Putting molecular biology into water

Complaints that molecular biology is indifferent to quantitative considerations are belied by brave efforts to understand the hydration of glucose.

THOSE who complain from time to time (and even often) that modern biology is insufficiently quantitative have a responsibility to look for signs that the wind is changing and then to say so. What follows is in that spirit. It is an evocation of heroic efforts recently made to tackle what may be thought one of the rudimentary problems of biological chemistry, that of the behaviour of the glucose molecule in solution in water. A little reading leaves no doubt of how much effort (and Cray-time) has already been expended on the task. It also confirms that there is much to be learned about glucose before the real polysaccharides with which cells are filled can be understood.

The complainants are fond of saying that one of the glaring deficiencies of molecular biology is that its descriptions of the behaviour of the interesting molecules of biology, from protein enzymes to DNA, almost always overlook their interaction with the sea of water molecules in which they are invariably immersed. The complaint, it goes without saying, is not a moral complaint against the character of those who practise molecular biology. Rather, it is a comment on the inherent difficulties of describing water interactions on a molecular scale. It might be different if there were a calculable theory of water itself. Since there is not, it may seem remarkable that so much can be said about glucose in solution.

Glucose, everybody knows, is a simple molecule with the formula $C_6H_{12}O_6$, which is hardly more complicated than the formula for benzene. Moreover, both molecules have a similar architecture, each being built around a six-membered ring of atoms. The difference is that the six-membered ring of glucose consists of five carbon atoms and an oxygen, and that it is held together by single and not double bonds. To one of the carbons next to the ring oxygen is attached a CH_2OH group — essentially methyl alcohol, while every other carbon atom in the ring carries a hydroxyl group characteristic of alcohols (plus a hydrogen atom to make up the carbon valency of four). The water interactions of the glucose molecule must plainly be central to its real-life properties.

But even in the absence of water, there are complications enough. For one thing, the ring-carbon next to the ring oxygen that carries both a hydrogen atom and a methyl alcohol residue is intrinsically asymmetrical, and so must be a centre of optical activity. (Real-life glucose consists of the D enantiomer.) So is the ring-carbon on the other side of the ring-oxygen (conventionally known as C1), which makes four distinct linkages with a ring-carbon, the ring-oxygen, a hydroxyl group and a hydrogen atom.

Worse, being aliphatic, the six-membered ring cannot be geometrically flat, like that of benzene, but must be puckered. In principle, there are three possible directions of puckering, corresponding to the three diameters across the roughly hexagonal ring, for each of which there may be two puckered forms, corresponding to the cases in which the atoms at the opposite ends of the diameters are displaced in the same or opposite directions above (or below) the plane formed by the remaining four atoms (the "boat" and "chair" forms of puckered rings respectively).

It seems common ground among glucosologists that the only form of the glucose molecule whose stability earns it a place in nature's scheme of things is that in which the oxygen atom of the methyl alcohol residue lies roughly in the plane of the puckered ring, which is the D enantiomer observed, and does not project away from it roughly at right angles. The other complication is the configuration of the groups attached to C1, which switch from one form (called α) to the other (called ß) naturally in solution. For some time, it seems to have been taken as a mark of progress in the field that there should be an explanation why, at equilibrium, there should be twice as much ß as α anomer in solution.

Simple handwaving has done much to keep the field alive. One line of argument has been the observation that intramolecular hydrogen-bonding may stabilize some forms at the expense of others. The idea is that some configurations of the whole molecule can place as many as all six of the hydroxyl hydrogen atoms near to other oxygen atoms, creating for the central ring of glucose a kind of simulation of immersion in water. The same line of argument favours the relative stability of the ß-anomer: the C1 hydroxyl group then lies roughly in the equatorial plane of the molecule, and so takes part more easily in internal hydrogen bonding. But that, of course, is only handwaving, and says nothing about external water interactions.

Now Christopher J. Cramer and Donald G. Truhlar from the University of Minnesota, Minneapolis, have given glucose what might be called the full Cray treatment (*Journal of the American Chemical Society* **115**, 5745–5753; 1993). The goal is to deal with all the aspects of the glucose molecule in one fell swoop — the electronic binding of the atoms together, their relative motion (vibration), the motion of the intact molecules and their interaction with the external aqueous environment. It is a *tour de force*, although no doubt merely a foretaste of what lies ahead.

One interesting conclusion is that the predictions of handwaving are not borne out. What seems to stabilize the ß relative to the α-anomer is not so much the intramolecular hydrogen bonding as the vibrational patterns of the two forms. Nobody could have guessed at that by handwaving. But clearly the number-crunching will have to be applied to many of the other isomeric forms of glucose before all the subtleties of that molecule, even in the 'gas phase' (which means no water), are fully understood.

And, for all the care that Cramer and Truhlar have taken, the water interactions remain the weak point of the calculation. Although it would be possible in principle to set up a molecular dynamics routine to deal with even the most ramified chains of interacting water molecules connecting pairs of hydroxyl groups on a glucose molecule, that would be equivalent to solving the whole water problem in especially taxing circumstances. The snag is that it is quite possible that some of the peculiarities of biologically significant molecules in solution derive from the persistence of extended interaction chains through water molecules in the medium.

Instead, Cramer and Truhlar (no doubt for the time being) are forced to rely on the solvation models they have developed in the past few years (see *Journal of Computer-Aided Molecular Design* **6**, 669; 1992), and which effectively treat the water surrounding a solute as a continuous medium beyond the first bound shell of water molecules. The calculated results are as precise as anybody could wish at this stage, but they do not yet describe real water.

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Correction: Bringing photosynthesis to the bench

Since the appearance of my article commenting on a paper by J. L. Martin *et al.* in the same issue of *Nature* (**363**, 297 & 320–325; 1993), it has come to light that Dr Douglas C. Youvan was solely responsible for the genetic engineering of the bacteria used in the study. This would have been more evident from the original article if more explicit reference and acknowledgement had been made.

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