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The role of water in drug–receptor interactions

GUILLAUME HUCHET[1], MELVIN R. EUERBY[2], SIMON P. MACKAY[1], & ROGER D. WAIGH[1]

[1]*Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK, and* [2]*AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, UK*

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Abstract
The idea that liquid water is not a uniform and random arrangement of molecules has been taken very seriously by the scientific community. Many experimental and computational investigations show that clathrate- or ice-like structures probably exist at a short time scale in solution. We have designed a new program to simulate water structure around solutes. Our model is based on the geometrical constraints of hydrogen bonding in order to be capable of producing clathrate-like structures. Simulations with small molecules and bio-molecules, using the new software, produce networks of water with specific patterns made of small water rings. The water structures built are consistent with the classification of molecules in terms of structure breaking and making. This approach may give insight into, and a more accurate description of, drug–receptor interactions. The results also suggest that water structure may impart sufficient energy to modify the conformational space of organic molecules through hydrogen bonding.

Keywords: *Water structure, structure maker, structure breaker, drug–receptor interaction, clathrate, simulation*

Introduction

Although water is our most familiar liquid, its structure is still the subject of debate [1–3]. Molecules of H_2O behave as sticky tetrahedra and the ways that these tetrahedra interact are of continuing interest, and may explain why water has so many anomalous properties [4,5], for example the boiling point. Although the special properties of water are universally acknowledged, the implications for the pharmaceutical sciences have been largely ignored. Many studies of drug–receptor interactions still avoid the question of water completely, pretending that the system is in a vacuum, or the water is treated as a continuum [6], characterised by a dielectric constant and little else.

A characteristic of sp^3 atom centres, as in carbon chemistry or in water, is the formation of 5- and 6-membered rings as a stable feature [7]. This is shown very clearly for water from the x-ray crystallography of ice [8] and water clathrates [9]. The x-ray crystallography of the clathrates is of special interest, because an interaction occurs between a 'guest' and the structured, ice-like water around it. This interaction is the conceptual basis of the 'hydrophobic' effect that occurs with proteins, holding non-polar parts of the molecule together in aqueous solution. The driving force is said to be the unfavourable entropy [10] of water structuring: when non-polar moieties come together, the amount of structured water is reduced. This effect is exploited in the use of guanidinium and urea to treat synthetic proteins, if they do not fold spontaneously in the way that is required to produce biological activity [11]. Both guanidinium and urea are 'water structure breakers' and their use sometimes allows the protein to refold in the 'correct' manner. Refolding can also be assisted by the use of sodium sulfate, which is a 'structure maker'. The concept of the structure maker or breaker is consistent with the work of Hofmeister [12] who classified various ions according to their ability to precipitate proteins. The so-called Hofmeister series is still used to evaluate the effect of ions on the structuring and denaturing of biological macromolecules [13]. By exploring the role

Correspondence: R.D. Waigh, Department of Pharmaceutical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK. Tel: 44 141 548 4355. Fax: 44 141 552 6443. E-mail: r.d.waigh@strath.ac.uk

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of simple molecules like urea and phosphate and their non-specific effects on protein structure, we can extrapolate to the influence of drugs on receptors. While a 'blocking agent' may simply occupy a receptor, without producing a dynamic effect, agonists must do more; after binding, there is an effect on the protein to change its conformation in a specific way. The interaction may have components involving van der Waals and Coulombic forces, but many cases must surely also involve the water that surrounds both interacting species.

Theory

Our work is based on the early 'mixture model' theory proposed by Frank and Evans [14] in 1945. Basically, liquid water is described as a mixture of two states: (a) highly ordered networks very similar to clathrates and ice and less dense than the macroscopic mean value, and (b) more randomly organized structures that are more dense. This description must be brought into a dynamic context where these structures have a short lifetime: a single hydrogen bond remains approximately stable between 1 to 20 ps [3] at room temperature. For this reason it is impossible to get a clear picture of water structure with the current analytical techniques. Consequently, most of our understanding of water relies on thermodynamic and macroscopic observations. This work extends the theory that some solutes like urea tend to destabilize these transient structures whilst others like phosphate promote them, through an effect that is probably limited by distance, by directly building and analysing water structure around organic molecules. Owing to experimental obstacles like the high dynamic rate, structure analyses of water have been widely conducted using force field approaches [15] such as TIP4, but none investigates the potent effect of structure making and breaking because existing modelling techniques failed to produce highly arranged water networks. *Ab initio* calculations [7] should give more accurate answers but unfortunately the calculations are so computationally expensive that we are still far from modelling a large enough sample. The purpose of our model was to build water structures in a reasonable time scale without using the usual force field methodology.

Materials and methods

Materials

The program has been designed using C++ within the Microsoft Visual C++.NET© environment. Microsoft Foundations Classes (MFC) is used for the window interface and the 3D graphics are generated from the library of functions within OpenGL®. Calculations are accelerated with the wide utilization of pointers and the axis rotations have been optimized with the mathematical help of a matrix.

In order to make the program user-friendly and flexible, it uses the interface support Protein Data Bank (PDB) format file, the most used format for organic and biological molecules. From the atom coordinates and the connectivity table, the program automatically calculates bond orders and advanced atom types (e.g. O_oh type for oxygen of an alcohol) specific to our model.

Optimal geometric H-bond

Our model is based on the structure of clathrates and the low-pressure polymorph of ice [8]. In these arrangements, the network is ruled by the geometry of H-bonds between water molecules. Two parameters mainly prevail: the directionality and the distance (see Figure 1). We have shown in a previous study [16] that the optimum distance for an O–O bond is 2.79 Å and the optimum angle O–H···O is 180°.

To reproduce exactly the geometry associated with the H-bond, the model needs to localize the position of the lone pairs (Lp). The most obvious geometry implies a totally tetrahedral water molecule where all angles H–O–Lp, H–O–H and Lp–O–Lp are fixed to 109.47°. For consistency, the distances X–H and X–Lp (X=O, N, S) are normalized to 1 Å as is usually done for crystal structures deduced from x-ray crystallography. Finally to optimize the directionality, a second angle has to be defined as optimal: O–Lp···O at 180°. The literature [17] and database of crystal structures show that the H-bond can fluctuate around a distance of ±0.1 Å and the angle can vary by ±20°.

The H-bond parameters also have to be defined between solute and surrounding water molecules. For the water dimer, analysis of H-bonds in the Cambridge Structural Database[18], using the software ConQuest 1.7, enabled optimal distances to be extracted and the positions of Lps were defined by relative geometry parameters depending on the

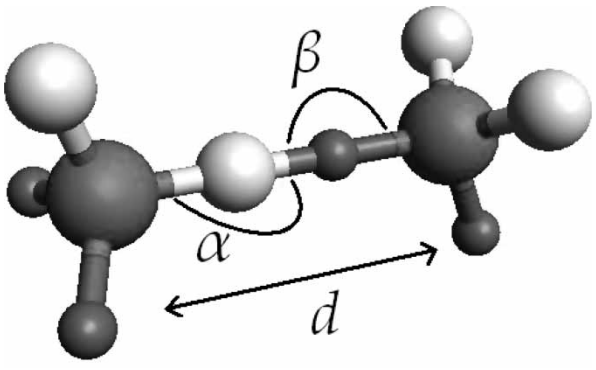

Figure 1. Geometric definition of H bond in the model. α is the angle O–H···Lp, β is the angle H···Lp–O and d is the distance O···O.

chemical group potential for the formation of the H-bond. These parameters are sometimes founded on *ab initio* studies (e.g. with phosphate [19]) when the position of the Lp is less obvious. Where the potential energy of the H-bond between water and solute is too weak [20], the substituent is considered as non-H-bonding.

Building water structure

With the geometry of the H-bond between a donor and an acceptor as the primary unit for our model, we developed a simple method to add sequential chains of water in order to obtain a complete network. Taking an initial structure, such as a water molecule or a solute, the program chooses randomly an available H-bond site and binds a water molecule to it based on the perfect geometrical parameters previously defined. One by one, water molecules are added to the chain of water and each axis of rotation of the H-bond newly created is rotated, usually at 10° intervals, to generate all the possible conformations of this chain. For each conformation, the program checks if an H-bond site (solute or water already attached to the structure) is available and assesses the predefined range where the geometry is acceptable. Where this is so, the conformation of the new chain of water is optimized geometrically, based on equation (1), with a *Monte Carlo* like algorithm and then saved as part of the structure. Where the geometry is not acceptable, the screening of the conformation continues as long as the size of the chain remains below six water molecules. In normal circumstances, when the chain reaches five water molecules, a 5-membered ring is formed but depending on the surrounding constrains this is not always the case, in the case where no suitable conformation is found, the chain is discarded. After these two cycles, the program restarts from a new H-bond site as before. During the process, the program takes into consideration any close interactions between the chain of water and the full structure, for example, steric repulsion represented by hard sphere atom types [21] can discard some of the conformations. Moreover, to improve the construction of water structure in terms of quality and density, the space studied around the solute is constrained to a certain solvent layer size or box.

$$D = 100\frac{\sum_{i=1}^{n}(d - d_{HB})^2}{n} + 36\frac{\sum_{i=1}^{n}(\alpha_i - \alpha_{HB})^2 + (\beta - \beta_{HB})^2}{2n} \quad (1)$$

Equation (1) Deviation from the perfect geometric H bonds in a water structure. d_{HB}, α_{HB} and β_{HB} are the reference values and n is the number of H bonds in the system.

The algorithm can be run for pure water and a solute with or without an H-bond site. In the first case, the system is initialized with a single molecule of water as the starting point. When the solute does not contain any available H-bond sites, a first water molecule is added randomly and positioned as close as possible to the surface of the hydrophobic solute using the hard sphere atom approach.

The previous program [16] relied on fixed geometry of a solute; we have since allowed flexibility in the initial structure of the solute by developing a second algorithm based on the same principle in order to study the impact of constrained water structure on the conformation of molecules with flexible torsion angles. Using this new program, both the chain of water and the torsion angles within a solute can rotate and once one water-solute conformation has been generated, it forms the basis of future water network construction. The program is limited to a maximum of four torsion angles within a solute for reasons of computational expediency.

Analysis of the water network

Each time the program is run produces a different result, reflecting the 'flickering clusters' of water molecules. The extended structure of water around a solute is relatively complex which makes it difficult to distinguish by eye the significance of the network of water molecules. Analysis with graphical and mathematical tools is conducted with the program to get more user-friendly displays of the generated structure. Displaying the 'first hydration shell' keeps only the arrangement of the water molecules linked to an H-bond site on the solute and it shows clearly which site is involved in an H-bond or not. The 'loop chain shell' can be displayed to indicate the chains attached by both head and the tail to the solute. The more loop chains the structure contains, the more stabilized the interaction: each loop chain reinforces the geometry of the solute. The smaller the loop chain, the better the stabilisation.

In order to depict the long-range arrangement of water structure, that these reduced structures do not, it is common to use the radial distribution of interatomic distances [22,23] to characterise the arrangement of water in the liquid and solid state for x-ray and neutron diffraction. This type of graph provides an opportunity to compare directly our simulations with experimental data where each peak reveals a special molecular arrangement of the water molecules together.

Water structure usually tends to form small rings of water, and the changes to the composition in the size of the rings can indicate a modification of the state of water by the solute. To count the number of rings without omission or duplication, our program employs an algorithm that incorporates ring testing.

The stability of a specific water structure is relatively dependent on the number of H-bonds. Each water molecule has got the possibility of forming four bonds but this potential is rarely completely exploited. Our program evaluates the density of the network of H-bonds through the occupancy of water. With our model, a water molecule is almost always bonded twice but can be fully 4-bonded as well. A simulation of a stable water network shows a dense structure with a great percentage of fully bonded water.

Results

In our previous work [16], we demonstrated the effect of structure makers and breakers at the molecular level around different ions using a similar model. It was shown how phosphate, a structure maker, can easily build water networks from its H-bond sites, whereas conversely, a structure breaker like urea is unable to do so. We report here a program more orientated towards the hydration of organic molecules to evaluate the possible implications of water structure for drug design. We have performed simulations to evaluate the reliability of the model on a wide range of molecules: hydrophilic and hydrophobic organic species, biological systems such as proteins and DNA and small flexible molecules.

Pure water

The model can be assessed on the basis of simulations with pure water for which many experimental data exist. The algorithm has been conceived to build chains of water based on the best possible H-bond geometry using an optimisation module. The program tends to form mainly 5- and 6-membered rings as expected: a planar conformation is observed for the former and boat, twist and chair for the latter. The literature contains many *ab initio* studies that have shown quasi systematically that 5- and 6-membered rings are the lowest in energy [7,24], which correlates perfectly with our geometrically based model. When the network is extended, larger structures naturally and frequently appear. For example, one is made of twenty water molecules each binding to three neighbours forming a small cavity. Its structure contains twelve pentagons of water and quite often merges together to form super structures and can be directly correlated with shapes observed in clathrate structures.

A key element in the assessment of our model is the comparison of the simulated radial distribution of inter-atomic distances between oxygens (the distance between H···H and O···H can also be used) with experimental data from the x-ray and neutron diffraction of liquid water (see Figure 2).

Fixed solute

Water interactions with hydrophobic molecules such as small alkanes (1 to 4 carbons), benzene and its

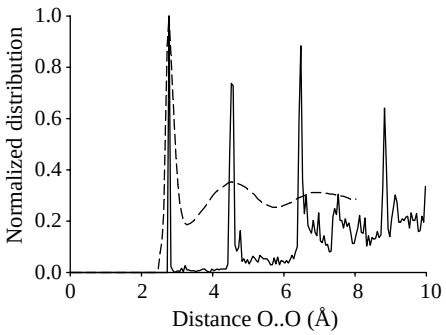

Figure 2. Radial distribution of interatomic distance between oxygen atoms: in solid line simulated structure of water with the model and in dash line x-ray data at 298K.

derivatives have been systematically simulated. The results reveal an increasing complexity of the type of cage created around bigger solutes. Most interestingly, we were able to reproduce a 12-pentagonal clathrate structure around methane when the first water molecule is correctly placed at its hydration surface (see Figure 3), which agrees with the observation that methane can be trapped in liquid water at high pressure in oceans [25]. Moreover, hydrophobic molecules seem to promote the formation of structured water as Franks proposed to describe the hydrophobic effect [26].

Around hydrophilic molecules, from mono-alcohol to 12-mer DNA, the program has successfully created realistic networks of water in a reasonable time scale. Alcohols and primary amines seem to behave similarly by generating clathrate cages of 5-membered rings. For ethers, the lone pair of the oxygen can hold a 4-membered chain that can be assimilated to a planar pentagonal ring. With polyethylene glycol, the branching differs because chains of water can link two consecutive oxygen atoms in the carbon skeleton

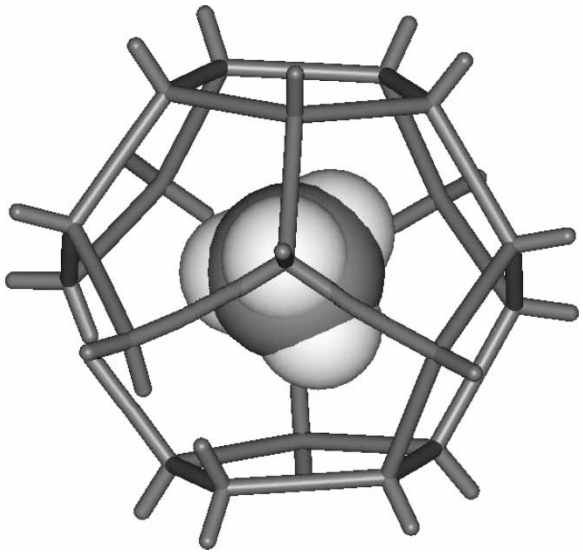

Figure 3. Methane clathrate generated from the model in which methane is encaged with a water structure based on 5-membered ring.

and more complicated networks appear, but these are quite repetitive through the systematic use of all available lone pairs. With sp^2 atoms like carbonyls or primary imines, patterns with 6-membered rings are preferred to smaller rings. With larger structures like DNA, it is possible to see special features highlighted. For example, we can reproduce the spine of hydration [27] within the minor groove of B-DNA and by simulating an 8 Å layer around A- and B-DNA, highlight significant differences [28] in the distribution of the size in the 'first loop chain' as well as in the distribution of the types of rings generated in the two cases.

Flexible molecules

Most organic molecules cannot be treated as rigid; flexibility plays an important role in medicinal chemistry because it allows conformational change to fit drugs to the targeted active site. Previously, we have looked at the effect of fixed-geometry molecules on the surrounding water structure [16], but by allowing flexibility, we can investigate how water and the solute can interact interdependently.

By studying small compounds containing two H-bonding groups such as ethanolamine, we have been able to demonstrate that its conformation can be locked by adding a chain of three water molecules (see Figure 4). Extended networks around a few of the locked conformations show clathrate-like arrangements with regular 5-membered rings. In these highly organised water structures, ethanolamine is slightly bent from the perfect linear conformation, which involves very small expenditure of energy largely balanced by the H-bonding of the water arrangement. Larger structures have been observed with aminopropanol (see Figure 5), where a complete clathrate cage can be built around each of the hydroxyl and amino groups. By changing the hydrocarbon conformation, it is possible to merge the two end cages with a third one in the middle to produce a very stable final structure, which constrains the aminopropanol to adopt an unusual shape.

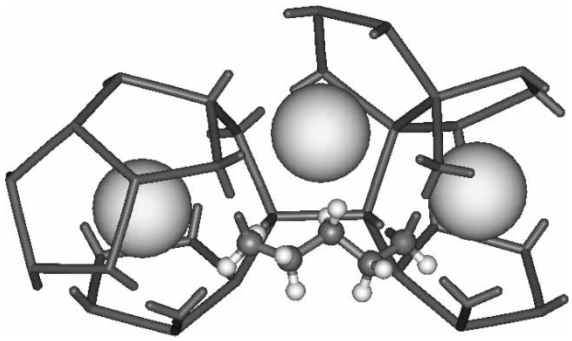

Figure 5. Conformation of propanolamine surrounded by three cages of water (indicated by big spheres) which are adjacent to the amine group, the hydroxyl group and the propyl moiety.

With both of the lower order amino alcohols, the formation of 5-membered rings was promoted but with hexan-2,4-dione we observed other topology (see Figure 6); the conformation tends to be fixed with a chain of two water molecules, the two methyls are in a *cis*-conformation, and all the simulations attempted generate a large majority of 6-membered rings.

Discussion

Simulations based only on the geometry of a perfect hydrogen bond (HB), can generate models that potentially give insight into the structure of liquid water. The hydration spine in DNA or water cages around methane can be produced easily with the program in a reasonable time scale. More interestingly,

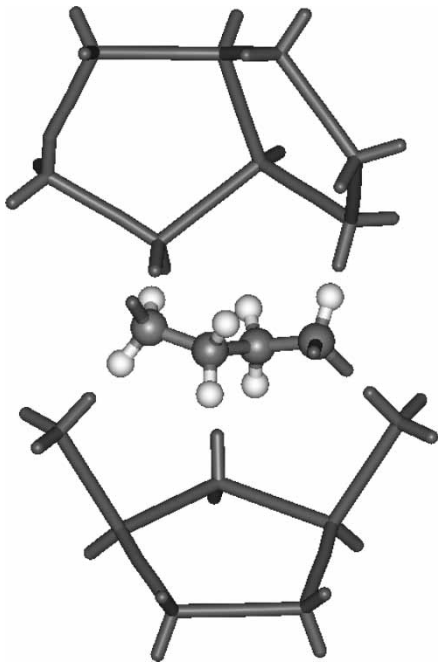

Figure 4. Conformation of ethanolamine surrounded by a clathrate like arrangement of water.

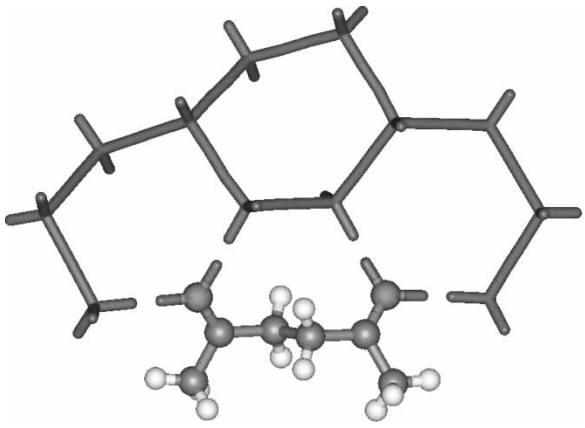

Figure 6. Conformation of hexan-2.4-dione hydrated by a 6-membered ring type of water.

this work demonstrates that the structure of water can be modified depending on the molecule in solution. Some molecules form essentially 5-membered rings or 6-membered rings or none at all, particularly the latter with structure breakers (urea, catechol). We offer a simple set of prediction rules for water structuring based on our modelling: sp^3 HB groups (hydroxyl, amine) tend to form complete clathrates made of 5-membered rings, sp^2 HB groups (primary imine, carbonyl) tend to form 6-membered rings that are ice-like, with a less dense structure. These predictions will be tested experimentally.

In terms of drug design, these results can potentially change the accepted perception of the interaction between molecules in aqueous media [29]. We can imagine that all three different water structures may not be compatible. A structure breaker environment is not compatible with a clathrate-like environment generated by ethanolamine, for example. The water arrangement inside an active site can be changed with the arrival of a drug, which, by altering water structure locally, is able to modify the structure of the receptor through induced conformational change. Mediating inhibition or activation, it is possible that many drug actions may be based on this principle of incompatibility of water structure between an active site and its ligand.

In conclusion, we have developed a computational model to build highly organized water structures around solutes based on the geometry of the hydrogen bond. The model has been assessed by comparison with experimental data and theoretical results. Simulations have been conducted to analyze the water networks with flexible and rigid molecules. Our results highlight the possibility that molecules can influence water structure and vice-versa. In term of drug design, this model could potentially be useful to help describe more accurately the interactions between bio-molecules or between drugs and active sites.

Supporting information

A short simulation has been recorded with polyethylene glycol (see the movie file WaighHuch-Sup.wmv).

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