Water structure theory and some implications for drug design

T. H. Plumridge and R. D. Waigh

Abstract

The development of theories of water structure has been hindered in the past by the difficulty of experimental measurement. Both measurement and computer modelling studies have now reached the stage where theoretical treatments of water structure are converging to a broadly acceptable model. In current understanding, water is a mixture of randomly hydrogen-bonded molecules and larger structures comprised of tetrahedral oxygen centres which, when hydrogen-bonded to each other, lead to five-membered and other rings which can aggregate to form three-dimensional structures. Evidence is taken from studies of the ices, from clathrates and other solid solutions, as well as from liquid solutions, that certain motifs occur very frequently and have relatively high stability, such as the $(H_2O)_{20}$ cavity-forming structure known from studies on clathrates. The implications of recent models of water structure for an understanding of biological events, including the interactions of drugs with receptors, are profound. It is becoming clear that modelling of aqueous solutions of any molecule must consider the explicit interactions with water molecules, which should not be regarded as a continuum: water itself is not a continuum. Solute molecules which possess hydrogen-bonding groups will provoke the formation of further hydrogen-bonding chains of water molecules: if these can form rings, such rings will tend to persist longer than chains, giving the solute a secondary identity of associated water which may play a role in molecular recognition. Solutes that do not have hydrogen-bonding capability, or regions of solutes which are non-polar, may also produce partial cage-like water structures that are characteristic of the solute.

The classification of many solutes as structure makers or structure breakers has relevance to the interactions between ligands and large biomolecules such as proteins. While it is generally accepted that sulfate and urea, respectively structure maker and breaker, may alter protein conformation through effects on water, it has not been recognised that bioactive ligands, which also change the conformation of proteins, may do so by a related, but more selective, mechanism. Very early studies of cell contents suggested that the associated water might be different from bulk water, a concept that lost support in the mid-20th century. Current theories of water structure may invite a reappraisal of this position, given the observation that structuring may extend for many molecular diameters from an ordered surface.

Introduction

Water is the dispersion medium for all the biochemical reactions that constitute the living process and takes part in many of these reactions. Despite the chemical simplicity of the water molecule, its bulk properties are very peculiar and have attracted a large amount of scientific attention. Recent physico-chemical studies, allied to increasingly sophisticated computer simulations, have reached the stage where many of the old controversies have been resolved. Structuring in liquid water is being described with increasing confidence, in the pure liquid, at interfaces and in solutions. This review attempts to draw together much current understanding of water and to place the interactions of small molecules (e.g. drugs) and large molecules (e.g. receptors) into that context.

Early theories of water structure

The immiscibility of water with extruded cytoplasm from plant and animal cells led some mid-19th-century biologists to believe that water in the cytoplasm is bound

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to the macromolecular constituents (Nageli 1855; Kuhne 1864). The suggestion was made that water may contain solid particles, and in 1891 Vernon postulated that maximum density effects could be explained by the aggregation of water molecules (Davidson 1973). Some water in protein solutions and in living cells refused to freeze at temperatures as low as $-20°C$ (Rubner 1922).

In the 1930s Gortner and co-workers carried out experiments dissolving sucrose into water in various environments and determining how much had dissolved by measuring lowering of the freezing point (Gortner & Gortner 1934). They found that bound water, for example cell water, dissolved less sucrose than free water did and hence inferred that water in these situations was more structured than the bulk.

At the same time, the first X-ray (Morgan & Warren 1938) and infrared studies (Magat 1936) on liquid water were carried out, and this new perspective allowed Bernal & Fowler (1933) to advance the first plausible model for liquid water – the uniform continuum model. In this model, all oxygen atoms retain their four-coordination, but the hydrogen bonds are bent to such an extent that an instantaneous view from the central oxygen would see no order beyond the nearest neighbours. A random network model with soft hydrogen bonds implies a water network with apex-linked polygons in rings of 4-, 5-, 6- or more-membered rings, similar to ices and clathrates, but randomly arranged.

Even at this early stage the bound water theories met with considerable opposition. In a 1940 review, Blanchard compiled evidence against the bound water viewpoint on the basis of vapour-pressure measurements and the fact that ethylene glycol will distribute itself evenly between erythrocytes and external solution (Blanchard 1940). He also stated that water can be supercooled to $-20°C$ and suggested that the complexity of the protein system made accurate determination of freezing point almost impossible. This review marked the end of the first phase of research into structured water as the membrane theory, where the cell was assumed to maintain a different composition from that of its surroundings by the use of sodium pumps located in the cell membrane, became generally accepted and the hydration theory of cell water was virtually abandoned.

Following on from work on the origin of negative partial entropies exhibited by various simple solutes in aqueous solution (Eley 1939), Frank & Evans (1945) introduced the concept of icebergs induced in water by solute molecules. On the basis of volume and entropy measurements, while studying the effects of non-polar molecules dissolving in water (noble gases, CO, methane, etc.), they found that these molecules affect the water around them making it less dense, at lower entropy and less mobile than bulk water, essentially making it more crystalline and more structured. They postulated that some species would act in the opposite way, making the water less structured and more dense than bulk. This was the first mixture model of liquid water. Subsequent models have generally been based either on this work or on Bernal & Fowler's continuum model (Bernal & Fowler 1933).

At the same time, Samoilov (1946) was investigating the nature of the interactions between water and ions in solution. Hall (1948), on the basis of acoustic relaxation in water, advanced the first detailed mixture model, based on the concept of water as a mixture of two distinguishable species. Pople (1951), working with the model proposed by Bernal & Fowler (1933) and his own studies into the properties of the hydrogen bond, suggested that hydrogen-bond bending could explain properties such as the temperature dependence of the dielectric constant of water.

The next major step forward for the structured water viewpoint occurred when Frank & Wen (1957) postulated that the existence of long-lived structures in liquid water was unlikely and that a more useful description might involve flickering clusters of hydrogen-bonded water molecules. They classified ions as either being structure makers or structure breakers, and suggested a mechanism by which this may occur. Basically, the ion is surrounded by three concentric regions: the innermost, in which all water is immobilised; the second in which water is less ice-like (i.e., more random than normal); and the third, normal water polarised in the normal way by the ionic field, which has become relatively weak this distance from the ion.

The ions originally classified by Frank & Wen were as follows:

structure makers: Li^+, F^-, Mg^{2+}, OH^-, $(n-C_4H_9)_4N^+$

structure breakers: K^+, Rb^+, Cs^+, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, SO_4^-

They postulated that the structure-breaking region was caused by the balance between the two competing orienting influences acting on any given water molecule in this region (i.e. the normal water influence from the outer zone and the spherically symmetrical ionic field dominating in the inner zone). They also attributed stronger structure-making ions with a high enough degree of incipient hydrolysis of the first water layer around the ion to produce hydrogen charge centres which would act as edge nuclei for cluster formation and stabilisation for clusters once formed. This increases the ice-likeness of the water in these environments.

Shortly afterwards, Kauzmann (1959) discussed the possible role of water in protein conformation and denaturation incorporating Frank and Evans' model. He reviewed the role of apolar group interactions or hydrophobic bonding in maintaining the tertiary structures of proteins, and suggested that comparative studies of aqueous and non aqueous solutions of simple analogues of apolar amino-acid side chains (alkanes, for example) may provide an insight into the peculiarities of water as a solvent. This sparked off a whole range of publications describing various aspects of hydrophobic bonding in biological systems.

Pauling (1959) proposed a model for the structure of water in which clathrate cages are present and may be occupied by interstitial non-bonded water molecules. This model was considered to be too crystalline (Stillinger 1980), and is not consistent with X-ray diffraction data, but less rigidly defined cavities, more disordered than the clathrates, would fit the experimental evidence available both in 1959 and today.

Most of the material arising from this vast amount of research in the 1960s and '70s is summarised in Felix Franks' 7-volume collection, *Water: a comprehensive treatise* (Franks 1972–1982).

At present, all models developed for the structure of liquid water still fall into two main classes: continuum models, originally proposed by Bernal & Fowler, and mixture models based on Franks' work. Speculation regarding the nature of molecular motions in water led to comparisons of various bulk and microscopic transport processes (e.g. viscosity, self-diffusion and dielectric and NMR spin-lattice relaxation). High-precision Raman studies of the hydrogen bonding modes in liquid water followed (Walrafen 1964), and infrared and Raman techniques have been applied to the study of intramolecular OH and OD stretching modes in H_2O and D_2O (Walrafen 1968; Senior & Verall 1969). This information was carefully analysed to deduce whether evidence suggested that water should be treated as a mixture and, although this was not fully resolved, mixture models were favoured. Frank & Evans' iceberg theory has now become accepted as the standard model of hydrophobic hydration (Blokzijl & Engberts 1993).

Structure-making and structure-breaking molecules

It has long been known that the native conformations of proteins, as assessed by enzyme activity, denaturation temperature and solubility, are stabilised by some solutes and destabilised by others (e.g. Collins & Washabaugh 1985). These effects are additive; a strongly denaturing (structure breaking) solute such as urea can be balanced by the action of a strongly stabilising (structure making) solute such as trimethylamine oxide. Two structure-breaking solutes will destabilise protein structure more than if either was used separately.

The structure-making (kosmotropic) or structure-breaking (chaotropic) action of solutes is determined in aqueous solution by:

A change in viscosity (structure breakers lower it)
The rate of exchange of water molecules (structure breakers lower energy of activation)
The longitudinal relaxation rate of water molecules as measured by NMR (structure breakers increase the rate).

The entropy of hydration is related to the structure-making and -breaking properties of solutes. Marcus (1986) estimated the entropy contribution of structured water effects by subtracting contributions due to compression, immobilisation and electrostatic effects from the standard molar entropies of hydration for a set of 50 ions. This structural entropy contribution is positive for structure breakers and negative for structure makers, and was found to be in accord with experimental findings despite the assumptions involved in the calculations.

The Hofmeister series, discovered over a hundred years ago (Hofmeister 1888) originates from the ranking of various ions based on their ability to precipitate hen egg-white proteins. This series has also been shown to affect the structuring (at one end of the series) or denaturing (at the other end of the series) of biological macromolecules in the same order (Collins 1997). The series shows opposite correlations for anions and cations with their degree of hydration.

$$citrate^{3-} > sulfate^{2-} > phosphate^{2-} > F^- > Cl^- > Br^- > I^- > NO_3^- > ClO_4^-$$

Strongly hydrated	Weakly hydrated
Protein stabilising	Protein denaturing

$$N(CH_3)_4^+ > NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > H^+ > Ca^{2+} > Mg^{2+} > Al^{3+}$$

Weakly hydrated	Strongly hydrated
Protein stabilising	Protein denaturing

Structure-breaking ions destroy the hydrogen-bonded water network in a manner which is similar to the effect of increased temperature or pressure (Leberman & Soper 1995). Anions hydrate more strongly than cations of the same ionic radius, as water hydrogens can approach about 0.8 Å more closely than the water oxygen. Small ions are strongly hydrated with small or negative hydration entropies creating local order. Large singly charged ions have larger positive entropies of hydration, and so act like hydrophobic molecules, their binding being dependent on van der Waals forces as well as their charge.

Very similar molecules often have totally opposite effects. Both *meta*- and *para*-hydroxybenzoic acids are structure makers, whereas the *ortho*-form is a structure breaker (Chatterjee & Seal 1992). Sulfate is almost exactly the same size and shape as perchlorate, but sulfate is a strong structure maker and perchlorate a strong structure breaker (Collins & Washabaugh 1985; Yokoyama et al 1992). The reasons for this still remain unclear.

The Walden product, which is the product of viscosity and conductivity at infinite dilution of a solution, has been shown to be a measure of the water-structuring activity of the solute. This has been used to quantify structure-making and -breaking effects of amino acids, in conjunction with viscosity and spectroscopic studies. Most amino acids exhibited some structure-breaking activity. L-Lysine, L-glutamic acid, L-aspartic acid and their salts showed stronger structure-breaking activity. Dextrose, however, behaved as a classic structure maker and reversed the structure-breaking action of L-lysine (Lutz et al 1994).

Pure water

Structure of the isolated water molecule

The structure of the isolated water molecule is known accurately from spectroscopic studies. The H—O—H angle and O—H bond lengths were first determined accurately by Darling & Dennison (1940). They found the O—H bond length to be 0.95718 Å and the H—O—H angle to be 104.523°. These values have since been validated by experimental (neutron scattering: Soper & Phillips 1986) and theoretical calculations (e.g. ab-initio: Goddard & Hunt 1974).
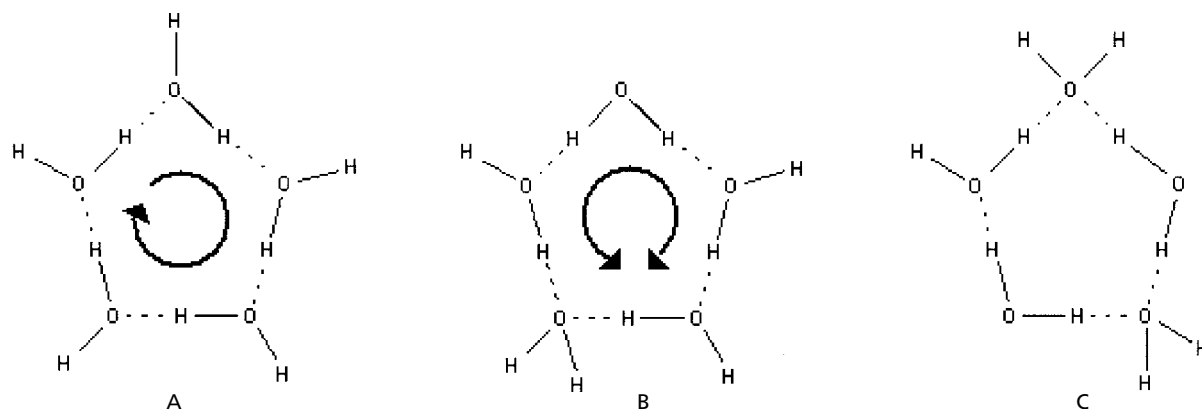
Figure 1   Three possible arrangements of cyclic hydrogen bonds: homodromic (A); antidromic (B); and heterodromic (C).

Each water molecule can form up to four hydrogen bonds in a tetrahedral arrangement: two acceptor bonds through the lone pairs and two donors through the hydrogens. This is a unique feature of the water molecule and leads to three-dimensional networks as, for example, in ice structures.

Cooperative effects
An important feature of hydrogen bonds is that one will tend to reinforce another. This is known as cooperativity, and allows large tetrahedral networks of hydrogen-bonded water to form. Cooperativity, or non-additivity of hydrogen bonds, was conceptually inferred from the early theories of liquid water and aqueous solutions (Frank 1958; Kavanaugh 1964), and first demonstrated by early ab-initio quantum mechanical calculations on water complexes (Del Bene & Pople 1970; Hankins et al 1970). Cooperative patterns were found in the first systematic studies of hydrogen bonding in carbohydrates (Jeffrey et al 1977). The water molecule acting as acceptor in a particular hydrogen bond will strengthen the other hydrogen bonds of the water molecule acting as a donor (Tombari et al 1999), and so in liquid water cooperativity strengthens the hydrogen bonds by up to 2.5 times the strength of the hydrogen bond in the water dimer (Luck 1998).

This effect is also apparent in rings of hydrogen-bonded water. For example in the five-membered rings shown in Figure 1 the homodromic system (a) where the hydrogens all point in the same direction (clockwise or anticlockwise) around the ring is more stable than the antidromic system (b) where some hydrogens point in the same direction, which is itself more stable than the heterodromic arrangement (c) with only two hydrogens aligned (Peeters 1995).

Pure water in the solid state
Much more information can be obtained by diffraction methods on the structure of solids than of liquids. In a liquid there are continuous rearrangements of structure occurring within a very short time. This means that only a mean environment can be determined, which is the number and spatial arrangements of nearest neighbours of a mol-

ecule averaged over both time and space. The only information obtainable by X-ray diffraction of a liquid at a given temperature is the radial distribution function. This is the probability of finding an atom at a radius, r, from the central atom, averaged over both space and time. For example, an X-ray diffraction study on water will yield only the probability of finding an oxygen atom at a certain distance from another oxygen.

With the advent of neutron diffraction, using deuterated samples, distribution factors can be determined which give the O...O, O...D and D...D spacings from which the hydrogen-bonding patterns in the first hydration shell can be inferred, but interpretation of these patterns in terms of nearest neighbours is very difficult.

On the other hand, X-ray diffraction on a solid sample will usually establish the crystal structure (i.e., will yield the coordinates of all non-hydrogen atoms). Using neutron diffraction techniques with deuterated samples, the positions of the hydrogens (deuterous) can also be found, allowing hydrogen-bonding patterns to be clearly and unequivocally elucidated in the solid state.

Ices
The ice structures reveal the structural patterns for the regular cohesion of water molecules in the absence of other species. Arrangements of four-connected tetrahedrally or nearly tetrahedrally coordinated hydrogen-bonded water molecules are found in all known ice structures. These ice structures are characterised by maximisation of the number of hydrogen bonds and minimisation of the short-range repulsive restraints.

At least 14 distinct ice structures exist, along with at least two distinct amorphous phases (Jeffrey & Saenger 1991). In all known ice structures each water molecule is hydrogen bonded to four neighbouring water molecules. Only ices Ih, III, V, VI and VII can be in equilibrium with liquid water. All other ices are not stable with liquid water under any conditions.

The high-pressure phase lines of ice-X and ice-XI are still subject to experimental verification. Two different forms of

ice-XI have been found, the high-pressure form having the hydrogen atoms equally spaced between the oxygen atoms (Benoit et al 1996) whereas the low-temperature form has ordered hydrogen bonding (Lobban et al 1998). Ices II, XIII, IX, X and XI (both forms) have ordered hydrogen bonding whereas in all other ices the hydrogen bonding is disordered even down to 0 K, if this is reachable (Suga 1997). As well as five- and six-membered water rings, the high-pressure ices contain four-, seven- and eight-membered rings (Saenger 1987).

It has been discovered that the structure of normal ice (Ih) is not as simple as originally thought. To explain two well-separated optic bands in a high-resolution inelastic neutron-scattering study of ice, two different interaction strengths of hydrogen bonds were proposed (Li & Ross 1993). It was estimated that the numerical ratio of strong-to-weak hydrogen bonds was of the order of 2:1. The two interaction strengths were attributed to dipole or other types of electronic interactions, caused by proton disorder and the relative strengths were estimated to be approximately 2:1 (Li et al 1994).

It has recently been found that water under extreme pressure at low temperature (493 atmospheres at $-40°C$), between two hydrophobic plates held a nanometre apart, will contract into a two-dimensional glass rather than expanding into a known ice form. This glass, known as Nebraska ice, is made up of approximately planar 5-, 6- and 7-membered rings (Koga et al 2000).

Hydrogen-bond geometry in water in the solid state
A wide-ranging survey of all the known ice structures, together with data for clathrates and simple hydrates, found that the length of the hydrogen bond is remarkably constant in all the ices and differs only slightly with a wide variety of hydrogen-bond donors and acceptors (Plumridge et al 2000). The hydrogen-bond angle normally assumes values in the range $180 \pm 25°$, consistent with maintenance of the O…O distance (Figure 2).

Pure water in the liquid state
While there is general agreement that the structure of liquid water is determined by hydrogen bonding, the structure has been notoriously difficult to describe. In liquids, atom topologies exist for very short lifetimes, so they can only be observed by techniques recording the structure on an even shorter timescale. The techniques available, in decreasing timescale resolution, are infrared and Raman spectroscopy, dielectrical absorption and NMR spectroscopy, and inelastic neutron scattering. Despite the increasing scope of these techniques, for example by using isotopic substitution to enhance neutron diffraction data, the very short lifetimes of particular liquid-phase topologies, estimated on the basis of relaxation times to be of the order of 10^{-12} s (Frank & Evans 1945), mean that the only structural information which can be obtained by any of these techniques has to be averaged over time. Thermodynamic properties can be derived from the averaged long-lifetime atom distributions obtained from X-ray and neutron scattering.
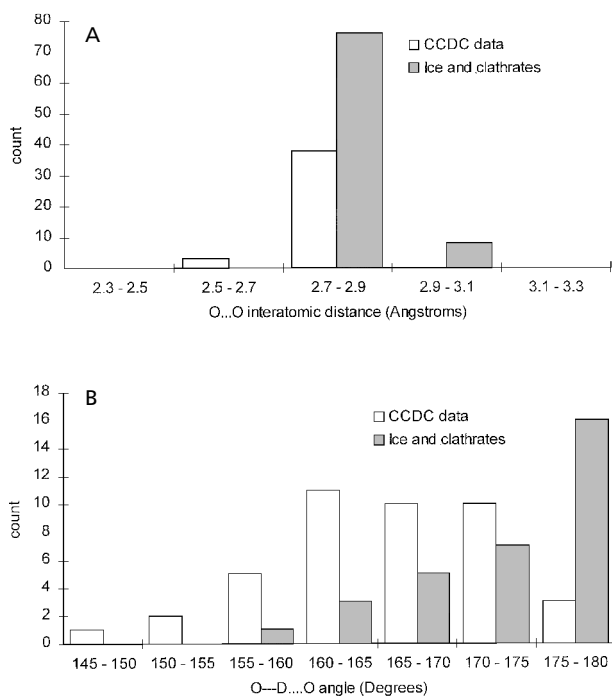

Figure 2 Survey of hydrogen-bond geometries in ices and clathrate hydrates, and small biological molecules from the CCDC. A. Distribution of O…O interatomic distances for ices and clathrate hydrates (grey bars) and hydrates of small biological molecules from the CCDC (clear bars). B. Distribution of O—D…O bond angle for ices and clathrate hydrates (grey bars) and hydrates of small biological molecules from the CCDC (clear bars).

In liquids, the molecules are in constant translational motion. Although this requires that long-range order is lost, there is still some short-range order. This short-range order has been found to extend to at least 8 Å even with totally inert, non-polar and spherically symmetrical molecules such as in liquid argon (Eisenstein & Gingrich 1942). The distance over which this short-range order exists should be greater when there is extensive hydrogen bonding, as in water.

There is a large number of anomalies in the physicochemical properties of water, including its unusually high melting point and boiling point, and the density anomaly. Whereas most liquids are less dense than their respective solids, water at 4°C is 8% more dense than ice. Upon melting, ice undergoes an increase in density that continues during heating until the temperature reaches 4°C. This contradicts the idea of thermal expansion, where most substances increase in volume with an increase in temperature. Above this maximum density, water behaves normally, decreasing in density with increasing temperature.

There are of the order of 38 such anomalies in the macroscopic properties of water and, while the physical bases of most are well understood, it has not been possible so far to devise a computer simulation method that can explain all of them.

Computer simulation of pure water

Since about 1980, increasing computing power has meant that complex simulations involving many hundreds of simulated water molecules have become possible. A large number of different models have been, and still are, proposed to represent water, and although each is capable of reproducing some of the macroscopic properties of water, no one model has explained all of them. Simulations involving just water molecules are now usually only run to test the ability of new water models to predict measurable thermodynamic properties of water and hence to refine the parameters involved in the simulation.

The models are usually used in conjunction with either molecular dynamics or Monte Carlo techniques, usually modelling dilute aqueous solutions involving of the order of a few hundred water molecules and usually only one or two solute molecules. These techniques are very powerful and are now routinely applied to increasingly complex systems such as docking ligands to hydrated protein structures.

Monte Carlo methods can be loosely described as statistical simulation methods where the simulation is driven by random numbers. Many simulations are performed (multiple trials or histories) using these random numbers and the desired result is taken as an average over the number of observations. In many practical applications, the statistical error can be predicted in this average result, and hence the number of Monte Carlo trials that are needed to achieve a given error can also be estimated.

The first Monte Carlo calculations on water that were able to simulate experimentally derived distribution functions with any degree of accuracy used 343 water molecules at 26°C (Ben-Naim 1971) using an improved Hartree-Fock potential with empirical corrections for the electron correlation energy. This success prompted Clementi and coworkers to lead a long and computationally expensive series of simulations beginning with the wave functions of water monomer, dimer, trimer and tetramer systems using potential functions from quantum mechanics, and Monte Carlo techniques to move the molecules classically (Clementi et al 1976). This study proved that accurate thermodynamic data could be obtained from ab-initio calculations in quantum chemistry (see Ab-initio section). At this time, no evidence from Monte Carlo studies had been found to support two-state or iceberg theories of liquid water.

Molecular dynamics simulation is a technique whereby successive configurations of the system are generated by integrating Newton's laws of motion, based on the positions and the velocities of the individual particles. In pictorial terms, atoms will move in the computer, bumping into each other, wandering around (if the system is fluid), oscillating in waves in concert with their neighbours, perhaps evaporating away from the system if there is a free surface, and so on, similarly to the way atoms in a real substance behave. This method allows the prediction of the static and dynamic properties of substances directly from the underlying interactions between the molecules.

Computer simulations have increased the demand for accuracy of the models. For instance, a molecular dynamics simulation allows the melting temperature of a material to be evaluated, and modelled with an interaction law. This is a difficult test for the theoretical model to pass so the simulation discloses critical areas and provides suggestions to improve the models.

When computer simulation results can be compared directly with experimental results, simulation becomes an extremely powerful tool, not only to understand and interpret the experiments at the microscopic level, but also to study regions which are not accessible experimentally, or which would need very expensive experiments.

Molecular mechanics studies of conformational preferences

Molecular mechanics treats molecules as atoms linked together with springs, allowing for harmonic bond stretching and bond angle bending. Each atom has finite spherical volume and relatively sharp boundaries with sinusoidal torsional energies. The force field for a typical molecule (e.g. a protein) can be given as a sum of the various components including bond stretching and bending, torsional potentials and non-bonded interactions. The resulting equations for the total energy can be combined with a variety of algorithms to locate energy minima on a conformational surface.

Models used to describe water

The TIP (transferable intermolecular potential) family of potentials has been used for modelling liquid water. TIP4P (Table 1) is a rigid four-site water molecule (Jorgensen et al 1983). This model attempts to capture the characteristics of a dipolar molecule by creating an imaginary fourth charged site on the bisector between the two hydrogen sites (Figure

Table 1 A comparison of common water models.

Model	Type	ε (kJ mol^{-1})	l_1 (Å)	l_2 (Å)	q_1	q_2	$\theta°$	$\Phi°$
SPC	A	0.6500	1.0000	–	+0.410	−0.8200	109.47	–
SPC/E	A	0.6500	1.0000	–	+0.4238	−0.8476	109.47	–
TIP3P	A	0.6364	0.9572	–	+0.4170	−0.8340	104.52	–
TIP4P	B	0.6480	0.9572	0.15	+0.5200	−1.0400	104.52	52.26
TIP5P	C	0.6694	0.9572	0.70	+0.2410	−0.2410	104.52	109.47

The type refers to the structures shown in Figure 7 (Jorgensen et al 1983; Mahoney & Jorgensen 2000).
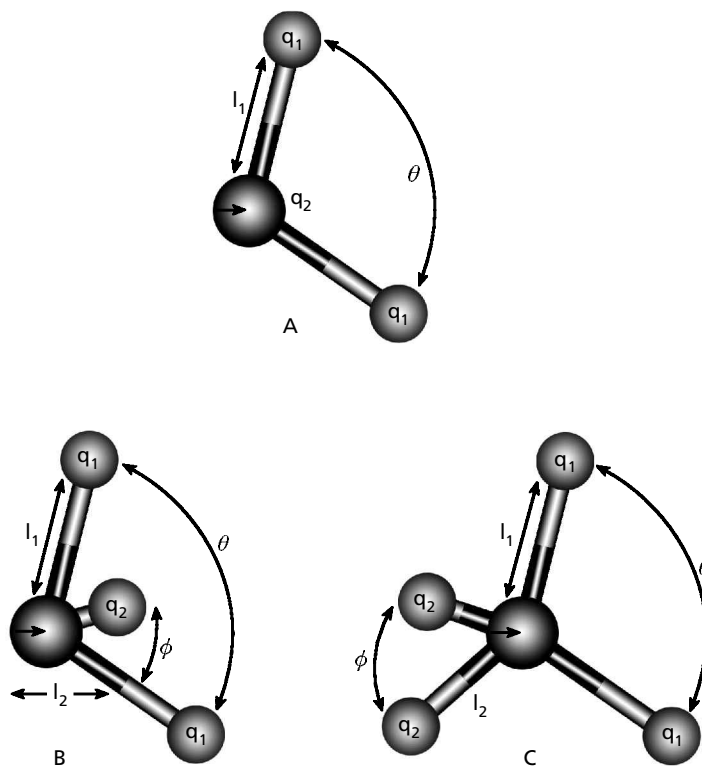
Figure 3 Models used to describe water.

3). The imaginary fourth site and the two hydrogens have an electrostatic charge, while the oxygen has only a Lennard-Jones potential. The parameters were obtained by fitting experimental, thermodynamic and X-ray structural data for liquid water at 298 K and 1 atm.

This model yields reasonable geometric and energetic results for the water dimer and is routinely used in simulations of aqueous solution (Barnes & Finney 1979).

SPC, the simple point charge model (Table 1), is an intermolecular potential function that gives good agreement with liquid water. This is a three-point charge model with a (6–12) Lennard-Jones potential on the oxygen, and charges of 0.41 on the hydrogens and -0.82 on the oxygen. The water geometry is shown in Figure 3, with O—H distances of 0.9572 Å, and an H—O—H angle of 104.52°. SPC/E is an extended version of this model.

Recently, the TIP5P model has been developed (Mahoney & Jorgensen 2000). This is a variation on the TIP3 and TIP4 families of models with five atom centres (i.e., the lone pairs are explicitly defined). Although this is a new model that has not yet been used widely, in-house development has shown that this model is more accurate than any previous simple models. The TIP5P model accurately predicts the diffusion constant of water at temperatures between -25 and 75°C and pressures between 1 and 300 atm. The predicted value is 2.62 ± 0.04 as compared with the experimental value of 2.30 (Mahoney & Jorgensen 2001). This is much better than any other method, with TIP4P predicting 3.29 ± 0.05, and signifi-

cantly improves the model's ability to describe the maximum density anomaly.

The water models are tested by how accurately they can reproduce quantitative information that can be determined experimentally. Table 2 shows that recent models can predict the physical properties of water with a reasonable degree of accuracy. However, the approximations involved mean that no present model can explain all the macroscopic properties of water (Finney 2001).

These and other similar models are routinely used in conjunction with either molecular dynamics or Monte Carlo simulation techniques to predict low energy configurations of aqueous solutions, or using ab-initio techniques if the system is simple enough to be solved by this method.

Ab-initio calculations
Ab-initio molecular orbital computation has reached the stage that in some areas the calculations are more accurate than experimental results. However, there are so many possible variables in terms of the position of the nuclei that even in a system such as $(H_2O)_3$ a vast amount of computer time is required to satisfy the statistical mechanics constraints.

It is possible to calculate extremely accurate wave functions for monomeric H_2O incorporating the effects of many thousands of excited configurations. Properties such as dipole moment, charge distribution and spectroscopic

Table 2 Comparison of physical properties of water calculated for each of the common water models with experimentally determined results.

Model	Dipole moment	Dielectric constant	Self diffusion $(10^{-5} cm^2 s^{-1})$	Average configurational energy $(kJ mol^{-1})$	Density maximum $(°C)$	Expansion coefficient $(10^{-4}°C^{-1})$
SPC	2.27	65	3.85	−41.0		
SPC/E	2.35	71	2.49	−41.5	−38	
TIP3P	2.35	82	5.19	−41.1	−13	9.2
TIP4P	2.18	53[a]	3.29	−41.8	−25	4.4
TIP5P	2.29	81.5	2.62	−41.3	+4	6.3
Expt	2.65, 3.0	78.4	2.30	−41.5	+3.984	2.53

Expt = experimentally determined. All the data are at 25°C and 1 atm, except [a]at 20°C (Jorgensen et al 1983; Kusalik & Svischev 1994; Mahoney & Jorgensen 2000).
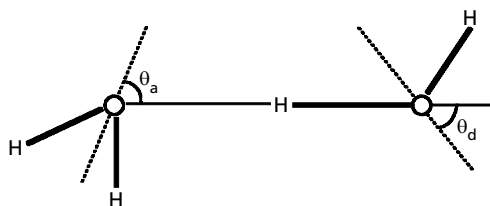

Figure 4 The structure of the water dimer. R_{OO} = 2.98 Å; θ_a = 58°; θ_d = 50° (Dyke et al 1977). Dotted lines are the bisectors of the H—O—H angles.

Figure 5 The global minimum conformation for five TIP4P molecules (Wales & Hodges 1998).

constants have been calculated many times in good agreement with experimentally determined values (see for example Goddard & Hunt 1974), but the techniques become much more computationally demanding when larger water complexes are considered.

The water dimer $(H_2O)_2$ has been studied spectroscopically (Dyke et al 1977) by expanding water vapour through a pinhole nozzle to produce a molecular beam of hydrogen-bonded dimers. Radio frequency and microwave transitions were observed, resulting from changes in the rotational energy of the dimer. These spectra were interpreted along with the dipole moment of the dimer to give the structure in Figure 4. This is the trans-linear complex and is in reasonable agreement with previous ab-initio calculations (Morokuma & Pedersen 1968; Lie & Clementi 1975), with the intermolecular distance in excellent agreement, good agreement with θ_d but only poor accord with the θ_a angle. Such comparisons between theoretically calculated structures without prior structural knowledge and experimentally determined structures allow models to be evaluated, and indeed many of the currently used models for liquid water are derived from potential functions tested on the water dimer. The best fit with the water dimer was given by a simple point charge model, similar to those proposed by Bernal & Fowler (1933).

The calculations become much more complex when considering three or more waters. The water trimer was investigated regularly in the 1970s and '80s (e.g. Del Bene 1971), but the structure remained unresolved; the investigators could not be certain whether it was an open structure
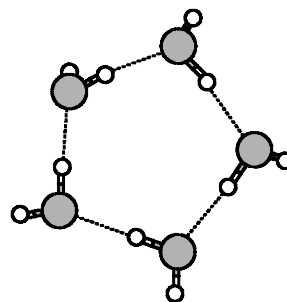
with two hydrogen bonds or a cyclic structure with three. McDonald & Klein (1978) concluded that there was little chance of reconciling the properties of the liquid and gaseous states on the basis of a single water dimer potential. The first accurate calculations on the structures of clusters involving three and four water molecules were carried out by Clementi and coworkers (Niesar et al 1990). They found that the extra waters make the clusters more stable than the dimer. Model potentials were derived which included many-body terms and when used in simulation of bulk water gave radial distribution functions that agreed well with those deduced from X-ray and neutron diffraction studies. Calculations using the best currently available potentials have indicated that a square ring of four water molecules and a cubic array of eight waters are particularly stable (Vegiri & Farantos 1993). Cooperative effects have also been demonstrated by ab-initio computation. It has been shown that a cyclic homodromic water tetramer has 30% more energy than four isolated dimers (Koehler et al 1987).

Various quantum mechanical calculations on randomly generated arrangements of water molecules have shown that rings are more stable than chains (Gregory & Clary 1996), and it has been experimentally verified that the predicted minimum energy structures are found in bulk water (Liu et al 1996). Figure 5 shows the global minimum energy conformation for five TIP4P water molecules.
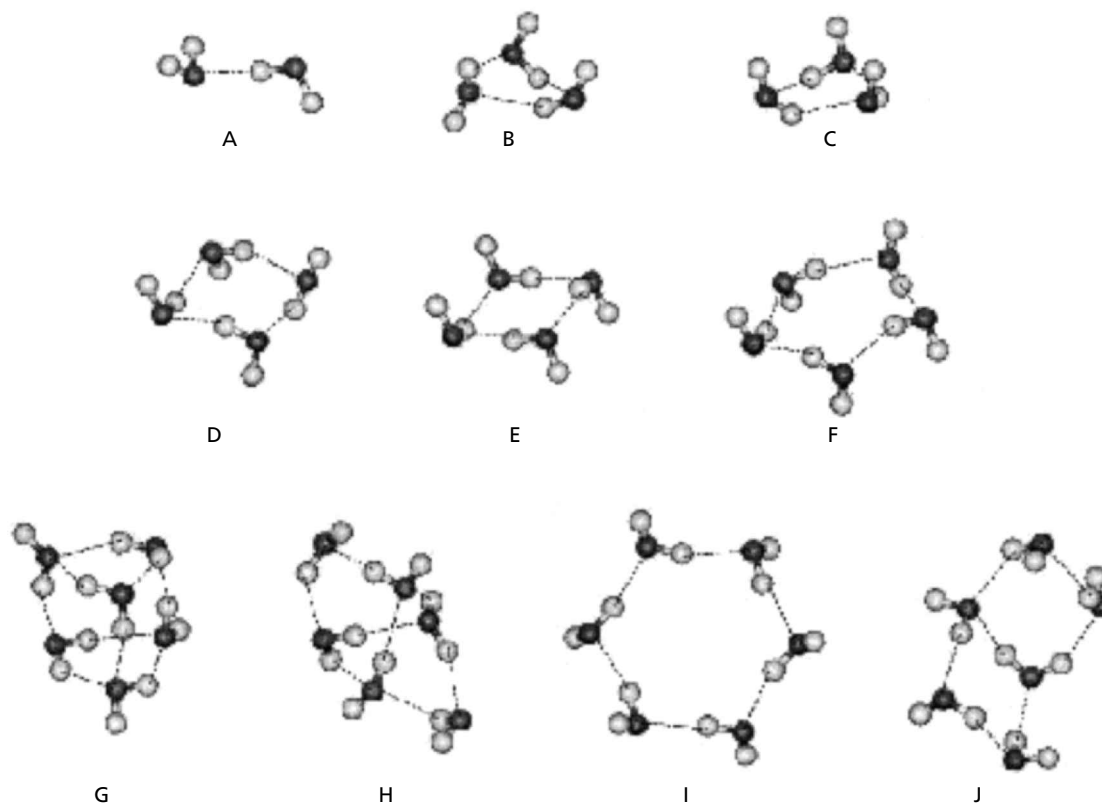
Weinhold (1998a) has used a combination of ab-initio

Figure 6   Geometry configurations, corresponding to the stationary points on the potential energy surface of the $(H_2O)_n$ clusters as predicted by the diatomics-in-molecules (DIM) method (reprinted from Grigorenko et al (2000) with permission from the American Institute of Physics and Dr Grigorenko).

computational techniques and Natural Bond Order analysis to shed light onto the electronic principles governing hydrogen-bonded clusters. The energetics of these clusters were found to be largely dependent on cooperative charge transfer which led to aggregation patterns markedly different from those predicted by the pairwise-additive potentials in common usage. Weinhold devised the quantum cluster equilibrium theory of hydrogen-bonded liquids in which the clusters are treated as the fundamental constituent units rather than individual water molecules. This method has been applied to water and has demonstrated numerical accuracy for a wide range of thermodynamic properties (Weinhold 1998b).

A related method by which potential energy surfaces of hydrogen-bonded aggregates are generated, the diatomics-in-molecules (DIM) theory has been developed to overcome some of the shortcomings of traditional molecular mechanics techniques using quantum mechanical calculations. The technique was applied to hydrogen fluoride clusters (Nemukhin et al 2000) and was found to give results in good agreement with both experimental information and previous simulations. The theory has been applied to water clusters of up to six molecules (Grigorenko et al 2000) and the minimum energy structures are shown in Figure 6.

This method is very computationally demanding, but the structures and binding energies of the complexes show a better correlation with experimentally derived terms than has previously been shown with any simulation method.

Various novel computational methods have been devised that bypass the conventional water models and methods. Chaplin (2000) attempted to explain water structure in terms of a network constructed from icosahedral water clusters. The 14-water structures (see Figure 7) can be joined together to form large networks. This model can explain some, though not all, of the properties of liquid water.

A further approach used the geometric information described in Figure 2 to constrain a simulation of water molecules with various solutes in which hydrogen bonds would be accepted if their length and angle fell within stated boundaries (Plumridge et al 2000). The simulation has built large ice-like structures and successfully distinguished between structure-making and structure-breaking solutes. This approach assumes that the hydrogen-bonding observed in solid water is similar to that in the liquid state, an assumption that appears more likely as modern techniques reveal the details of liquid water structure. This simulation would not succeed with a continuum model of water structure.

Water in contact with interfaces
Thermal expansion of water in glass capillaries shows normal behaviour in tubes of inner diameter greater than
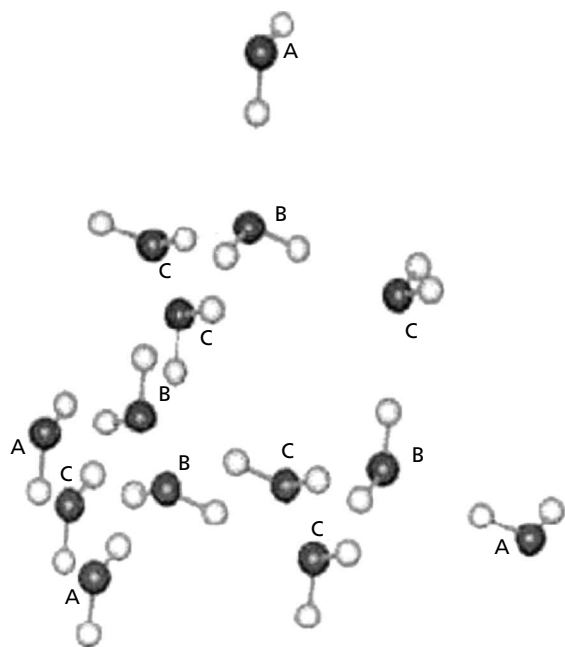
Figure 7   Icosahedral water cluster (reprinted from Chaplin (2000) with permission from Elsevier Science and Prof. Chaplin).

1 μm, but in narrower tubes the curves began to deviate. In fine capillaries the minimum specific volume at 4°C disappears and the coefficient of expansion becomes constant (Fedyakin 1962). Metsik studied the thermal conductivity of water between mica sheets, and found normal conductivity in films wider than 1 μm (Derjaguin 1970). However, as the film becomes thinner the conductivity rises sharply. At a thickness of 0.1 μm, the thermal conductivity is ten times that of normal water (Derjaguin 1970). It is worth noting that a 1-μm film is more than 3000 water molecules thick.

Hori (1956) measured the freezing point of water between mica sheets and polished glass surfaces. He found that if the film is between 1 mm and 10 μm, the freezing point is between −10 and −30°C. At a thickness less than 10 μm, he found no evidence of freezing down to −90°C. Hori also studied the equilibrium vapour pressure of thin water films held between a flat and a curved glass or quartz surface. When the film was thicker than 1 μm, the vapour pressure decreased with increasing temperature. Below this the vapour pressure was lower than that of the thicker film at the same temperature, and when the films were as thin as 0.1 μm there was no detectable vapour pressure at temperatures up to 300°C.

DeBoer & Zwikker (1929), in an early study of the condensation of water on glass surfaces, presented a theory whereby multi-layer condensation was attributed to a propagation of the electric polarisation at the polar solid surface. They also found that thicker layers built up if the solid surface contained alternating positive and negative charges. Ling (1971) found that if a surface contains positive and negative charges in a regular array, and in

particular if two of these surfaces are brought together, then deep layers of water will exist in an ordered array of dipolar lattices. A stabilised three-dimensional matrix of polarised water molecules may then be established.

Soviet soil scientists have studied water in soils, and categorised it as either being suspended (i.e., similar to bulk), loosely bound or bound. Bound water is the liquid in air-dried soils, and is no more than one or two molecules thick. The loosely bound water has been described as being formed by a process of multilayer sorption by successive polarisation of water dipoles. The thickness of such envelopes may be hundreds or even thousands of molecular diameters. Loosely bound water has a diminished capacity for dissolving electrolytes. (Forslind & Jacobsson 1975). NMR studies by Ducros (1960) and Woessner & Snowden (1969) have shown signal splitting in the deuteron signal of D_2O–clay systems, indicating that the dipole–dipole interactions do not average out as in liquid water, indicating deep layers of structured water.

Ever since the discovery (Derjaguin 1966) and subsequent demise of polywater (Rousseau & Porto 1970), most novel work on water has been viewed very sceptically by the scientific community. In fact, the analyses which disproved the polywater findings were also found to be flawed. The samples were only available in miniscule volumes and there were delays in the analysis. Some samples had no impurities, and some samples contained silica that was thought to be insoluble in water. An unrelated high-density form of liquid water has since been proved to exist at low temperatures (Mishima & Stanley 1998).

It is only recently that neutron diffraction and spectroscopic techniques have reached high enough resolutions to investigate pure water in confined conditions. A neutron diffraction study of water confined in Vycor glass, with pores of 40 Å on average, showed that the orientational preferences of the confined water molecules are very different from those of bulk water (Bruni et al 1998). Molecular dynamics using data from this experiment predict that the waters have very slow relaxation times, and this was thought to indicate the presence of longer-lived cavity structures encaging other waters (Starr et al 1999).

Recently, several groups have investigated water in confined conditions by means of ultrafast laser spectroscopy to shed light on dielectric relaxation times (Bhattacharyya & Bagchi 2000). The most striking of the results is that water, in a variety of conditions, exhibits a bimodal response, with not only a normal fast sub-picosecond response as would be expected from bulk water, but also a slow component in the timescale between hundreds and thousands of picoseconds. This slow component constitutes 10–30% of the total response and is totally absent in pure water. The confined environments in these studies were a range of molecular assemblies, such as reverse micelles and microemulsions (Riter et al 1998), cyclodextrin (Vajda et al 1995), micelles (Telgmann & Kaatze 2000), lipids (Datta et al 1998), proteins (Jordanides et al 1999) and DNA (Halle & Denisov 1998), as well as several macroscopic solids which trap water (e.g. hydrogels (Datta et al 1997)). In all cases, the slow component was detected and, after discussion between the groups, its origin was attributed to a

dynamic exchange between free and bound water. A comprehensive understanding of the mechanism and implications of this phenomenon is not yet available.

Overall, it is apparent that water in contact with regular hydrogen-bonding surfaces can adopt the pattern expressed at the surface and extend that pattern out into the bulk water, at least to several hundred molecular diameters. Such well-characterised effects are reminiscent of the early ideas on cellular water (see above). It would be ironic if the very earliest ideas on cellular water were found to have merit, nearly 150 years after they were first expressed.

Aqueous solutions

Solid solutions

The advantage of studies on solids is that all the intra- and inter-molecular parameters can be measured with confidence. While extrapolation to liquids must be approached with caution, some valuable conclusions can be drawn concerning the intrinsic properties of aggregates of water molecules. These aggregates may be present as simple or complex hydrates, or clathrates. There is increasing evidence for the formation of comparable structures in liquid water.

Hydrate inclusion compounds

Hydrate inclusion compounds are solid crystalline complexes in which water molecules are hydrogen bonded to form regular three-dimensional four-connected networks. Guest molecules are included either in void spaces in the network, or hydrogen bonded to the water network. At present, four classes of hydrate inclusion compounds are known: clathrate hydrates, alkylamine hydrates, alkylammonium salt hydrates and the polyhydrates of some strong acids.

Clathrate hydrates are a type of hydrate inclusion compound in which a small, usually non-polar, guest molecule is encaged in a host network of hydrogen-bonded water. The guest in true clathrate hydrates will only interact with the host through van der Waals interactions with no other guest–host bonding involved. In the alkylamine hydrates, hydrogen bonds are formed between the amine group and the water network. Alkylammonium salt hydrates are formed with an anionic guest inside a cationic host lattice.

The first reported preparations of a crystalline clathrate hydrate were made by Davy in 1811 and then by Faraday in 1823, who were both investigating the hydrate of chlorine. The term clathrate was introduced by Powell (1948), who deduced that these were, in fact, inclusion compounds and named them from the Greek for lattice. Concurrent work by three teams, von Stackleburg & Muller (1951), Pauling & Marsh (1952), and Claussen (1951a, b) established the common 12-Å and 17-Å clathrate structures. These structures, now referred to as structures I and II, accounted for the stoichiometry and powder diffraction patterns for the majority of known hydrates. The most common clathrate cavity types are shown in Figure 8. Structure-I clathrates are comprised of pentagonal dodecahedra and tetrakaidecahedra (A, B) while structure-II clathrates are built from pentagonal dodecahedra and hexakaidecahedra (A, C). The pentagonal dodecahedron corresponding to $(H_2O)_{20}$ occurs in all true clathrate structures and many related inclusion compounds.

Clathrates are known to occur in natural environments, from deep-sea sediments (Appenzeller 1991) to nucleation processes in the atmosphere (Yang & Castleman 1991). Upon decomposition they can cause explosions in high-pressure gas pipelines (Kelkar et al 1998).

A clathrate of CH_3CCl_2F (CFC R-141b) has been observed to form at the interface between liquid water and liquid CH_3CCl_2F (Ohmura et al 1999). The system was observed with a video camera with a micrographic zoom lens, and plate-like crystals were formed over a number of hours, that dissolved if the temperature was raised. The water shell was apparently left unchanged, as the crystals reformed if the temperature was lowered again.

A series of alkylammonium salt hydrates were first prepared by Fowler and co-workers in 1940 (Fowler et al 1940). Unlike the clathrates, each guest molecule produced a different host water lattice. At the request of Henry Frank, McMullan & Jeffrey (1959) carried out an initial X-ray diffraction study of these compounds. This work revealed structures very similar to the clathrates and a subsequent detailed study showed close structural
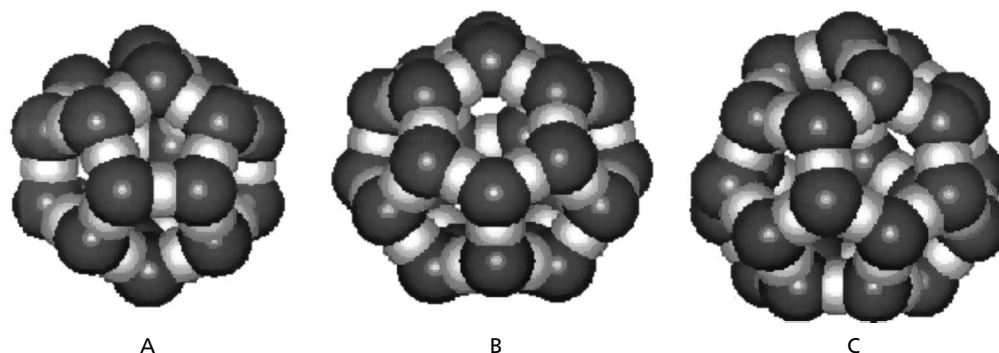

Figure 8 The cavity types in structure-I and -II clathrate hydrates. A. Pentagonal dodecahedron $(H_2O)_{20}$. B. Tetrakaidecahedron $(H_2O)_{24}$. C. Hexakaidecahedron $(H_2O)_{26}$.

analogies, with some alkylammonium salt hydrates being isostructural with clathrate hydrates.

Jeffrey and co-workers carried out similar diffraction studies of alkylamine hydrates, which again had different lattice structures for each guest molecule (McMullan et al 1966). These structures included hydrogen bonding between the guest and the water lattice, but again there were many similarities with the clathrates. Using results from these three types of compound, a common structural theme was discovered, based on the $(H_2O)_{20}$ pentagonal dodecahedron.

Some strong acids (HPF_6, HBF_6 and $HClO_4$) were found to form crystalline hydrates at lowered temperatures, and X-ray diffraction confirmed that they were iso-structural with the 12-Å cubic structure-I clathrate hydrates. These were the first clathrate structures where cationic hosts were found (Mootz et al 1987).

In inorganic hydrates, the water molecules generally complete the coordination shell of the cations, and hydrogen bond to the anions. This means that the structures formed are governed largely by the stereochemical requirements of the ions rather than those of water.

Organic hydrates can be subdivided into hydrates with isolated water molecules, in which the structure is determined principally by the ionic coordination or hydrogen-bonding function of the solute, and water framework structures. Water framework structures include the hydrate inclusion compounds, and also systems in which water molecules form nets, sheets, columns, ribbons or chains, with the functional groups of the organic molecules strongly influencing the structure, and no recognisable clathrate voids (Luck 1998).

The three-dimensional structures of all biological macromolecules are intimately associated with water. The solid-state hydration structures of small biological molecules (carbohydrates, purines, pyrimidines, nucleosides and nucleotides) are determined mainly by packing forces, and hydrogen bonding between the functional groups of the organic molecules. Water plays a secondary role in these structures, occupying the space between the organics, and adding to the hydrogen bond energy of the lattice while in competition with the molecular packing in the absence of water. If biological molecules aggregate, or if a substrate enters the active site of an enzyme, the water molecules have to move from the contact surface of the biological molecule in a coordinated manner with the least expenditure of energy. Hydrogen bonding must play an important role in this substitution process (Jeffrey & Saenger 1991).

Aqueous solutions in the liquid state
As has been stated above, the structural information that can be obtained from diffraction studies on liquid species is limited because of the continual translational and rotational movement of the molecules. However, the increasing sophistication of computer simulation techniques along with accurate models of the water molecule has allowed the structural aspects of aqueous solutions to be investigated.

Computer simulations
Molecular dynamics studies on aqueous solutions Molecular dynamics has been used to study the hydrophobic hydration of noble gases (Tanaka & Nakanishi 1991). It was found that the introduction of a xenon solute gives rise to a restructuring of the water to form a clathrate-type structure. This is shown by an increase in population of the cyclic water pentamer, which gives rise to an exothermic hydration process.

A molecular dynamics simulation (Skipper 1993) of a system containing four methane molecules and 256 waters at around room temperature found solvent-separated methane interactions which were compatible with a first hydration shell in which the water dipole vectors were predominantly tangential, and a second hydration shell where they were predominantly radial. This orientational information and the sizes of the cavities are compatible with a simple clathrate-type structure. A similar, though more exhaustive and computationally more complex, study (Marcera et al 1997) confirmed these findings.

Molecular dynamics has also been used to study the hydrogen-bonded network in liquid water. In a study of liquid water, topological defects were found which directly affected the mobility of the water. The network defects act as catalysts, providing lower energy pathways between different tetrahedral local arrangements (Sciortino et al 1992).

Molecular dynamics has been used to investigate water clusters. $(H_2O)_n$ clusters, where n = 3–6, were investigated by Dang & Chang (1997). They found cyclic planar structures to be the most stable for n = 3–5. For the water hexamer, several structures were suggested, very close together in energy. A prism-like structure was found to be the lowest energy structure, with cyclic boat and cage structures at slightly higher energy. Using the same methods, Dang (1999) characterised the water octamer, nonamer and decamer, and investigated the water–iodide cluster. The lowest energy octamer was found to be a rough cubic structure with four-membered rings, the nonamer was a similar system with a five-membered ring, and the decamer was found to be a system with two five-membered rings one above the other with hydrogen bonding between the rings. The iodide–water clusters are in excellent agreement with optimised structures developed from accurate electronic structure theory calculations (Combariza et al 1993).

Molecular dynamics has been used together with a number of novel water potentials (van Maaren & van der Spoel 2001) designed to investigate biomolecular hydration. Flexible, as well as fixed, water models were tested and it was found that varying the water potentials based on their distance from the biomolecule improves the accuracy of the method to reproduce measurable liquid-phase properties. This work, though still in its early stages, could help to elucidate the role of water in biological processes.

Another water potential has been devised to reproduce long-range effects by putting diffuse charges on the hydrogens and oxygens as well as the normal point charges (Guillot & Guissani 2001). This method can accurately

model polarisation effects when calculating the dipole moment, and so the technique can accurately predict the dielectric constant at any state point. This supports the idea that induction effects in water are more important than previously expected.

Monte Carlo simulations of aqueous solutions. The first Monte Carlo simulations of aqueous solutions were reported for the systems Li^+F^- and Li^+Cl^- (Fromm et al 1975) and Na^+F^- and K^+F^- (Clementi et al 1976). The coordination and shell radii were found to be in good agreement with neutron diffraction experiments.

The first Monte Carlo simulation claiming to reproduce the iceberg model was carried out on pure water and an infinitely dilute solution of methanol (Nakanishi et al 1981). Water molecules in the vicinity of the hydrophobic methyl group are energetically more stable than those in the hydrophilic region. This is clear evidence of conventional hydrophobic hydration where a water cage structure forms around the hydrophobic group.

A study of the aqueous hydration of benzene has shown a complex with two water molecules attached, one either side of the π-cloud, and a hydration shell with 21 water molecules around this (Ravishanker et al 1984). The structure involves puckered 5-membered rings of waters, and also larger ring structures. Water–π interactions have been observed in aqueous benzene using X-ray diffraction (Atwood et al 1991). This system has been re-investigated (Urahata & Canuto 1999) with more advanced techniques. The results confirmed these findings, the authors concluding that the first hydration shell must be similar to a clathrate cavity.

In a similar study of the hydration of small alkanes, the hydration numbers in the first hydration shell were determined using a TIP4P model of water together with an OPLS set of Lennard-Jones parameters optimised for liquid hydrocarbons (Jorgensen et al 1985). The hydration shells were consistent with clathrate structures with more irregular forms. Methane formed a structure-I clathrate with an $(H_2O)_{20}$ cavity in close agreement with the Monte Carlo value of $(H_2O)_{20.3}$ (Jeffrey & Saenger 1991).

A study of the hydration of ethanol with TIP3P waters using Monte Carlo techniques predicted the hydration energy of -57.1 ± 6.5 kJ mol^{-1}, agreeing well with the experimental value of -54 kJ mol^{-1} (Levchuk et al 1991). This meant that more complex problems could be tackled. In an attempt to understand the interactions between protein denaturants and aromatic rings, the approach of urea and guanidinium to benzene, and the approach of urea to naphthalene in aqueous solution were studied by Monte Carlo techniques (Duffy et al 1993). Single minima were observed at around 4 Å in all cases, which indicated the existence of direct interactions between the structure breaker and the aromatic ring with unstructured water in the vicinity.

Recent Monte Carlo studies have shown that water structuring around hydrophobic solutes is very sensitive to solute size and shape (Fang et al 1997). The hydration structure around hypothetical spherical ions was shown to generate clathrate-type structures if the radius of the ion was within specific limits, whereas different sized and shaped ions produced much more irregular structures. A similar investigation (Martinez et al 1999) showed that, when considering small highly charged ions such as Cr^{3+}, it is more useful to consider the hydrated ion's interactions with bulk water rather than the naked ion (e.g. $[Cr(H_2O)_6]^{3+}$ is used rather than Cr^{3+}). This has been shown to reproduce experimental radial distribution functions more accurately.

A Monte Carlo study of the hydration of methane (Hernandez-Cobos et al 2001) gave essentially the same results as the molecular dynamics simulation (Skipper 1993), and the direct experimental methods (Bowron et al 1998c – see EXAFS spectroscopy section, below) with disordered hydration shells around the methane. The radial distribution functions from the simulation compared very favourably with those for aqueous methane (Soper & Finney 1993).

Advanced Monte Carlo techniques involving specialised potentials have been applied to the hydration of Li^+ (Lyubartsev et al 2001). The results show almost identical radial distribution functions to those derived from neutron diffraction (Howell & Neilson 1996) with four waters arranged tetrahedrally around the ion.

Reverse Monte Carlo (McGreevy 1990) is a computer simulation technique which works with scattering patterns derived from, for example, neutron diffraction or EXAFS patterns (see below). The process then generates configurations that will fit the radial distribution curve. No potentials are used, and the system works by optimising the difference between the experimental pattern and the pattern generated by the trial system. This has been applied to liquid water (da Silva et al 2001) and has successfully reproduced energy terms and hydrogen-bond configurations, but flaws in the existing water models have been highlighted in this study.

Experimental evidence for the structure of liquid aqueous solutions

X-ray and neutron diffraction. In an X-ray diffraction study of aqueous *t*-butanol at 20°C, Nishikawa & Iijima (1990) found that, at mole fractions around 0.17, some *t*-butanol molecules were encaged by water molecules in clusters, which tended to clump together in larger aggregates of structured water by sharing their polyhedra. They found that for compositions $(TBA)_m(H_2O)_l$ the ratio l/m is between 17 and 25, consistent with the structure-II clathrate structure. At a higher temperature (55°C) they found evidence of clusters, but at this temperature the structures were looser and more distorted.

In an attempt to understand the structure-breaking properties of nitrate and perchlorate ions, neutron diffraction experiments have been carried out on concentrated aqueous solutions using isotopic substitution on the chlorine. The results showed that both species have a reduced number of waters in the first hydration shell: 3–4 for NO_3^- (Neilson & Enderby 1982) and 4–5 for ClO_4^- (Neilson et al 1985) compared with, for example, the value

obtained for Cl^- of 7–8 (Neilson & Enderby 1983). The distances between the central atom and the deuteriums and oxygens of water are very similar in all three cases (carbon–deuterium distance = 2.9–3.1 Å; carbon–oxygen = 3.6–3.8 Å). This is a consequence of the strength of the structure-breaking properties, nitrate > perchlorate > chloride.

A more recent higher resolution neutron diffraction study on the hydration of chloride (Yamaguchi & Soper 1999), using a more advanced procedure to refine the data, found 9–10 waters in the first hydration shell, but found that only 5–6 pointed a deuterium at the chloride atom (i.e., were involved in conventional hydration).

The use of hydrogen/deuterium (H/D) isotopic substitution in structural studies of water and organic liquids provides more information, particularly when hydrogen bonding is involved (Soper & Egelstaff 1981). Separated partial distribution functions can be obtained from the radial distribution function when partially deuterated species (e.g. HDO) are used which can give direct information on local hydrogen-bond configuration. This technique can also be used to acquire more structural information when dealing with aqueous solutions (Turner et al 1990; Bowron et al 1998c).

A neutron diffraction experiment on a 1:9 molar ratio methanol–water mixture, in which isotopic substitution was used to isolate the methyl-hydrogen to water-hydrogen pair correlation function from the other interactions, confirmed the existence of a definite hydration shell of water molecules at a distance of 3.7 Å from the methyl carbon atom (Soper & Finney 1993). The water molecules in this shell formed a disordered cage but retained the roughly tetrahedral local coordination found in pure water. Some degree of polarisation of the water molecules was also apparent, and the shape of the shell implied a weak alignment of the water molecule dipoles with that of the methanol. This was the first experimental evidence of a hydration shell around methanol, and served to reinforce Frank's theories, as well as closely agreeing with the results of various simulation studies (Okazaki et al 1984; Ferrario et al 1990; Guillot et al 1991; Skipper 1993).

Neutron diffraction with isotopic substitution was used to study the hydration structure of methane in aqueous solution (De Jong et al 1997). H/D substitution was used on both the solute and the solvent to determine the partial pair correlation functions. Analysis of these functions showed that the methane molecule is surrounded by 19 ± 2 water molecules, and showed that these waters were tangentially oriented with respect to the methane. This figure is in excellent agreement with the pentagonal dodecahedron, with twenty waters found in clathrates and many simulations.

A recent study, on concentrated sodium hydroxide in the liquid state using neutron diffraction with isotopic substitution (Bruni et al 2001), showed that a large change in the water structure relative to that of pure water occurred at around 300 K. The peaks corresponding to orientational correlation between neighbouring water molecules were considerably shifted relative to those of pure water and this was thought to indicate that ions in aqueous solutions induce a change in water structure equivalent to the application of high pressure.

Dielectric measurements. It is known that the dielectric relaxation times of liquids are influenced by temperature and concentration. The relaxation times can be derived from time domain reflectometry in the microwave region (commonly 500 MHz to 25 GHz) at different temperatures and concentrations. In considering interactions in water mixtures, accurately measured dielectric relaxation times allow the activation enthalpy (ΔH) and entropy (ΔS) to be separated from the activation free energy (ΔG), and hence allow excess partial molar activation free energy, enthalpy and entropy to be calculated for the water and for the other component as a function of temperature and concentration. Maxima in the excess enthalpy and entropy are due to structural enhancement of the water network by the other component.

In a study of ethanol–water mixtures using these methods (Sato et al 1999), two maxima were found in both the excess enthalpy and entropy of ethanol in the dilute region. These maxima occurred at mole fractions of ethanol in water of 0.04 and 0.08 which were clearly attributed to structural enhancement of the water network by the ethanol. The mixing schemes for the two points appear to be qualitatively different. The 0.04 mole fraction corresponds to a ratio of one ethanol to 24 water molecules, possibly indicating a clathrate-type cage. The 0.08 mole fraction refers to a ratio of one ethanol molecule to 12 waters; the type of structural enhancement associated with this maximum is unknown. At mole fractions of ethanol as low as 0.005 (one ethanol to around 200 waters), positive values in the rates of change of the excess enthalpy and entropy indicate that ethanol–ethanol interaction through the water network is operative with each ethanol surrounded in a spherical fashion by waters. At a mole fraction of approximately 0.15, a minimum is observed in the excess enthalpy and entropy of ethanol and a maximum in those of water. This can be attributed to the formation of a structure-I clathrate hydrate of the formula $6C_2H_5OH.46H_2O$. This is also suggested by adiabatic compressibility results on the ethanol–water system (Onori 1988).

In a further study of methanol–water mixtures using the same techniques (Sato at al 2000), again two maxima were found in the methanol excess enthalpy and entropy in the dilute region, both attributed to structural enhancement of the water network.

Microwave dielectric relaxation measurements have been used to study water structure around larger molecules. In a study of polysaccharides, all molecules showed two dielectric relaxation peaks, except for glucose which only showed one (Mashimo et al 1992). One peak is due to the orientation of the water molecules whereas the other is due to the orientation of the saccharide molecules. Glucose can occupy a site in an ice lattice corresponding to the position of a six-membered water ring. From this it was inferred that glucose behaves in a similar manner in liquid water, with several hydrogen bonds between glucose and waters, and that the glucose can only reorient itself when the water structure is broken.

Similar studies have been carried out using a sample of DNA (Mashimo 1994). The sample was dissolved in a buffer and subjected to microwave dielectric measurements at different temperatures. The DNA solution exhibited two peaks at all temperatures, attributed to bound and bulk water. However, measurements between -15 and $-65°C$, where all bulk water was frozen, showed definite evidence of the participation of two types of bound water. Part of the water would freeze gradually in the temperature range -15 to $-35°C$, but some water would not freeze at $-65°C$. These phases were shown to be different from bulk water, and each other, so were designated as tightly bound and loosely bound.

NMR. Deuterium spin-lattice relaxation times were reported for undercooled (180–300 K) solutions of tetra-alkylammonium ions (Bradl & Lang 1993). A comparison with undercooled alkali-metal halide solutions allowed the competing influence of coulombic, hydrophobic and hydrogen-bond interactions on the dynamic structure of the transient hydrogen-bonded network to be studied. Solvent dynamics were found to be closely related to the glass-forming tendency of undercooled Me_4N^+ and Pr_4N^+ solutions, and the clathrate-forming tendency of the Bu_4N^+ solutions.

The NMR rotational correlation times for heavy water molecules have been measured in aqueous solutions of benzene and phenol. The measurements were made by the integrated capillary method and showed that the correlation time for shell water molecules was 4 times as long in benzene as in phenol. Benzene is a known structure maker, while phenol is a structure breaker. The increased correlation time indicates that the hydrophobic hydration of benzene is associated with a remarkable rotational slowdown, or increase in local viscosity (Nakahara & Yoshimoto 1995).

In a similar study, the ^{17}O NMR spin-lattice relaxation times of the solvent water have been determined in aqueous solutions of nine alcohols and five diols at 25°C (Ishihara et al 1997). From these data, the coordination numbers and rotation correlation times compared with pure water have been estimated. The correlation times are inversely related to the alcohol's structure-breaking capability, so *t*-butanol provides the longest-lived water structure.

Fourier transform infrared spectroscopy. Fourier transform infrared (FTIR) spectroscopy can be used to give insight into the structural perturbations which occur when hydrophobic solutes are introduced into water by examining the vibrational transitions occurring in the solute-induced hydration shells. Infrared spectroscopy has been used to detect the vibrations of DOH molecules in the solvation shells of inorganic salts (Kristiansson et al 1988). By a calculated difference spectrum, where the spectrum of pure water is subtracted from that of a solute in water, the water-structuring capacity of solutes can be quantitatively compared. Shifts in absorbance intensity and frequency reflect changes in populations of water molecules with different hydrogen-bonding patterns (Lindgren & Tegenfeldt 1974). In a study of methane, ethane, propane, butane, pentane and hexane sulfonates, Hecht et al (1992) compared the difference plots obtained from the FTIR analysis, and found shifts in the vibrational intensity and frequency which were consistent with increased ordering of the water shell as the hydrocarbon length was increased. These results are consistent with theories of hydrophobic interactions as well as other experimental techniques such as excess molar heat capacity measurements (Muller 1990) and relative solubility in aqueous and non-aqueous solutions (Nozaki & Tanford 1971).

Heat capacity measurements. The analysis of heat capacity changes is of central importance in understanding the thermodynamics of protein folding, protein–protein binding, protein–nucleic acid binding and hydrophobic effects (e.g. Murphy & Freire 1992). The improved sensitivity and accuracy of calorimeters has provided much heat capacity data. Heat capacity is useful as it relates enthalpy, entropy and free energy to each other. Processes such as protein folding or unfolding produce large changes in heat capacity due to the desolvation of polar and nonpolar groups (Sturtevant 1977). The change in heat capacity involved on hydration is large and positive for nonpolar groups, and negative for polar groups (Marcus 1994). This is more useful than entropy and enthalpy measurements as these hydration processes can be either entropy- or enthalpy-driven. Computer simulation has suggested that heat capacity changes can be explained by non-polar solutes causing a concerted decrease in the hydrogen-bond length in the first hydration shell and polar solutes increasing the oxygen–oxygen distance, and causing the hydrogen bonds to bend (Sharp & Madan 1997).

HPLC. The molar free energy, enthalpy and entropy of transfer of a non-polar solute from a highly polar liquid (e.g. poly(dimethylsiloxane)) into water can be deduced from HPLC measurements (Shinoda et al 1987). In a study of toluene and ethylbenzene in water, Silveston & Kronberg (1989) found a large positive free energy when the two probes were transferred from the polymer liquid into water, along with a large negative entropy change. This was interpreted as a consequence of water structuring around the toluene and ethylbenzene molecules.

EXAFS spectroscopy. Recent work on the hydrophobic hydration of krypton, using extended X-ray absorption fine structure (EXAFS) spectroscopy (Filipponi et al 1997; Bowron et al 1998a), has shown that the liquid-to-solid transition produces a clathrate hydrate, indicating that Frank's structuring of liquid water by non-polar solutes may be very similar to clathrate hydrate structures.

The EXAFS probe is intrinsically short-ranged and element specific, so is ideal for comparing local structure in the solid and liquid phases due to its insensitivity to long-range order in the crystalline phase. In this case the structural signal was dominated by $Kr–O_{(water)}$ correlations.

The size of the liquid hydration shell was found to correlate very well with the small structure-II clathrate cage, but this cage was more loosely defined than in the solid state. The experimentally determined coordination numbers indicated that one water molecule was drawn into the 4.5–5.1 Å range to form the large clathrate cavity, although the number of water molecules in the 2.6–5.7 Å region remained constant at 22.5 in both states. This figure agrees very well with the values for structure-II clathrates, where the small cavity is composed of 20 waters and the large cavity 26.

In a further EXAFS study, the temperature dependence of the hydrophobic hydration shell was investigated for krypton and xenon in the range 277–368 K (Bowron et al 1998b). The experiment provided clear evidence that as the temperature is raised, systematic thermal perturbation leads to increased entropy, and the cage becomes less clearly defined.

The hydration of Ca^{2+} was investigated in a combined EXAFS, large angle X-ray scattering and molecular dynamics study (Jalilehvand et al 2001). The results accurately confirmed the radii of the first and second hydration shells at 2.46 and 4.58 Å, as well as the number of waters in the first shell (8). These findings agreed well with existing experimental data on the system and the molecular dynamics results.

Structured water in the gaseous state

Clusters are weakly bound aggregates of atoms or molecules. Water clusters occur in the gaseous phase and have been studied extensively to further an understanding of hydrogen bonding, and solvation and nucleation phenomena in general. In the mass spectra of polymeric compounds or complexes the appearance of prominent peaks in an otherwise continuous distribution of signals is called a magic number cluster, and may indicate the existence of species with enhanced stability.

In water systems, it is well known that the cluster corresponding to $(H_2O)_{21}H^+$ always exhibits a pronounced magic number under different experimental conditions (e.g. expansion of ionised vapour (Beuhler & Friedman 1982), ion bombardment of ice surfaces (Haberland 1984); electron impact ionisation (Echt et al 1989) and vacuum photoionisation of neutral clusters (Shinohara et al 1985)).

Shinohara et al (1985) employed a neutral supersonic nozzle linked to a molecular-beam mass spectrometer supplied with premixed water–ammonia gas, to investigate the formation of mixed binary water–ammonium clusters. Evidence was found for exceptional structural stability of protonated clusters corresponding to $(H_2O)_{20}(NH_3)_mH^+$ (m = 1–6) and $(H_2O)_{27}NH_4^+$. A parallel Monte Carlo simulation yielded larger binding energies for these structures compared with their close neighbours, in agreement with the mass spectrometry results, and a deformed pentagonal dodecahedron enclosing an NH_4^+ ion was proposed, with the stability due to strong coulombic interactions (ionic hydrogen bonding) between the NH_4^+ and the 20 waters, as well as the inherent stability of pentagonal rings and the pentagonal dodecahedron. The authors suggested that this

may also explain the exceptional stability of the $(H_2O)_{21}H^+$ cluster. To account for the exceptional stability of this cluster, a pentagonal dodecahedral structure has been proposed (Miller et al 1983) in which H_3O^+ is encaged. If this structure were present, then it is known from clathrate studies that ten non-bonded hydrogens would extend outward from the cage.

In a mass spectrometry study of water–trimethylamine clusters, Wei et al (1991) found that the maximum number of trimethylamines that can bond to an $(H_2O)_{21}H^+$ cluster is ten, indicating that the pentagonal dodecahedron is present.

The same group carried out a similar experiment in which methanol was introduced into the system. Methanol can replace the water molecules in the $(H_2O)_{21}H^+$ cluster only where the CH_3 group can extend outward from the cage, so it was expected that a maximum of ten methanol molecules would replace water molecules in a single cluster. It was found that this was the case, as $(H_2O)_n(CH_3OH)_mH^+$ showed maximum intensity at n + m = 21 for m = 1–9 (Shi et al 1992).

Other cavity types found in clathrate hydrates have also been found as clusters in the gaseous phase. Selinger & Castleman (1991) found that magic numbers were exhibited in clusters surrounding small ions such as Cs^+, corresponding to $Cs^+(H_2O)_{20}$, but also found magic numbers at 22, 24, 27 and 29 water molecules. All these systems can be rationalised using clathrate-type cages involving 5- and 6-membered rings of water molecules. $Cs^+(H_2O)_{24}$ corresponds to the large cavity in structure-I clathrates, and $Cs^+(H_2O)_{27}$ corresponds to the large cavity in structure-II clathrates with Cs^+-H_2O enclosed.

Similar structures have been observed around other small ions such as K^+, Li^+, Na^+ and Rb^+ (Steel et al 1995).

Ionic clathrates have been observed by mass spectrometry from aqueous solution using laser-induced liquid-beam ionisation/desorption (LILBID) techniques (Sobott et al 1999) around K^+, Cs^+ and ammonium ions. The largest peak was observed at $(H_2O)_{20}$ as has been demonstrated above. Other peaks were observed corresponding to other clathrate cages encaging ions or ion–water pairs, but the ammonium ion showed a single peak at $NH_4^+(H_2O)_{20}$, corresponding to the pentagonal dodecahedron.

Biological systems

Crystallographic studies have revealed a wide range of hydration processes in biological macromolecules, ranging from neo-clathrates down to single hydrogen-bonded waters. The formation of the more complex hydrates may give some insight into the processes occurring in solution.

The most direct method of examining the hydration of a macromolecule is by using X-ray or neutron diffraction at a resolution better than 1.8 Å. In the crystal structures of proteins the macromolecules are heavily hydrated; 20–90% of the total volume is water. The amino-acid atoms at the periphery of the molecules display larger thermal motion than those in the interior and, as the hydrating water molecules are attached to these, they sometimes have even

larger thermal motions and this can lead to ambiguities in the analysis concerning the water positions (Soper & Phillips 1986).

In biological systems, water is in a wide range of environments. Far away from a protein, for example, water should have a structure similar to the bulk, unless the protein produces an effect comparable with that of a silica surface, in which case the effect could be felt many molecular diameters away from the macromolecule.

Ordered water molecules at protein surfaces – clusters and pentagons

Most water molecules identified from X-ray electron density maps are individually bound to the protein with one or more hydrogen bonds formed with main-chain and side-chain functional groups. Two-water chains are also found in significant numbers bound to the surface of the protein. Larger clusters occur rarely but are of special interest as hydration phenomena. Clathrate hydrates form with small guest molecules that have little or no hydrogen-bonding character. In this sense the water structure around proteins must be different as the strong hydrogen-bonding groups on the side chains will determine the initial hydration shell, and whether this shell is compatible with partial clathrate cage structures will differ markedly with subtle changes in conformation. As may be expected, in the majority of protein hydration structures, the strong hydrogen-bonding groups are not compatible with clathrate geometry, and the hydration structures are characterised by single water molecules hydrogen-bonded to the functional groups. Although a minority, arrangements of hydrogen-bonded waters resembling partial clathrate structures have been found in some biological hydration structures.

In human lysozyme, the methyl group of Ala92 is surrounded by four hydrogen-bonded water molecules in a semicircle (Blake et al 1983). These waters are buried deep within the protein. If such hydration schemes were to occur at the periphery of a protein, the waters would probably not be seen in the analyses due to thermal motion.

Two fused water quadrilaterals are found between variable domains in the Bence-Jones protein Rhe (Furey et al 1983). The rings have an edge in common and are bonded to two tightly bound water molecules, and to two peptide N-H sites. Comparable quadrilateral water structures are found in structure-H clathrates, and also in cyclodextrin hydrates.

A chain of two triangles, five quadrilaterals and one pentagon of water molecules is found in a cavity between two subunits in glutathione reductase (Karplus & Schulz 1987). The water molecules are all connected to the protein main-chain or side-chain functional groups, or to other water molecules acting as anchor points. This structure does not share any structural motifs with the clathrates, and three-membered rings are not a feature of clathrates or ices. The five-membered ring in this system has two extended sides.

Three fused water pentagons are found capping an apolar valine3 side chain of the A-chain in insulin (Baker et al 1985). They are anchored by two-ring water molecules and

are further stabilised by the nearby His28 side chain. The three pentagons have near-ideal pentagonal dodecahedron geometry.

Five fused pentagons forming a clathrate-like structure are the predominant feature of the hydration of the small (MW 4720) hydrophobic plant protein, crambin (Teeter 1984). Again, these rings have very close to ideal clathrate-like geometry. The network of pentagons covers a hydrophobic patch on the surface of crambin.

The crystals of crambin diffract to at least 0.88 Å, which is a far better resolution than has been obtained with any other known protein. The analysis of insulin is based on 1.5-Å data, which is also rather better resolution than most similar studies. In both these cases, the anchor points for the water structure are very tightly defined. This suggests that there may well be structures in which clathrate-like structured water may occur, but sufficient resolution to see the structure may not be achievable.

In X-ray diffraction studies on [Phe[4]Val[6]] antamanide.12H_2O (Karle 1986), all 12 water oxygens have been located, and pentagonal water rings have been found.

Substrates can sometimes mimic the hydration water structure of enzymes. An example of this is *S. griseus* protease A, which in the native state has a cluster of water molecules associated with the active site (James et al 1980). These waters are replaced by the product tetrapeptide, Ac-Pro-Ala-Pro-Tyr, and the water cluster closely resembles the shape of the product in the hydrophilic and hydrophobic areas.

Hydration of nucleic acids

The conformation of DNA is determined by water activity that can be altered by the addition of salts. If the DNA is fully hydrated, there are about twenty water molecules per nucleotide. If the hydration is reduced, the minimum number of waters per nucleotide is 3.6. The hydration of DNA can be most simply described by two hydration shells (Cohen & Eisenberg 1968), as suggested by sedimentation equilibrium studies. The first hydration shell is impermeable to ions and does not freeze into an ice-like state. Of the twenty waters per nucleotide, 11 or 12 are directly bound to the DNA. The waters have binding affinity for phosphate, phosphodiester plus sugar oxygen atoms and functional groups of bases, in order of decreasing affinity. These waters are observed in crystal structure analyses, and are hydrogen-bonded to DNA oxygen and nitrogen atoms. The second hydration shell is permeable to cations and freezes to ice I. In this respect, this shell resembles bulk water, but as Donnan-type equilibria could have an influence on the structure of this water layer around the DNA polyelectrolyte, it is believed that this layer is subtly different from bulk water far away from the DNA. In considering the hydration of A-, B-, and Z-DNA, characteristic hydration patterns are observed which can be broadly grouped into sequence-dependent and sequence-independent motifs.

Most sequence-independent motifs can be found in the minor grooves, at the sugars and at the phosphates (Jeffrey & Saenger 1991). The water molecules can bridge sites in

the same nucleotide (e.g. purine N(7) with O(6)/N(6) groups) or they can occur between different nucleotides (e.g. between a free phosphate atom and a base atom in A-DNA). Such intranucleotide bridges usually involve only one or two waters, and can bridge between strands. The separation between adjacent base pairs (~ 3.4 Å) is comparable with the separation of water–water hydrogen bonds; the waters associated with one base pair can hydrogen bond to the water associated with the adjacent base pair. In this way, extended filaments and nets of water can be built up which will cover major and minor grooves. Hydration of the phosphate backbone is sequence independent, but is structure dependent. The different puckering modes in the three DNA structures significantly affect the phosphate–phosphate distances, and hence affect the hydration structure.

At present, there are only a few cases known of sequence-specific hydration of double helical nucleic acids. In the crystal structure analyses of the two isomorphous octa-nucleotides, d(GGTATACC) and d(GG^{Br}UA^{Br}UACC), the same characteristic hydration pattern is observed, comprising four fused five-membered rings of water molecules (Kennard et al 1986). It seems that the sequence TATA induces the formation of these pentagons in the major groove of A-DNA. In other sequences, such as TTAA or G/C sequences, pentagons are unlikely to form because of steric reasons, and have not been observed. An isolated 5-membered ring of water molecules has been observed hydrogen bonded to G(3) O6, G(3) N3, C(4) N4 and G(13) O6 in an A-conformation octamer of d(GTGCGCAC) (Bingman et al 1992).

A spine of hydration has been observed in the minor groove of B-DNA in the central AATT sequence of the dodecamer d(CGCGAATTCGCG) (Kopla et al 1983). The waters span O(2) and N(3) atoms of bases in adjacent base pairs. These water molecules form the first hydration layer, but are connected to waters in the second hydration layer so that each water in the first layer is tetrahedrally coordinated. The N(2) amino groups in G/C interfere sterically with this regular structure; it is disrupted at both ends of the AATT sequence. In sequences other than A/T, the minor groove is wider and adjacent nucleotides are bridged by intra-chain waters between O(4′) and either purine-N(3) or pyrimidine-O(2). A further X-ray diffraction study of the same system (Tereshko et al 1999) resolved the water positions, showing four fused water hexagons dissecting the central portion of the minor groove, with the inner corners of the hexagons coinciding with the original spine water positions, and so it may be more appropriate to refer to this as a ribbon of hydration.

A spine of hydration is also observed in the minor groove of Z-DNA (Chevrier et al 1986), in which the water molecules are primarily hydrogen bonded to the O(2) atoms of the cytosine bases. These are cross-linked and there are additional water molecules bridging these waters with the phosphate oxygen atoms and with the guanine N(2) amino group.

In a room temperature X-ray diffraction study of a deoxynucleotide phosphate d(CpG)-proflavine complex.27H$_2$O (Niedle et al 1980), most water oxygens were located, showing a hydrogen-bonding scheme involving a system of four pentagonal rings. In a low-temperature study on the same complex (Schneider et al 1992) at $-2°C$ and $-130°C$, similar structures were observed, but additional water molecules were located. Nine of the waters bonded only with other waters, and these allowed the formation of water pentagons in the structure, which formed new polyhedra, giving an infinite tetrahedrally coordinated three-dimensional water network. This network was mainly constructed of pentagons, but also included 6- and 7-membered rings.

Other biological molecules

A vitamin B$_{12}$ coenzyme (C$_{72}$H$_{100}$CoN$_{18}$O$_{17}$P) was analysed at 279 K in a combined X-ray and neutron diffraction experiment in which more than 140 water oxygens and 4 acetones were located (Savage et al 1987). The hydrogen-bonding scheme was disordered and an unambiguous representation of the water structure was impossible. A further study at 15 K, using a high-resolution (0.9 Å) neutron data set (Bouquiere et al 1994), showed two discrete water networks, each made up of 17 waters. One is a channel comprising statically disordered water molecules, and leading into this channel is a pocket region of highly ordered water molecules. This pocket region is mainly made up of five-membered rings in geometries concordant with clathrate structures. Although the channel region is disordered, the water molecules are arranged tetrahedrally in geometries similar to ices and clathrates.

Cyclodextrin hydrate

In the crystal structure of α-cyclodextrin hexahydrate, the hydrogen-bonding scheme has infinite chains extending throughout the crystal, and cyclic motifs are present, consisting of 4-, 5- and 6-membered hydrogen-bonded rings. Both the water molecules and the O—H groups of the cyclodextrin participate in these rings (Saenger 1979). This crystal structure has homodromic rings throughout, except where a water has to donate two hydrogen bonds to the same ring. This shows clearly that cooperative effects may be important in stabilising hydrogen-bonded rings. Rings in which the hydrogen bonds are randomly oriented (heterodromic) do not occur in any cyclodextrin hydrate structures.

Cryoprotective agents

Sugars, polyhydric alcohols and oligosaccharides are widely used as excipients in solid pharmaceutical preparations involving proteins and liposomes (Crowe et al 1996a). They are used not only because of their compatibility with, for example, proteins, but also because they are reluctant to crystallise during the drying process (Aldous et al 1995) and so increase the stability of the biomolecule.

Trehalose is found widely in plants and animals and performs a protective role, allowing survival during periods of total dehydration (Crowe et al 1996b). The exact mechanism by which trehalose exerts its protective effect is not fully known, but it is thought that the extensive hydrogen-

bonding functionality allows it to replace water molecules in the biological membranes (Miller et al 1997). This increases resistance to air-drying, and also to freeze-drying, as the number of water molecules present will be reduced and their arrangements around the trehalose molecules will prevent the formation of an extensive ice lattice. Many cryoprotective solutions have been shown to produce a glass on cooling, and a stable, wholly amorphous, state on warming (Baudot et al 2000), and this is thought to relate to their cryoprotective function. A study of trehalose solutions by Raman spectroscopy and viscosity measurements (Branca et al 1999) suggested that trehalose promotes a destructuring effect on the tetrahedral hydrogen-bond network of pure water, imposing on adjacent water molecules positions and orientations incompatible with the crystallization process.

The structural and functional features of antifreeze proteins enable them to protect living organisms by depressing freezing temperatures, modifying or suppressing ice-crystal growth, inhibiting ice recrystallization and protecting cell membranes from cold-induced damage (Fletcher et al 1999). The proteins, which are found in northern cod and antarctic fish, are up to 500 times more effective at lowering the freezing temperature than any other known solute molecule because of unique aspects of their tertiary structures, and act by specifically adsorbing to the surface of ice crystals as they form, thereby preventing their growth (Fletcher et al 2001).

Implications for drug–receptor interactions

As has been demonstrated from both experiment and computer modelling, water is composed of tetrahedral molecules which, when bonded to each other, tend to promote further bonding. The most stable resulting structures are rings and there is considerable evidence for the formation of higher-order assemblies which have pronounced geometric shapes in repeating motifs.

In considering the role of water in drug–receptor interactions, it is clear that a model that regards this solvent as a continuum is inadequate since water does more than simply separate the solute molecules. Formation of a hydrogen bond between a drug molecule and water will polarise the water, resulting in further hydrogen bonding to other water molecules. In many cases, geometry permitting, this will lead to the formation of at least one ring, which is likely to persist longer than other structures. It is apparent that the drug will tend to be hydrogen bonded with a pattern of water molecules that may play a role in receptor identification. Even in the absence of hydrogen bonding, non-polar drug molecules will tend to cause water structuring – formation of partial or total cages – which again means that the molecule will have an identifiable water 'signature'.

In the vast majority of cases, the drug will have a combination of hydrogen-bonding and non-polar groups, which will tend to give it a unique secondary identity when surrounded by water: it is possible that the primary recognition process is through the water surrounding the drug

and the water surrounding the receptor. One consequence of this idea is that a drug substituent does not need to come into direct contact with the receptor to affect recognition; even when pointing outwards, towards the bulk solvent, a non-polar group which is too large may disrupt a water cage and change the recognition face. Similarly, a destructuring substituent will have a dramatic effect on the surrounding water, as will a polar group which is hydrogen-bonding but not destructuring.

The simplest kind of ligand–'receptor' interaction, which is almost entirely non-specific, is the denaturation of proteins and peptides by structure breakers such as urea and guanidinium. Denaturation is primarily a change of 3-dimensional geometry and does not require breaking of covalent bonds. The effect can sometimes be reversed by addition of a structure maker, such as sulfate. Both these effects are most easily explained by an effect on the water surrounding the macromolecule, primarily in regions where there is water structuring, which may hold the shape by virtue of 'hydrophobic bonding' between non-polar parts of the molecule. The driving force for 'hydrophobic bonding' is normally assumed to be the gain in entropy when structured water is liberated between two hydrophobic regions. Addition of urea or guanidinium disrupts the water structure and removes the driving force for close association of hydrophobic residues.

It is normally accepted that the binding of a drug to its receptor is mediated by ion–ion interactions, hydrogen bonding, dipole–dipole interactions, lipophilicity and shape complementarity (the latter being an optimisation of the first four effects), although the relative contribution of each is poorly understood. It has been shown that the optimisation of hydrophobic interactions can produce tight binding, even at the expense of possible hydrogen bonds, and this plays a large part in the 'induced fit' of receptors to ligands, allowing molecules of apparently different shapes to bind to the same part of the same receptor (Williams et al 1993). By the same token, the drug molecule may also be coerced into a certain conformation by the receptor protein (Davis & Teague 1999). These concepts are probably sufficient to account for the biological effects of many antagonists of natural ligands, since tight binding is a prerequisite to passive receptor occupancy. However, where the ligand induces a conformational change in the receptor, sufficient to trigger a biological response, passive receptor occupancy is insufficient. In this case, it may be useful to analyse the interaction as a specialised example of denaturation.

An agonist is recognised by the receptor and provokes a conformational change in the macromolecule. The change in conformation and possibly the recognition are likely to be mediated through the water which surrounds both partners. Receptor agonists such as adrenaline (epinephrine; Figure 9A) and 5-hydroxytryptamine (Figure 9B) have identifiable recognition sites around the amino group and structure-breaking moieties on the benzene ring. It is not difficult to perceive an interaction that depends on the structure-breaking effects of the catechol or phenol moiety to induce a change in receptor geometry. In comparison, analogues of the neurotransmitters that have no phenolic
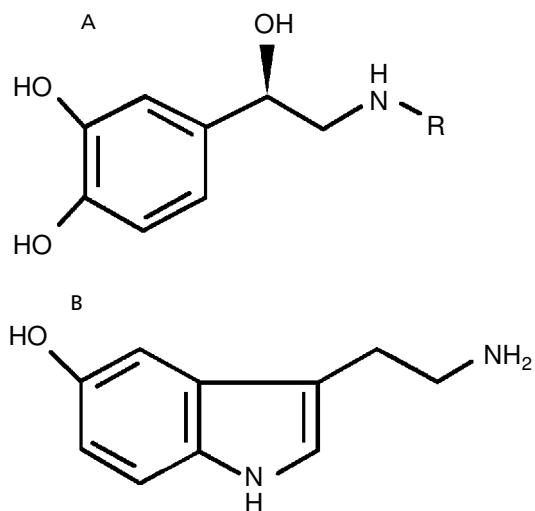
Figure 9   The structure of adrenaline (epinephrine; R = CH$_3$) and noradrenaline (norepinephrine; R = H) (A) and 5-hydroxytryptamine (B).

or similar structure-breaking groups will potentially be unable to induce the required conformational change and will, at best, be antagonists. It should be noted that this concept does not require the structure-breaking moiety to fit the receptor, merely to be brought into fairly close proximity, and that the effect is essentially directed outwards, towards the solvent sheath. If this idea is correct, it will be necessary, in designing a bioactive molecule, to take note of the parts of the molecule which are not in direct contact with the receptor and to explore the effect of structural changes in those regions; these changes may not only affect biodistribution and metabolism.

There are likely to be at least four ways in which water may influence the structure of a biological macromolecule (Figure 10). The first is simple bridging between two hydrogen-bonding substituents; there are several examples where this is known to occur and such effects are normal. The second is known as hydrophobic bonding, where two or more non-polar regions come together, releasing the water that is structured around both. The third involves a polar residue that is geometrically incompatible with structured water, in which case the effect may be transmitted by water molecules to neighbouring groups, disrupting hydrophobic bonding. The fourth, which does not appear to have been discussed elsewhere, would involve the connection of two domains of structured water, where these are geometrically compatible. This could occur over a relatively long range, by analogy, with the water at interfaces. Such interactions could occur in the water between non-polar groups, or could be promoted by, for example, phosphate residues: introduction of these by 'kinases' has a profound effect on the structure and function of proteins.

A vast amount of effort has been devoted to elucidation of the interactions of water with various solutes. The weight of evidence points strongly to the preference for water molecules to bond together in structures, allowing the O—O bond distance and the O—H—O bond angle to be maintained close to their optimum values. Water 'bucky
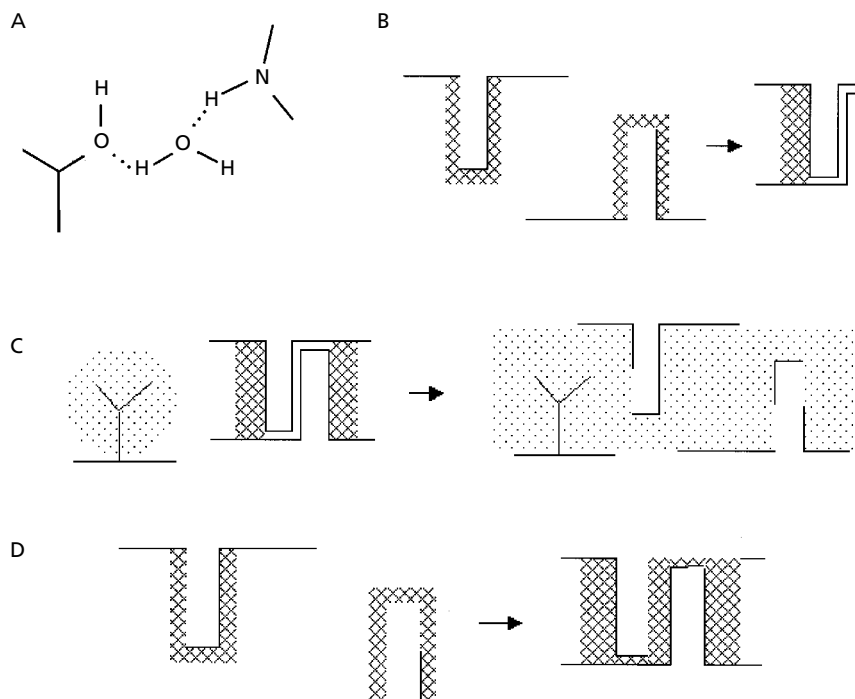

Figure 10   Types of water interaction possibly important in biological processes. A. Water bridging two hydrogen-bonding sites; note that the water is more likely to bridge between a hydrogen-bond donor and an acceptor. B. Hydrophobic effects hold the two substituents together. C. Structure-breaking substituent disrupts the hydrophobic interaction. D. Structured water between two groups holds them together.

balls', for example, are found very frequently in gas pipelines and inside mass spectrometers. However, water is not a continuum: there are regions of structuring and destructuring, dictated largely by the geometry of the system, particularly the relative disposition of functional groups on solutes. If these ideas are to be pursued, modelling of ligand–receptor interactions will have to include specific geometric considerations related to the associated water structure. This does not mean that the water around drug molecules has to be 'frozen', merely that certain structures may be favoured in a rapidly changing dynamic system, in the same way that certain conformations of the drug molecule may be favoured.

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