SHORT PAPER

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Do NOE studies of water in DNA-histone units give significant information regarding the strength of solvation?†

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A few water molecules attached to DNA have much longer residence times than expected, as established by NOE studies. It is now suggested that this arises because of the presence of long lived protein 'caps' spanning across the edges of the two DNA strands, thus trapping the water molecules underneath.

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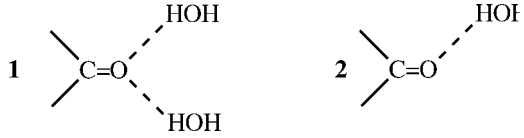
In some recent, interesting, nuclear Overhauser effect (NOE) studies of protein–water[1], DNA–water[2] and protein–DNA–water[3] interactions, it has been possible, using refined techniques and making due allowance for exchanging protons, to establish that a very small number of water molecules have detectably long average life-times close to certain hydrogen atoms of the biopolymers. The time for detection is in the region of 1 ns, but since only one feature for water was detectable, exchange must nevertheless be quite fast. Thus these unique water molecules are bound to the biopolymer within the range *ca* 10^{-4} –10^{-9} s. This time is long relative to those for most solvating water molecules, including those on the outer regions of proteins.[1] It is significant that, for the DNA–protein complexes, this time range is short compared with the lifetimes of the complexes.[4]

What further significance can be placed on these results? Certainly, they may indicate regions where long-lived water occurs. However, several workers have implied that they also give a measure of the water–protein, or water–DNA bond strengths. My aim is to suggest that this extra inference is probably unjustified.

Many properties of water need to be considered, including the range of correlation times for pure water. Water loves water, most molecules forming four strong tetrahedrally oriented hydrogen bonds. Those that fail to achieve this have relatively high reactivities, either as H-bond acceptors or donors.[5] Also water tends to bring out the maximum hydrogen bonding ability of any solute[6] bonds in 4-bonded water are much stronger than those in water dimers (*ca* ×2) as a result of the co-operative effect. However, when a polar group or ion acts only as an H-bond acceptor, the H-bonds it forms with water get weaker as the number of bonds increases (the anti-co-operativity effect[7]). Thus, for example, the C=O group of an amide forms two H-bonds as in structure **1**, but these are both weaker than the single bond in structure **2**, although the solvation energy for **2** is less than that for **1**.



A nice demonstration of anti-co-operativity comes from an infrared study of the progressive solvation of chloride ions, the O–H stretching frequency moving strongly to higher values as the number of bonds increases.[8] Such spectroscopic studies give H-bond solvation numbers, and relative H-bond strengths. These correlate with NMR shift data.[5] Also, because of its size, water is very good at space-filling, but even in constrained spaces, each molecule will still endeavour to form four H-bonds, either to the biopolymer or to water, or generally to both.

Lifetimes of water-water units are short, despite the strong bonding, because of the low mass of the molecule, and the tendency for bonds to make and break co-operatively. Life-times for water bound to polar units or to ions may be shorter or longer depending on their number, the mechanisms for exchange and, in particular, steric factors. In a broad sense, the chelate effect may often tend to increase the life-times of specific units.

For biomolecules, steric factors must often be important in determining residence times for solvent molecules. This can be illustrated with the following simple example. Imagine a group of say, four water molecules solvating four donor or acceptor units on DNA bases. If a protein 'cap' is then placed across the top, contact with bulk water is greatly reduced, and the exchange rate must also be greatly reduced. The water molecules are in a 'tunnel' and can now only exchange by moving in and out in a greatly restricted manner. Bonding between these molecules and the protein *may* occur, but even if it does not, the exchange rate will be orders of magnitude less than usual. Thus the actual bonding may remain identical, and the increase in residence time need not indicate any *increase* in such bonding. Indeed, steric constraints could easily reduce the bond strengths, but still the residence times will remain long.

Equally important is the fact that water links that occur within sterically restricted areas may or may not increase the stability of the units that comprise this region. In the above example, they have little effect. Obviously, they may sometimes contribute a positive factor by bridging between groups that cannot come close enough for direct bonding, or between groups of the same type, that normally avoid each other. However, such links may be very weak because of an anti-co-operativity effect, and hence may contribute little to the overall bonding. The fact that water exchange occurs many times during the life-time of the protein–DNA unit implies that these water links are not a major part of the structure of the unit.

Thus whilst accepting that the NOE studies give previously unknown residence times for water molecules in certain regions of biopolymers, the reason for this is largely one of steric constraints which restrict exchange, rather than being a measure of enhanced bonding. Thus a given unit may form

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.

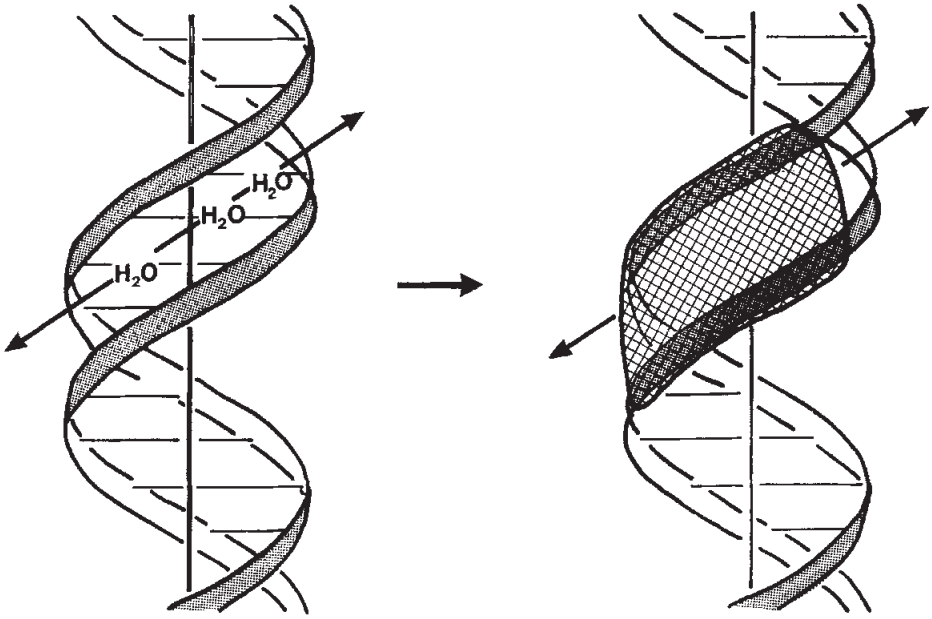

Fig. 1 Pictorial representation of (**a**) four water molecules (W) held by H-bonding to basic portions of DNA bases in a minor groove, and linked by dashed lines to outer water molecules. In (**b**) a protein 'cap' has been added by four H-bond links to the DNA thereby excluding the four water molecules from contact with outer water molecules and constraining them to the minor grove tunnel, from which escape is slow.

very strong H-bonds to one or more water molecules, but exchange may be in the ps time range, or they may not even bond at all, and yet their residence times can be in the ns range.

Finally, it is worth noting that residence times of water molecules may be longer when they are bound to poorly solvated units than when these units are well solvated. In such a case, the hydrogen bond *is* stronger, but the danger lies in reasoning that reduced solvation should imply weaker hydrogen bonds. Thus, steric factors might block the ability of a group such as >C=O to form two hydrogen bonds, but then the bond that *can* form will be much stronger than usual.

In the past, a lot of weight has been placed on the importance of crystallographically detected water molecules in biopolymers. As stressed by Wuthrich and his co-workers, these are, in general, not the water molecules that show up in the NOE studies, and hence their role is probably primarily a packing or space-filling role, dictated by the crystal structures.

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