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The Stereochemistry of the Water Molecules in the Hydrates of Small Biological Molecules

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Abstract

An examination of the stereochemistry of the water molecules in the hydrates of amino acids and peptides, carbohydrates, purines and pyrimidines, and nucleosides and nucleotides, reveals a variety of hydrogen-bonded configurations within a radius of 3·0 Å from the water oxygen atom. Water molecules which accept one hydrogen bond are more common than those that accept two, by a factor of 1·4. There are nine examples where the water is not a hydrogen-bond acceptor, but only one where it does not donate two hydrogen bonds. Of the 621 $O_WH\cdots A$ bonds examined, 15% were three centered and 2% were four centered or three-center bifurcated. The amino-acid and peptide hydrates displayed the greatest variety with 15 different hydrogen-bond configurations. The coordination of the donor and acceptor atoms within 3·0 Å of the water oxygen atom ranged from two to seven.

Introduction

The water molecule is unique in its hydrogen-bond functionality, which allows a wide range of orientational flexibility about a central atom with relatively minor differences in hydrogen-bond energy. It is for this reason that it has been, and still is, notoriously difficult to model convincingly the hydrogen-bond structure of water, aqueous solutions and the highly hydrated regions of crystalline macromolecules, or any assembly of water molecules when direct evidence relating to the hydrogen positions is not available.

In the low hydrates of the small molecules discussed in this article, the water molecules fill space in the packing of the more awkwardly shaped organic molecules and contribute additional hydrogen-bond energy. They are seldom disordered because their hydrogen-bonding versatility is such that they can adapt to the variety of different environments provided by the packing of the solute molecules.

An early presumption that, with a few exceptions, hydrogen bonds are formed to a single acceptor atom and that the X—H$\cdots A$ geometry is approximately linear was shown to be incorrect and frequently misleading by all the more-recent surveys (Kroon, Kanters, van Duijneveldt-van der Rijdt, van Duijneveldt & Vliegenthart, 1975; Olovsson & Jönsson, 1976; Mitra & Ramakrishnan, 1977; Ceccarelli, Jeffrey & Taylor, 1981; Jeffrey & Maluszynska, 1982, 1986; Taylor, Kennard & Versichel, 1984; Jeffrey, Maluszynska & Mitra, 1985). Another misleading early concept was that it is only necessary to investigate hydrogen bonding to interatomic distances which are less than sums of van der Waals radii. This is clearly inconsistent with the concept that the interaction for moderate or weak hydrogen bonds, as distinct from very strong hydrogen bonds (*cf.* Emsley, 1980), has a major Coulombic component that attenuates as r^{-1}.

In this article we report the hydrogen-bonded stereochemistry of the water molecule in the crystal structures of 311 hydrates of the amino acids, peptides, carbohydrates, purines and pyrimidines, and nucleosides and nucleotides where the crystallographic data provided acceptable hydrogen atomic coordinates. These structures provided a total of 1063 hydrogen bonds where the water molecule was either a donor or an acceptor.

Method

The source of the data is classes 44, 45, 47 and 48 of the Cambridge Structural Database (1988). Included in the survey are only the crystal structure analyses that satisfy the following criteria.

(1) All HO_WH, —OH, —NH_2 and —N^+H_3 hydrogen-atom coordinates are determined experimentally. In X-ray analyses, the hydrogen coordinates of $>$NH and $\geqslant$NH groups can be determined as, or often more, reliably from the atomic coordinates of the adjacent non-hydrogen atoms.

(2) The disagreement R factors are $<0·08$.

(3) The reported H—O_W—H angles are <130 and $>90°$.

For the X-ray analyses, the O—H and N—H covalent-bond lengths were normalized to 0·97 and 1·00 Å respectively, by extension in the direction of the bond. This is to correct for the charge-density shortening and for consistency with the internuclear

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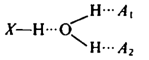
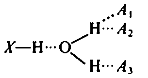
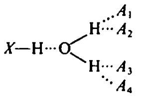
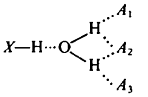
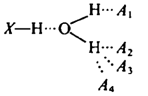
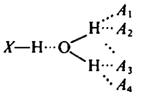
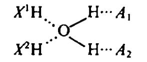
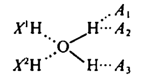
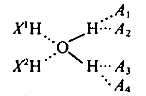
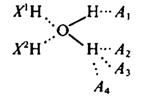
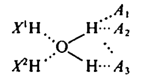
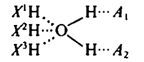
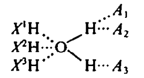
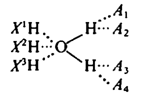
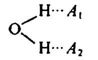
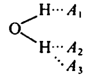
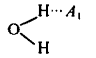
Table 1. *Types and distribution of water-hydrogen-bond stereochemistries*

	Type	Amino acids and peptides	Purines and pyrimidines	Carbohydrates	Nucleosides and nucleotides	Total
I*A*		40	26	7	34	107
I*B*		18	9	3	8	38
I*C*		3	7	0	1	11
I*D**		3	2	1	1	7
I*E*		3	0	0	0	3
I*F*		2	0	0	0	2
Totals (type I)		69	44	11	44	168
II*A*		31	14	8	16	69
II*B*		18	3	3	5	29
II*C*		7	6	2	3	18
II*E*		3	0	0	0	3
II*F*		0	0	1	0	1
Totals (type II)		59	23	14	24	120
III*A*		2				2
III*B*				2		2
III*C*		2		2		4
IV*A*		8			1	9
IV*B*		4	1			5
V		1				1
Totals (types III, IV and V)		17	1	4	1	23

* In this configuration, planes $H^1A_1A_2$, $H^2A_2A_3$ make angles $\simeq 60°$; $H\cdots A_1 \simeq H^1\cdots A_3 < 2{\cdot}0$ Å; $X—H\cdots A_1 \simeq X—H^1\cdots A_3 \simeq 160°$; $H^1\cdots A_3 \simeq H^2\cdots A_3 > 2{\cdot}5$ Å.

distances from neutron diffraction analyses (Jeffrey & Lewis, 1978). This procedure has been shown to significantly reduce the 'errors' in C—H, N—H and O—H bond lengths when X-ray analyses are compared with neutron analyses of the same crystal structure (Allen, 1986).

All interatomic distances and angles were calculated within a radius of 3·0 Å from the water oxygen atom. Those distances which involved the acceptor groups (A or AY), O_W, $\mathrm{O{=}C^-}$, $\mathrm{O{=}C}$, OH, $\mathrm{O}<$, $\mathrm{N}\lessgtr$, $\mathrm{Cl^-}$, $\mathrm{Br^-}$, $\mathrm{I^-}$ were identified as hydrogen bonds when $\mathrm{O}_W\text{—H}\cdots A < 3{\cdot}0$ Å and $\mathrm{O}_W\text{—H}\cdots A$ or $\text{N—H}\cdots A > 90°$.There are a few examples of $>$NH and $\mathrm{NH_2}$ groups acting as hydrogen-bond acceptors; these involved H···N distances $>2{\cdot}5$ Å.

The following definitions were used:

A *two-center bond*: a configuration with one acceptor atom, A, within 3·0 Å of the hydrogen atom making an $X\text{—H}\cdots A$ angle $>90°$.

A *three-center bond* (bifurcated donor): a configuration with two acceptor atoms, A_1, A_2, within 3·0 Å of the hydrogen atom making $X\text{—H}\cdots A_1$ or $X\text{—H}\cdots A_2$ angles $>90°$. In addition, the hydrogen atom is within 0·3 Å from the plane defined by X, A_1 and A_2, or the sum of the angles $X\text{—H—}A_1$, $X\text{—H—}A_2$ and $A_1\cdots\text{H}\cdots A_2 \simeq 360°$ (Parthasarathy, 1969; Taylor, Kennard & Versichel, 1984).

A *bifurcated bond* (bifurcated acceptor): a configuration where both water hydrogen atoms hydrogen bond to a single acceptor (Pimental & McClellan, 1960). This configuration is not observed in these hydrates except in combination with a three-center bond, *i.e.* a three-center/bifurcated bond (*e.g.* types I*D*, I*F*, II*F* in Table 1).

A *four-center hydrogen bond*: a configuration with three acceptors A_1, A_2, A_3, within 3·0 Å of the hydrogen atom with all three $X\text{—H}\cdots A$ angles $>90°$.

Results

The sixteen different hydrogen-bond configurations observed are shown in Table 1, with the populations in the four different classes of compounds. The number of three-coordinated waters, type I, exceeds the number of four-coordinated waters, type II, by a factor of 1·4. The configurations involving only two-center $\mathrm{O}_W\text{H}\cdots A$ bonds, *i.e.* I*A*, II*A*, III*A*, IV*A* and V constitute 60·5% of the total. Those involving one or two three-center $\mathrm{O}_W\text{H}\cdots A$ bonds constitute 34·5% of the total, the four-center bonds and three-center/bifurcated bonds are 5%. The proportion of three-centered water-donor bonds, 15%, is less than the 24% observed in the neutron diffraction crystal structures of the simple carbohydrates, by Ceccarelli *et al.* (1981), and the 70% in the amino-acid crystal structures, by Jeffrey & Mitra (1984).

In a general survey of $\text{NH}\cdots\text{O{=}C}$ bonds irrespective of molecular type, by Taylor *et al.* (1984), the proportion of three-center bonds was 20%. Water with its double-donor/single-acceptor functionality tends to reduce the proton deficiency, *i.e.* more acceptors than donor protons, which has been associated with three-center bonding in the carbohydrates and amino acids.

In this survey of crystal structures, there were nine examples where the water oxygen did not accept a hydrogen bond, but only one (MEGLAC) where the water did not exhibit its double-donor potential.

Water molecules tend to be stronger hydrogen-bond acceptors than donors. This is illustrated in the histograms shown in Fig. 1.

The numerical data for the individual bonds are given in Table 2.* The trend in $\mathrm{O}_W\text{H}\cdots A$ bond lengths for the acceptor group is $\mathrm{O{=}P^-} < \mathrm{O}_W < \mathrm{O{=}C^-} < \mathrm{O{=}C} < \mathrm{O(H)C} < \mathrm{N}\lessgtr < \mathrm{O}<$.

For $X\text{—H}\cdots\mathrm{O}_W$ bonds, the trend for donor groups is $\text{P—OH} < \mathrm{N^+(H_2)H} \simeq\, \geqslant\!\text{NH} < \text{C—OH} < \,>\!\text{NH} < \mathrm{O}_W\text{H} < \text{N(H)H}$.

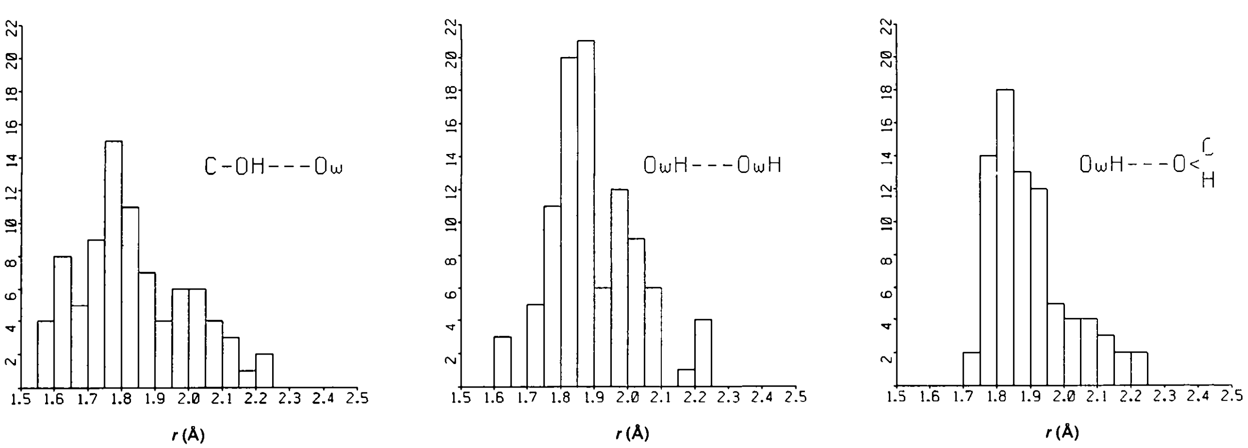


Fig. 1. Distribution of $\text{C—OH}\cdots\mathrm{O}_W$, $\mathrm{O}_W\text{H}\cdots\mathrm{O}_W\text{H}$, $\mathrm{O}_W\text{H}\cdots\text{O(H)C}$ hydrogen-bond lengths in the hydrates of small biological molecules.

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* Table 2 (hydrogen-bond distances, angles and sums of angles around O_W's) has been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52750 (44 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

The NH_2 group is rarely an acceptor. The exception is when it is the minor component of a three- or four-center bond with H···N > 2·5 Å.

The three-center bond lengths are most commonly unsymmetrical with the major component in the range 1·7 to 2·0 Å with angles 150–180°, while the minor component is 2·0 to 3·0 Å with angles in the range 140 to 90°.

There is a general correlation between hydrogen-bond lengths and hydrogen-bond angles, as shown in Fig. 2. As pointed out by Savage & Finney (1986), this is a consequence of the local repulsive forces between non-hydrogen-bonded atoms, which limits the orientation of the water molecules.

Discussion

When the water molecule is a single acceptor (type I), the coordination with respect to the water covalent O—H bonds may be planar or pyramidal. The sum of the bond angles around the water oxygen range from 360 to 300° with no bimodal distribution. This is consistent with the view that the water oxygen lone-pair electrons have little, if any, directional character. Similarly, the coordination when the oxygen is a double acceptor is frequently far from tetrahedral, with H···O_WH angles down to 90°. This absence of acceptor directionality has been noted previously in the study of the water stereochemistry in mainly inorganic salt hydrates by Chidambaram, Sequiera & Sikka (1964), Ferraris & Franchini-Angela (1972) and Chiari & Ferraris (1982). The repulsive interactions referred to previously (Savage & Finney, 1986) are clearly the dominant factors in determining the acceptor directions.

The greatest variety of configurations is found with the amino acids and peptides. This is a consequence of two factors. One is the higher proportion of charge groups, *i.e.* $N^+(H_2)H$, $\gtrsim$NH and O=C$^-$, which leads to more three-center bonding (*cf.* Jeffrey & Mitra, 1984). The second is the greater conformational constraints with the peptides, particularly the cyclic peptides. 12 of the 14 examples in which the waters are not hydrogen-bond acceptors (type IV) are peptide hydrates. In these crystal structures, the water appears to play only a minor role in the hydrogen-bonding scheme and non-stoichiometry is sometimes observed (*cf.* Marsh & Glusker, 1961).

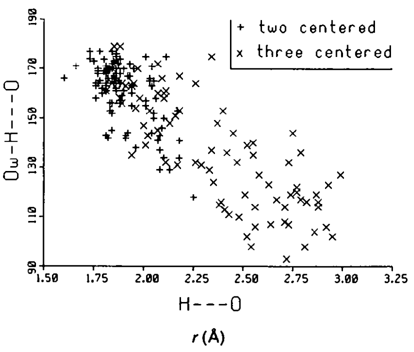

Fig. 2. Correlation between O_WH···O distances and O_WH···O angles for the components of the two-center and three-center bonds in the hydrates of small biological molecules.

In the absence of information concerning the hydrogen-atom positions, as in hydrated macromolecular crystal structures, these stereochemistries provide a menu of possible nearest neighbor environments, within 3·0 Å of a central water molecule, which range from two in type IV*A*, to seven in type III*C*. Combined with the constraints arising from the repulsive interactions (Savage & Finney, 1986), this information should assist in assigning hydrogen bonds to arrays of non-hydrogen atoms in the boundary and solvent regions of hydrated macromolecules.

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