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Water perturbation close to non-polar groups in aqueous solutions

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Abstract
The perturbation of water structure and/or dynamics close to biomolecules has often been considered to give rise to thermodynamic contributions to free energies of stabilisation, association, and substrate binding. Of particular interest is water perturbation close to non-polar groups, which has been argued for many years to give rise to the so-called hydrophobic interaction. Recent developments in neutron scattering instrumentation and techniques now allow us to probe directly possible water perturbations close to non-polar groups. The results presented suggest the conventional wisdom of the hydrophobic effect is significantly wanting.

BACKGROUND

Solvent interactions in biomolecular processes

It is now generally recognised that the forces controlling many important biomolecular processes - for example protein folding and enzyme-substrate binding - involve intimately solvent interactions (refs 1, 2). Since the historical paper of Kauzmann (ref 3), much emphasis has been placed on the hydrophobic effect, in which entropic effects are argued to drive together non-polar groups. It is frequently stated that this "hydrophobic interaction" dominates the protein folding process. Despite significant work more recently that has argued that to assert hydrophobic dominance may be an oversimplification (refs 1, 2, 4, 5), this assumption still pervades much of the chemical and molecular biological literature. The source of the hydrophobic effect is also conventionally stated to result from an entropic gain consequent upon expelling to the bulk solvent those water molecules that previously were in contact with the non-polar groups exposed to solvent in the unfolded protein. For this mechanism to be realistic, the water "hydrating" an exposed non-polar group must be in some way restricted compared to bulk water, and hence give a lower entropy contribution. Thus has grown up the idea that water close to non-polar groups is somehow "more ordered" than in bulk water, and idealised models related to clathrate cage structures have often been invoked.

There is, however, no direct structural evidence for solvent ordering. With the advent of pulsed spallation neutron sources and appropriate instrumentation, it has now become possible to investigate directly water perturbations close to not only non-polar groups, but also charged and polar entities. We summarise here the results of recent work on two kinds of system, namely the tetramethylammonium ion, and a series of alcohols. The results are interestingly at variance with long-held conventional wisdom, and suggest a reassessment of the molecular origin of the hydrophobic effect is needed.

Neutron scattering from solutions

The neutron is a particularly useful probe for studying the structures of aqueous systems. First, unlike x-rays, neutrons are scattered strongly by hydrogen, in particular by the deuterium isotope. Secondly, the neutron scattering power 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, behave differently towards the neutron scattering probe. By making judicious use of this "isotope substitution" technique first developed in application to electrolyte solutions by Enderby and Neilson (ref 6), 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 (refs 6, 7), 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 as a function of scattering vector Q

(relating to scattering angle and wavelength, 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 Fig 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 distances and angles, while peak areas tell us the number of neighbours, or "coordination number".

The systems of interest here, however, are much more complex. Even for just a solution of eg. 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. Taking the difference results in a correlation function 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, we can discriminate also the identity of the neighbouring atoms.

We thus have a very powerful technique which can be applied to solution systems of significant complexity. By judiciously applying isotope substitution techniques to atoms in the solute, and/or atoms in the solvent, we can begin to answer some important questions in solution chemistry. Among these are questions of the hydration of particular solute molecules, of perturbation of solvent close to charged, polar, and non-polar groups, as well as the distribution of solute molecules which can give information on any (solvent- or otherwise - induced) solute pairing or aggregation.

THE TETRAMETHYAMMONIUM ION

We first summarise some results on the tetramethylammonium chloride system; details can be obtained from refs 8-10. Isotope substitution can be used to determine how the TMA ion hydrates (as a cation with the water dipoles tending to point towards the quaternary nitrogen or as a non-polar molecule?), how the water is perturbed in the hydration shell (is it more ordered than in the bulk?), and whether or not there is any solvent-induced ion pairing as has been suggested in the literature. Finally, the method is sufficiently flexible for us to add a further molecule which classically we might expect to perturb the solvent structure in the direction of increasing disorder, and to monitor any such perturbations. This latter study is conditioned by the often-held view that protein denaturants operate by breaking down solvent structures close to non-polar groups.

The following experiments have been performed on several concentrations of aqueous TMACl.

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. In addition to reproducing the TMA structure from the nitrogen's viewpoint (a useful check on our experiment), a broad peak centred between 4 and 5Å from the nitrogen can be interpreted as a shell of about 20 water molecules. This is about the number we would expect for a classical non-polar "cage" hydration model 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.

2. Nitrogen substitution on TMA, with a 30% H_2O/D_2O solvent. This again yields the nitrogen-centred pair correlation functions, but, because neutrons are scattered with a different strength by H than 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.

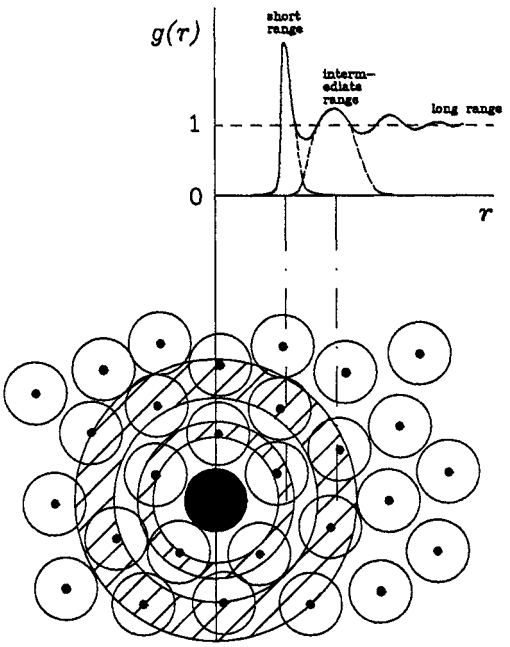

Fig. 1. The pair correlation function of a two dimensional liquid.

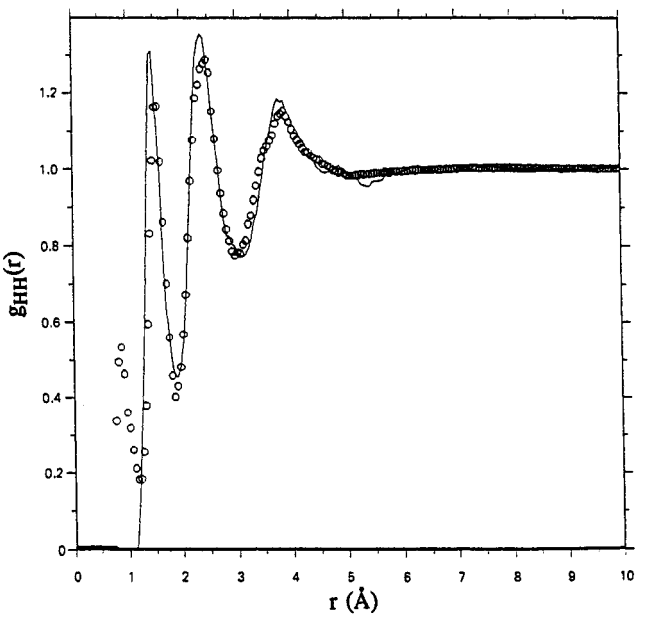

Fig. 2. The hydrogen-hydrogen pair correlation function for 1.0 molal TMACl (circles) compared with the result from pure water (line).

3. H:D substitution on the solvent. This allows us to extract particularly interesting partial pair correlation functions for the solvent alone, namely $g_{HH}(r)$, which is 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 2 shows this comparison. The peak at around 1.55Å denotes the intramolecular H-H distance, and again acts as an internal check of the data: they should be the same for both 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, 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.

4. H:D substitution on the solute. This allows us to extract the TMA-TMA pair correlation function shown in Fig 3 for a 4 molal solution. The broad peak at about 8.2Å 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.

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 (often termed a "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 Fig 4. These results are consistent with other work on urea-water solutions, in which urea, far from "breaking down" water structure, was seen to fit very comfortably in it.

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, and the

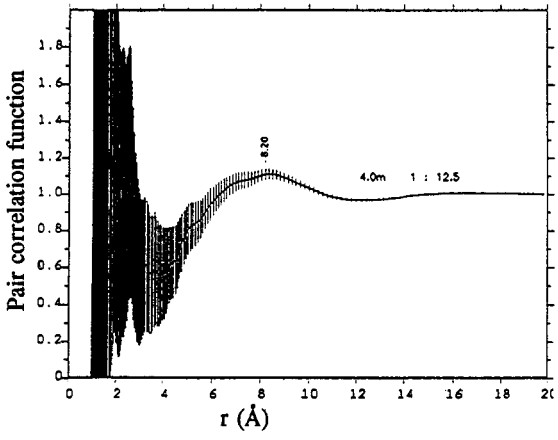

Fig. 3. TMA-TMA pair correlation function in a 4.00 molal solution in D_2O. The vertical lines represent error estimates.

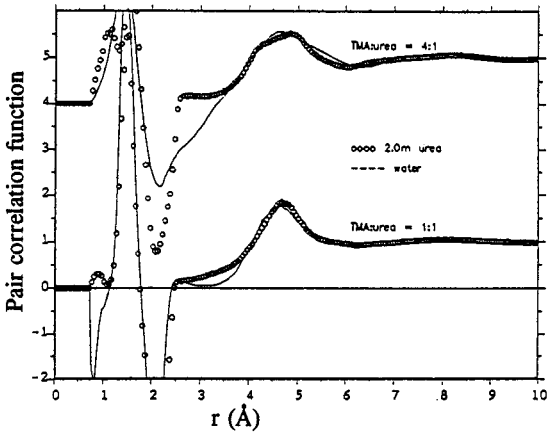

Fig. 4. Partial pair correlation functions, "sitting on" the nitrogen in TMA, in 2.0 molal urea solution (circles) compared to solution in pure water (line), at two TMA concentrations.

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 the non-polar hydration structure is very much one in which water is structurally very comfortable - a not 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 is happy to accommodate these. We have no evidence for any structural ordering which can be used to support the conventional explanation of the hydrophobic interaction.

ALCOHOL–WATER SYSTEMS

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 (ref 12), 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 (ref 13), 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 organisation? Is, as has been suggested, the hydration water of a structure equivalent to the bulk 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 summarise very briefly the conclusions of very recent work which will be published in detail elsewhere. 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.

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 construct a pair correlation 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

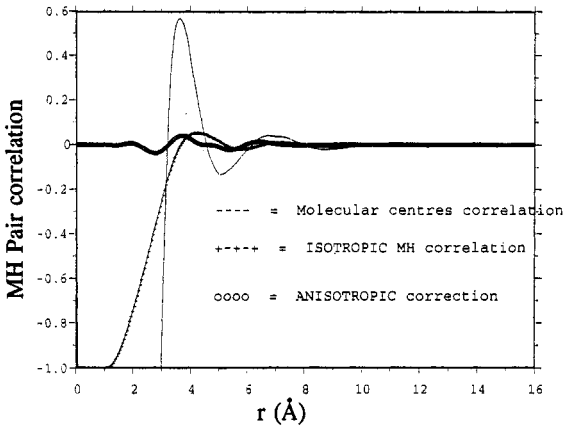

Fig. 5. The MH pair correlation in 1:9 methanol: water mixture.

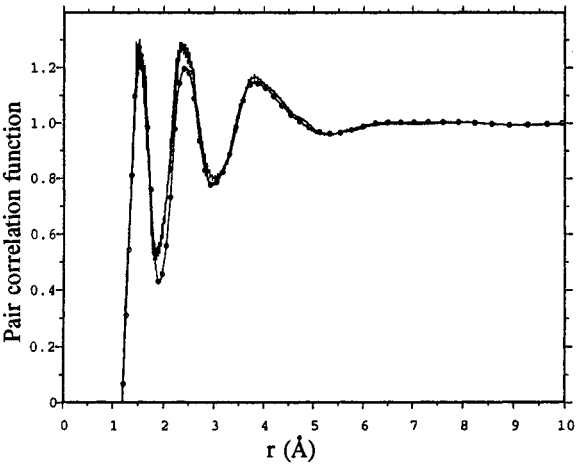

Fig. 6. The hydrogen-hydrogen pair correlation function for 1:19 EtOH:water and 1:32 tBuOH: water (lines with error bars), compared with pure water (circles).

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 (ref 14) 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 5 shows (solid line) the pair correlation function of the (methanol-centred) molecular centres. The peak at 3.6-3.7Å 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 3.6Å shows "excess" water hydrogens at this distance, that is, at about the same distance (actually slightly less) from the methyl group as the water molecular 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. Furthermore, the dip in the difference curve of Fig 5 at around 2.5-3.0Å shows there are fewer hydrogens in this region - that is, a 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 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.

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 "stabilise" 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 is small). We can then compare that with the same function for bulk water.

Figure 6 shows the $g_{HH}(r)$ correlation for both ethanol-water and t-butanol: 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 Fig 6 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 Fig 6, 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.

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 summarised above results 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 both cases, we conclude that the neutron diffraction results are consistent with a disordered "cage" structure around the methyl group(s). Although this 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 a similar environment 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 supposed "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. Indeed, instrumentation has improved significantly since the data reported here were taken and higher quality data is now possible. In addition, other next steps are indicated including 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.

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