Proteins and water structure

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One might think that, in order to understand how the structure of a protein affects its function and mechanism, all you need to know is the positions of the protein's atoms. These, after all, define the position of the secondary structure elements that make up the protein's 'scaffolding' and thus the location of its binding-site residues and functional groups. Yet this can be only part of the story. No living organisms known can exist without water, and most proteins can only function within a solution that is essentially, at the atomic level, little different from water. Most X-ray crystal structures of proteins show water molecules in fixed positions around the exterior of the protein molecules, forming hydrogen-bonding networks that include hydrophilic groups in the protein main and side chains.

Most often, these 'structured' water molecules do not seem to play a part in the function of the protein. In a few cases, however, one or more water molecules, held in place through hydrogen bonds, play a really key role in the protein's function. The HIV protease is one of the best-known examples of this. This enzyme is a dimer, with the active site formed between the two monomers and including both copies of the essential tripeptide Asp-Thr-Gly (DTG). A water molecule located between, and hydrogen-bonded to, the two aspartate residues (one of which is protonated) is thought to act as a key nucleophile in the reaction, attacking the carbonyl carbon of the bond that is cleaved during proteolysis. Many of the protease inhibitors that have contributed to the transformation of life chances for HIV-infected individuals throughout much of the world are structural analogues of the transition state formed by this reaction.

Although 'structural' water molecules have been observed since the very earliest protein structures, the study of the structure of water and its interaction with biomolecules goes back further than the half-century of structural biology. One of its most distinguished early exponents was Professor J.D. 'Sage' Bernal, founder and first head of my own department, the School of Crystallography at Birkbeck College, London, UK. Bernal's many other achievements include, as a young lecturer at Cambridge, and with Dorothy Crowfoot, later Hodgkin, as his PhD student, being the first to observe an X-ray diffraction pattern from a protein. His work on the structure of water is much less well known, but possibly equally seminal: he devised and proposed mathematical models for water that could explain many of its physical features, including its X-ray diffraction pattern. Even in the 1960s, when Bernal finished his work, computer modelling was in its infancy, and he developed many of his ideas using physical models with rods and rubber balls that would look very odd to any modern molecular scientist.

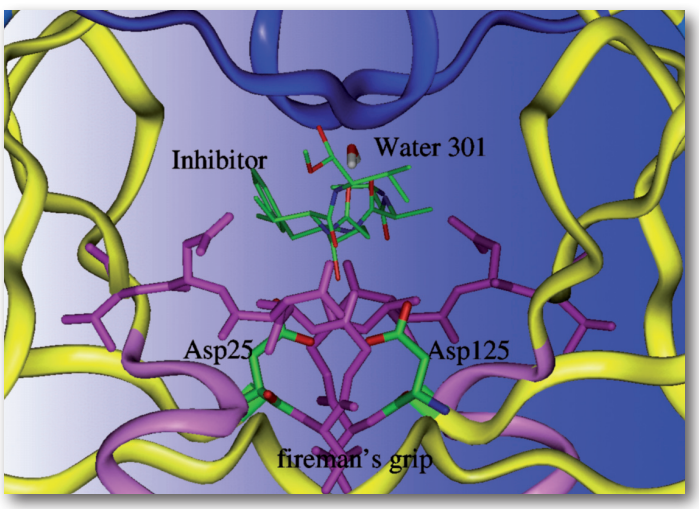

Figure 1. HIV protease-binding site with structured water molecule (from http://xpdb.nist.gov/hivsdb/gallery.html)

Bernal's younger colleagues and their co-workers at Birkbeck and, later, University College London, continued his work on the structure of water and its interaction with other molecules. John Finney and Ian Cherry were followed by Julia Goodfellow, who later became chair of the BBSRC and is now vice-chancellor of the University of Kent at Canterbury. For many years, when still a full-time researcher, she studied the distribution of water molecules around the surfaces of macromolecules, and in cavities inside proteins. Her group mapped the patterns of water molecules around amino acids in high-resolution protein structures and found that they had an ordered distribution around hydrophobic as well as hydrophilic amino acids. Water molecules were more common around the edges of aromatic rings than stacked parallel to their faces, although when waters were seen stacked above aromatic rings their positions were consistent with known pat-

terns for water–phenyl hydrogen bonds[1].

Most recent structural studies of water around protein molecules have concerned its dynamics, particularly how water contributes to the protein folding process and to protein stability. It has been known for decades that the drive of hydrophobic residues to be in contact with each other in the protein's core and away from solvent molecules is one of the most important forces controlling protein folding. This process must necessarily cause hydrophilic groups, even if only the main-chain amino and carbonyl groups, to become buried, and one of the ways in which these groups can be stabilized is if water molecules buried in cavities within the protein can hydrogen bond to them. Buried water molecules have been found more often close to turns and loops than to helices and strands in which all main-chain groups are hydrogen-bonded[2]. Now, 20 years after the HIV protease structure paved the way for what is still possibly the most important success of structure-based drug design, molecular dynamics studies of protein folding, and of differences between wild-type and mutated proteins[2] are underlining the importance of ordered water in predicting ligand binding. Understanding water structure may be almost as important as understanding protein structure for drug design. ■

References

1. Flanagan, K., Walshaw, J., Price, S.L. and Goodfellow, J.M. (1995) Protein Eng. **8**, 109–116
2. Park, S. and Saven, S.G. (2005) Proteins **60**, 450–463

Best of the Web

Mark Burgess

The main bit of safety advice I remember when I started in a laboratory was that one should not use a mouth pipette with what were then called 'strong mineral acids' (HCl, HNO_3, H_2SO_4), and quite right too. But there is a suspicion that things have now gone too far. I recently bought 100 g of magnesium chloride, useful for anaesthetizing tardigrades, and it came with a Material Safety Data Sheet of five pages.

This state of affairs is parodied in a website devoted to the menace of dihydrogen monoxide (DHMO), or hydric acid (www.dhmo.org). This compound has as its basis: "the highly reactive hydroxyl radical, a species shown to mutate DNA, denature proteins, disrupt cell membranes, and chemically alter critical neurotransmitters. The atomic components of DHMO are found in a number of caustic, explosive and poisonous compounds such as sulfuric acid, nitroglycerine and ethyl alcohol."

The site points out the danger of death following accidental inhalation of DHMO, even in small quantities, that prolonged exposure to solid DHMO causes severe tissue damage, that DHMO is a major component of acid rain, that gaseous DHMO can cause severe burns, that it leads to the corrosion and oxidation of many metals, that it is found in biopsies of pre-cancerous tumours and lesions- and much more. It has links to Material Safety Data Sheets, some real, some suspect, and sportingly links to an antithetical organization, the Friends of Hydrogen Hydroxide (www.armory.com/~crisper/DHMO/).

The whole thing is run by Dr Tom Way, a research scientist in Newark, Delaware, "to educate, and to promote cautious consumption of information and an active scepticism about what we read, see and hear." It is very well done and bears a strong resemblance to various research division websites at the Environmental Protection Agency.

Out of interest, I looked up the real Material Safety Data Sheet for sodium chloride (http://avogadro.chem.iastate.edu/MSDS/NaCl.htm is one of the best free sites): "Potential Health Effects — **Eye**: May cause eye irritation; **Skin**: May cause skin irritation; **Ingestion**: Ingestion of large amounts may cause gastrointestinal irritation. Ingestion of large amounts may cause nausea and vomiting, rigidity or convulsions. Continued exposure can produce coma, dehydration, and internal organ congestion; **Inhalation**: May cause respiratory tract irritation … **First Aid Measures**: Ingestion: If victim is conscious and alert, give 2–4 cupfuls of milk or water. Get medical aid. Wash mouth out with water."

Reality is always close on the heels of parody. ■