directions to lie approximately tangential to the circumscribing sphere surface, but there is very considerable disorder in this arrangement The hydration 'shell' is not, definitely not, the proverbial 'iceberg', either structurally or dynamically

6.2 The Water's Viewpoint

As in the TMA case, we can implement H/D substitution on the water molecules to extract a series of partial pair correlation functions which report to us the water (orientational) structure We can thus try to answer the second question set out above, namely do these low concentrations of alcohols 'stabilize' in some way the water network The $g_{HH}(r)$ correlation function is essentially that seen if we sit on a water hydrogen and look around us at all other water hydrogens (There is a small contribution from the alcoholic OH hydrogen, but at these concentrations, this can be neglected) We can then compare that with the same function for bulk water

Figure 13 shows the $g_{HH}(r)$ correlation for both ethanol–water and t-butanol–water solutions within the error bars, there is no difference, telling us that from the water's viewpoint, it does not – from this measure – know which of the two alcohols it is next to This is an interesting conclusion in itself Also plotted on Figure 13 is the same function for liquid water If there were an enhancement of the order in the solvent next to the alkyl groups of the alcohols, we would perhaps expect the second and perhaps the third peaks to be sharper than in the bulk water case (the first peak is, as explained in the TMA case, the intramolecular H–H distance, and is less likely to be affected) Looking at the second and third peaks of Figure 13, no such sharpening is found If anything, the effect is the reverse of what might be expected Although the error estimates suggest it would be dangerous to make a strong claim at this stage, the peaks for the alcohols are perhaps *less* sharp than for the bulk, implying that the water close to the alkyl groups may perhaps be '*more disordered*' than in the bulk

These results on alcohol systems are similar to those discussed above for TMA The 'bottom line' is clear, even at the present stage of development of the experimental techniques First, the

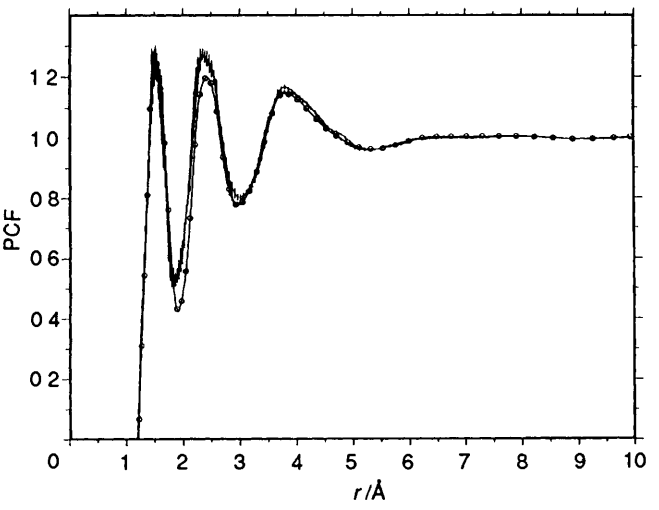


Figure 13 The hydrogen–hydrogen pair correlation function for 1 19 EtOH water and 1 32 t-BuOH water (lines with error bars), compared to pure water (circles)

methyl group hydration structure *is* related to the expected 'clathrate-cage' type model, though one that is considerably disordered Moreover, this disorder can be quantified through the orientational pair correlation between the methyl group and the surrounding water molecules Secondly, and from the viewpoint of the conventional wisdom, these *direct* structural data give *no* evidence that the water which hydrates methyl groups is structurally more ordered than is bulk water at the same temperature For the entropic contribution to the hydrophobic interaction, these results suggests we have to look elsewhere

7 Summary

Using neutron scattering methods and exploiting isotopic substitution, we are now becoming able to address some important questions concerning hydration of molecular groups important in both chemistry and biomolecular processes We have exampled above some results on molecules containing polar groups, and on two kinds of system in which non-polar methyl groups are exposed to the solvent, namely the TMACl system and a series of alcohols, looking at hydration from the viewpoints of both the solute molecule, and the surrounding water

In the case of DMSO, there was evidence for preferential DMSO–water over water–water interaction, and at high concentration of DMSO, the water structure was perturbed such that it was similar to pure water at a higher temperature For urea, there was *no* evidence that it disordered the water structure in any way, even at high concentrations, the urea molecule seems to fit very comfortably into the water structure

In both cases where the hydration of non-polar groups has been examined, we conclude that the neutron diffraction results are consistent with a disordered 'cage' structure around the methyl group(s) Although this hydration structure may be topologically related to the clathrate-like cages often proposed in discussions of so-called hydrophobic hydration, our results are able to quantify the disorder in these structures, and we find the degree of disorder considerable Secondly, we find that from the water's viewpoint, it sees itself as being in an environment similar to bulk water There is no evidence that the hydration water is in any way 'more ordered' than in the bulk, and this conclusion raises problems for traditional explanations of hydrophobic interactions which account for an entropic gain by expelling water molecules from the supposedly 'more-ordered' environment close to the non-polar group to the 'less-ordered' bulk If anything, our results suggest increased *disorder* for the hydration water, although further work is needed before this suggestion should be given credence

It is, however, early days for this kind of work, and so far we

have done no more than scratch the surface of the science that is now approachable Indeed, instrumentation has improved significantly since the data reported here were taken and higher quality data are now possible The techniques have now been developed to the level at which real structural data of some complexity can help us to understand for the first time the structural basis of solution chemistry We can perhaps now begin to do the solution equivalent of structural crystallography, and obtain high quality data on local structures in solutions of quite high complexity Some of the obvious next steps include the following of temperature and concentration dependence, the study of other alcohols, and further exploration of the effects on solvent of so-called structure-makers and structure-breakers Complementary work on the dynamics of hydration, again exploiting the advantages of neutron scattering as well as other techniques, is also called for and there is the whole field of non-aqueous solutions There is much exciting work to be done

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