Solvent interactions stabilising nucleic acid conformers

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ABSTRACT

The transition of oligonucleotides from the B to the A conformation has been studied by the use of simple geometric calculations aimed at finding possible hydration sites which could stabilize these conformations. The method involves the classification of equally spaced grid points, surrounding the oligonucleotide, into groups depending on whether a water molecule, so placed, could form single, multiple or nil contacts to polar oligonucleotide atoms. The occurrence of the multiple, and therefore bridging, sites is more extensive for the 'A' than the 'B' conformation. Thus, more general evidence is presented in support of the economy of hydration hypothesis in which phosphate groups, in the low humidity 'A' form, have been seen to be bridged by single water molecules. Similar calculations for the 'Z' DNA conformation show a different preference for multiple bridging sites.

INTRODUCTION

Deoxyribonucleic acid (DNA) exists in a number of conformations, the observed structure depending on the ambient conditions. DNAs with random sequences are found only in A, B and C forms, while material with strictly repetitive oligonucleotide sequences can adopt, in addition, D, E and Z forms. Z-family structures have left-handed double helices. Fibre diffraction studies (1,2) show that transitions between the A and B as well as the C and D forms are a function of both relative humidity and salt concentration. At high relative humidity, DNA adopts the B form, which is usually considered the biologically important conformer in vivo. If the relative humidity is lowered a transformation takes place to the A and C forms; the latter is an intermediate between B and A.

It has been suggested that the ability of A-DNA to achieve stability at low

humidity may be due to "economy of hydration" in this form relative to the B conformation (3). In B-DNA there is a wide interphosphate separation of 6.6Å. Each phosphate needs to be individually hydrated and it is only at relative humidities of 80% or higher that this can be achieved. In A-DNA, the sugar puckering is confined to the C(3')-endo form so the interphosphate separation is short, about 6Å. This means that two adjacent phosphate groups could be within hydrogen bonding range of the same water molecule. Bridges could then be envisaged where this water molecule simultaneously solvates both phosphate groups, and the total number of water molecules required to solvate the whole phosphate backbone is much smaller than would be the case for B-DNA. Several X-ray structures are available for hydrated A- and B-type oligonucleotides which give insight into the solvation structure, although only fully or partially ordered water molecule positions are determined by crystallographic techniques. An A-DNA octamer (4) is found to be highly solvated, particularly the phosphate-sugar backbone and the functional groups of the bases. The major groove contains a ribbon of water molecules forming closed pentagons with shared edges. These are linked to both O and N atoms and to the solvent chains connecting the O1 phosphate oxygens on each strand. The minor groove is extensively hydrated with a continuous network in the central region. An A-type tetramer (5,6) shows much less regular hydration; in particular, the minor groove is sparsely hydrated. This was attributed mainly to "blockage" of the minor groove by neighbouring atoms in the crystal. A B-DNA dodecamer (7,8,9) shows extensive hydration along the phosphate backbone. 65 peaks are associated with the backbone (an average of 3 per phosphate group). 19 others form a first shell of hydration to base edge N and O atoms within the major groove. The minor groove displays a zig-zag spine of hydration, believed to be mainly responsible for stabilising the B-form. In percentage terms, fewer water molecules are detected in the B-form dodecamer than in the A-form octamer. This was mainly attributed to the less ordered nature of the B-form crystal (10), reflected in much higher temperature factors. The temperature factors of the sugar-phosphate backbone of the B-form are especially high, implying either static or dynamic disorder. A low temperature study showed that in the dodecamer, disorder is mainly of static origin (11).

Saenger (12) has classified DNA hydration in terms of sequence independent and sequence dependent effects. Sequence independent hydration includes chains of water molecules along the base pairs in the major and minor grooves. Bridging of the phosphate groups in A-DNA can also lead to a sequence independent

hydration, comprising a systematic bridging with directional specificity, and involving O1-O1 and O1-O2 links. No such effect is thought to operate in B-DNA. Sequence dependent hydration is illustrated by the spine of hydration in the B-DNA dodecamer and in water pentagons in the A-type octamer, both referred to above.

A detailed comparison of the hydration sites in the A-octamer and B-dodecamer (13) shows that the average number of contacts a water molecule makes with other water molecules increases from the B-form to the A-form but the average numbers of contacts to nucleic acid atoms are identical. In both structures, waters make more contacts with the phosphates than with the bases and have infrequent interactions with the sugar rings, probably due to the shielding of the sugar oxygen by adjacent bases. Esterified O3' and O5 phosphate oxygen atoms appear to have a genuine lack of attraction for water molecules. Preferred solvation sites in model B-DNA structures have been predicted (14). The interaction surface between DNA and a water molecule is studied by calculating the electrostatic energy of interaction for each accessible position, based on ab initio fragment charge distributions. The author lists the sites where water can be simultaneously in contact with more than one DNA atom at a minimum point on the surface.

In this paper we describe a simple geometrical method for finding possible solvent sites around a macromolecule, and classifying such sites in terms of their capacity for bridging polar groups. Results from calculations of this type are presented for repeating base pair sequences in A, B and Z conformations, and for crystallographically determined A and B oligonucleotide sequences described above. The results demonstrate the "economy of hydration" in A-DNA relative to B-DNA and provide a qualitative explanation for the difference in stability of the two forms at different humidities.

METHODS

(A) OLIGONUCLEOTIDE STRUCTURES

Initially, coordinates for oligonucleotides were produced by a standard helix generation program, originally written by Dr S Neidle of the Institute of Cancer Research. 20 base pair lengths are used, encompassing about two complete turns of the double helix. We chose five sequences for study, d(CpGpCpGpCpGpCpGpCpG) in the A, B and Z conformations and d(ApTpApTpApTpApTpApT) in the A and B conformations.

Next, two crystallographically determined structures are used, the coordinates being taken from the Brookhaven Data Bank. These are an A-DNA tetramer at

2.0Å resolution (6), with 86 water molecule positions determined, and a B-DNA dodecamer at 2.3Å resolution (9) with 114 water positions. The former has the sequence d(CpGpCpG) and crystallises in space group $P4_12_1$ with eight asymmetric units per unit cell while the B-DNA has the sequence d(CpGpCpGpApApTpTp^{Br}CpGpCpG), space group $P2_12_1$ and four asymmetric units per unit cell.

(B) CALCULATIONS

The oligonucleotide is enclosed in a box equal to it in height (z-direction), and having a free area in the xy plane such that no atom is closer than 4Å to the sides of the box. The box is thereby ensured to be big enough to include all possible first neighbour solvent interactions. A regular grid of points is generated, spanning the volume of the box containing the central base pairs of each sequence, i.e. 10 base pairs in the case of the calculated structures, 2 for the experimental A structure and 6 for the experimental B structure. End effects are thereby eliminated. Grid points at 0.4Å apart and 0.8Å apart are sampled in turn looking for points which if occupied by solvent could interact with the macromolecule. The criterion used is that the point should not be closer than 2.6Å to any non-hydrogen atom, nor within 3.2Å of a non-polar atom, but should be within 3.2Å of a polar DNA atom. 'Polar' in this context refers to an oxygen or nitrogen atom and excludes carbon, halogen and phosphorous.

(C) ANALYSIS

Some grid points interact with more than one solute atom and are referred to as multicontact sites. For each sequence the numbers of single contacts, 2-fold, 3-fold contacts, etc. are tabulated. In addition the single contacts are subdivided into sugar, ester, phosphate and base atom contacts. The pair interactions arising from the multicontact sites are classified as phosphate/phosphate, base/base, phosphate/base etc. Whether these multicontacts are between atoms of the same or different phosphate/sugar groups or between atoms of the same or different strands, is noted. An additional type of calculation is performed for the experimental structures. Instead of generating grid points the experimental water molecule positions themselves, together with their symmetry related positions in other asymmetric units, are taken and classified as single or multicontacts in the same way as above.

Grid sampling leads to several neighbouring grid points in contact with a similar number and type of solute atoms. The contact sites are therefore collected into clusters, using a simple cluster analysis program, such that

TABLE I : Grid point contacts (percentages), at 0.4Å and 0.8Å for A-type Poly(dC-dG).poly(dC.dG)

GRID SIZE	0.4Å	0.8Å
<u>Single Contacts</u>		
SUGAR	15.1	15.0
ESTER	11.5	13.1
PHOSPHATE	53.5	51.6
CYTOSINE	8.5	9.1
GUANINE	11.4	11.2
<u>Multicontacts</u>		
SAME PHOSPHATE	13.6	12.8
INTER-PHOSPHATE	18.2	17.3
PHOS./ADJACENT ESTER	17.6	17.3
PHOS./FOLLOWING ESTER	9.8	9.7
PHOS./SUGAR	0	0
PHOS./BASE	3.8	3.7
BASE/BASE	28.9	30.4
BASE/SUGAR	6.5	6.6
BASE/ESTER	0.4	0.1
OTHERS	1.2	2.1

every contact in a given cluster is connected to every other via neighbouring grid points, while no grid point is neighbour to any point in another cluster. In this way mutually exclusive regions where contact with the solute is possible are generated. The size of these mutually exclusive regions gives a measure of the freedom of a water molecule to find such a position. Numerical calculations are performed on a VAX 11/750 computer. Graphical displays are produced on an Evans & Sutherland PS-300, using the FRODO program of Alwyn Jones.

RESULTS

Initially, the effect of varying the grid size was tested. A comparison between results at 0.4 and 0.8Å shows that the greater separation does not lead to any loss of information (Table I). The total number of contacts with

TABLE II : Numbers of Contacts Made by Grid Points with DNA Polar Atoms

SEQUENCE	NUMBER OF CONTACTS WITH N POLAR ATOMS						
	N=1	N=2	N=3	N=4	N=5	N=6	N=7
A-TYPE POLY(dC-dG).POLY(dC-dG)	3082	518	104	24	7	0	0
B-TYPE POLY(dC-dG).POLY(dC-dG)	2800	489	79	12	0	0	0
C-TYPE POLY(dA-dT).POLY(dA-dT)	2983	463	82	19	7	0	0
B-TYPE POLY(dA-dT).POLY(dA-dT)	2796	491	47	10	0	0	0
Z-TYPE POLY(dC-dG).POLY(dC-dG)	1973	363	34	0	0	0	0

the calculated structures decreases from around 15,000 to 3,000 for both A-DNA and B-DNA with increasing grid size but the proportions of contacts with each atom type are scarcely affected. It was therefore decided to use a 0.8Å grid size for all subsequent work, in order to reduce requirements for computer time.

Table II shows the total numbers of contacts found for each model sequence and Table III gives a breakdown in terms of contact type. The differences between the results for A and B sequences are quite marked. Multicontacts bridging more than two atoms are far more numerous for A than for B. In a given sequence (CG or AT) around half the single contacts are with phosphate oxygens in the case of A-DNA, while for B-DNA this rises to 66%. In contrast, looking at the multicontact pair interactions, A-DNA has up to 19% of these bridging different phosphate groups while B-DNA has no such bridging interactions at all. Considerable phosphate/ester bridging, i.e. between a phosphate oxygen and O3' or O5', can occur in this method for both forms. Substantial inter-strand bridges occur (bottom rows of Table III); these are more numerous in the case of CG sequences (15 and 17% for A- and B-DNA respectively) than in AT sequences (9 and 11% respectively). Some of them are phosphate-phosphate bridges in the case of A-DNA (always O2-O2), but by far the highest percentage are base-base contacts.

The results for the Z-DNA sequence show a number of distinctive features. There are far fewer contact sites : the total number calculated is 35% lower than the A-DNA value, see Table II. No multicontacts are found to bridge more than three atoms. Phosphate oxygens form as high a proportion of the single contact sites as they do for B-DNA. Unlike B-DNA, a significant proportion of multi-contacts bridge phosphate groups (about half the A-DNA value). However, bridges between a phosphate oxygen and an ester oxygen from the consecutive group are relatively scarce, unlike both A- and B-DNA. All of the

TABLE III : Grid point contacts (percentages) for model A-, B- and Z-type oligonucleotides.

CONFORMATION	A		B		Z
SEQUENCE	CG	AT	CG	AT	CG
<u>Single Contacts</u>					
SUGAR	15.0	15.9	6.8	7.7	2.2
ESTER	13.	13.6	10.8	10.7	11.4
PHOSPHATE	51.6	51.5	66.1	65.8	64.6
CYTOSINE (THYMINE)	9.1	10.2	5.9	5.9	7.0
GUANINE (ADENINE)	11.2	8.8	10.4	9.9	14.8
<u>Multicontacts</u>					
SAME PHOSPHATE	12.8	15.0	12.5	14.5	14.2
INTER-PHOSPHATE	17.3	19.3	0	0	8.0
PHOS./ADJACENT ESTER	17.3	20.3	17.6	20.4	22.8
PHOS./FOLLOWING ESTER	9.7	10.0	14.5	16.8	1.9
PHOS./SUGAR	0	0	0	0	0
PHOS./BASE	3.7	2.8	0.4	0	12.5
BASE/BASE	30.4	23.3	29.9	22.3	32.7
BASE/SUGAR	6.6	6.7	14.0	13.9	0
BASE/ESTER	0.1	0	1.1	1.2	3.2
OTHERS	2.1	2.6	10.0	10.9	4.7
<u>Inter-Strand</u>					
PHOS./PHOS.	6.3	11.6	0	0	33.9
BASE/BASE	87.3	88.4	90.6	100	66.1
BASE/SUGAR	6.3	0	9.4	0	0

inter-phosphate multicontacts are inter-strand bridges. 23% of multicontacts are inter-strand, a much higher proportion than for either A- or B-DNA. Two further striking differences between Z-DNA and the other forms may be noted; in Z-DNA, base-sugar multicontacts are completely absent and phosphate-base are relatively frequent.

TABLE IV : Grid point contacts (percentages) and numbers of water contacts for oligonucleotide structures

CONFORMATION	A (a)		B (b)	
METHOD	GRID	WATER	GRID	WATER
<u>Single Contacts</u>				
SUGAR	0	0	0	0
ESTER	38.0	8	14.5	3
PHOSPHATE	25.0	12	71.0	14
CYTOSINE	12.5	3	3.5	3
GUANINE	24.5	3	5.2	5
ADENINE	-	-	3.2	1
THYMINE	-	-	2.6	2
<u>Multicontacts</u>				
SAME PHOSPHATE	5.3	4	18.7	0
INTER-PHOSPHATE	5.3	1	0.5	0
PHOS./ADJACENT ESTER	6.4	7	16.0	1
PHOS./FOLLOWING ESTER	13.8	13	24.1	1
PHOS./SUGAR	0	1	0	0
PHOS./BASE	3.2	1	1.1	0
BASE/BASE	48.9	60	23.8	5
BASE/SUGAR	13.8	13	10.5	7
BASE/ESTER	1.1	1	0.3	0
OTHERS	2.2	15	5.0	0
<u>Inter-Strand</u>				
PHOS./PHOS.	0	0	0	0
BASE/BASE	89.5	15	83.0	3
BASE/SUGAR	10.5	3	17.0	2

(a) Conner et al, 1983

(b) Kopka et al, 1983

The contact sites found for the experimental structures are shown in Table IV. Again the preponderance of single phosphate contacts and absence of phosphate-phosphate bridging contacts is seen in the grid point analysis of B-DNA. 20% of multicontacts are inter-strand for A-DNA, 15% for B-DNA. Also shown in

Table IV is the contact analysis for the experimental water positions. This is given in terms of raw numbers of contacts since the total number of waters is too small for percentage values to be meaningful.

The distribution of multicontact sites is such that bridges between certain pairs of DNA atoms occur repeatedly. Taking the B-form poly(dC-dG).poly(dC-dG) sequence first, we find that the base-sugar bridges mainly involve O2 of cytosine, N3 of guanine and N2 of guanine. In each case the contact is to the sugar bound to the consecutive cytosine. Base-base bridges are formed between N2 and N3 or between N7 and O6 of the same guanine, also between O6(G)-N4(C), N7(G)-N4(C) and N2(G)-O2(C). Phosphate-base bridges are exclusively O1-N7(G). Inter-strand pair contacts are usually N2(G)-O2(C), N3(G)-N2(G), N4(C)-O6(G) and O6(G)-O6(G). All of these listed bridges occur also in A-DNA, and to a greater extent. Additionally, A-DNA has other miscellaneous base-base and base-sugar bridges which occur to a small extent, and the bridges between different phosphates, which are split between O1-O2, O1-O1 and O2-O2 in the ratio 5:2:1. However inter-strand contacts are always O2-O2 (half of the O2-O2s are inter-strand). Phosphate-ester bridges occur between all combinations of O1/O2 with O3'/O5'.

In Z-DNA the base-base and phosphate-ester sites are similar to those described above, except that N2(G)-N3(G) does not occur. Bridges between different phosphates are always inter-strand, and always O1-O1. Phosphate-base bridges include O2-N7(G) and O2-O6(G) as well as O1-N7(G). Base-sugar bridges do not occur.

Turning now to poly(dA-dT).poly(dA-dT), we find that in both forms O2 of thymine or N3 of adenine may be linked via a water bridge to O1' of the consecutive sugar, while in B-DNA additionally there are links of this type within the same sugar. Base-base contacts N6(A)-N7(A) and N7(A)-O4(T) are found frequently in both forms, additionally O4(T)-N6(A) occurs extensively in A-DNA but not in B-DNA, while N3(A)-O2(T) is peculiar to B-DNA.

Phosphate-base bridges are found only in A-DNA, and are O1-N7(A).

Inter-strand bridges (except phosphates) are most often between atoms of the same type, e.g. O4(T), N3(A), N6(A). This is true for both A- and B-DNA. In addition, substantial N6(A)-O4(T) inter-strand bridges are found. As far as phosphate-phosphate bridges are concerned, exactly the same features are found here as for poly(dC-dG).poly(dC-dG).

The distribution of the three-fold multicontact sites produces a sharp contrast between A- and B-DNA. In the A-type AT sequences, large numbers of assorted combinations of phosphate and ester atoms, such as O1-O2-O3',

Figure 1 : Centroids of contact site clusters around A-type poly(dA-dT).poly(dA-dT). Large clusters (more than 100 sites) are labelled.

01-02-05', 01-01-02 etc., are found to be in simultaneous contact with a single grid point. Then there are numerous examples of two atoms from vertically adjacent bases bridged together with a sugar oxygen atom, e.g. N1(T)-N3(A)-01', N9(A)-02(T)-01'. Three-fold base atom contacts bridging adjacent layers are N6(A)-N7(A)-04(T) or N3(A)-N9(A)-02(T), and there are inter-strand contacts involving N6(A) from both strands with 04(T), or 04(T) from both strands with N6(A). In the B-DNA sequence, the phosphate-ester type multicontacts are far fewer in number, the base-base-sugar contacts are not observed, and the base-base-base contacts are only found in inter-strand bridges such as N3(A)-N3(A)-02(T) and 04(T)-04(T)-N6(A), that is bases from different layers are not bridged. Numerous base-base-sugar bridges are seen in the B-DNA sequence; these involve either 02(T) or N3(A) with two 01' atoms. A similar pattern emerges from the CG sequences, except that some base-base-sugar bridges are seen in the B-DNA sequence; these involve either 02(T) or N3(A) with two 01' atoms. A similar pattern emerges from the CG sequences, except that some base-base-sugar contacts are now observed in the case of B-DNA. Also some

Figure 2 : Centroids of contact site clusters around B-type $d(CpGpCpGpApApTpTp\cdots CpGpCpG)$.

base-base-base contacts in the A-form have no analogue in AT sequences, being of type $06(G)-N7(G)-N4(C)$ between a cytosine and a guanine from adjacent layers.

Three-fold multicontacts in the Z-form $\text{poly}(dC\cdots dG).\text{poly}(dC\cdots dG)$ are of three main types : two oxygens from the same phosphate bridged with N7 from a neighbouring guanine, phosphate O2 with $06(G)$ and $N7(G)$, and inter-strand base-base-base contacts. $06(G)$ from both strands are bridged to $N4(C)$, or $02(C)$ from both strands are bridged to $N2(G)$.

Pictures of the calculated and observed hydration structures are shown in plates 1-7. Plates 1 to 3 show the centroids of clusters of grid point contacts around the A, B and Z structures. These are labelled according to their size : 'S' means a small cluster, containing less than 10 points, medium sized 'M' clusters are those with between 10 and 100 points, and 'L' represents a larger cluster containing over 100 grid points. Similar impressions are obtained from both the model and experimental oligonucleotide structures : for B-DNA, apart from one or two small clusters, the centroids produce a helical image of the backbone atoms. The larger and medium sized clusters are more likely to be in the vicinity of the phosphate groups. In A-DNA the centroids are more scattered, a helical image being more difficult to perceive, but a clear effect is the predominance of clusters within the

Figure 3 : Centroids of contact site clusters around z-type poly(dC-dG).poly(dC-dG).

grooves and not close to the backbone atoms themselves. Larger sized clusters are to be found near the bases and sugars; no clusters stand out as being near to individual phosphate groups. In Z-DNA the clusters are again distributed in an irregular way. They are, however, seen to be very prominent within the narrow minor groove.

Pictures of the hydrogen bonding contacts made by the crystallographic water positions (plates 4 and 5) further illustrate the differences between A- and B-DNA hydration; the individual solvation of phosphate groups in B-DNA and bridging effects in A-DNA are shown in close-up in plates 6 and 7.

DISCUSSION

Our results from the grid point calculations clearly support the idea of more economical hydration in A-DNA than in B-DNA (3). The bridging of phosphate groups and of bases from adjacent layers are found to be significant effects in the solvation of A-DNA, while B-DNA has individually hydrated phosphate

Figure 4 : Hydrogen bonding contacts in a crystallographically determined A-type tetramer.

groups and no examples of vertical base-base bridging by a single water molecule. Clusters of solvation sites line the atomic backbone of B-DNA but are found within the grooves of A-DNA.

The A-DNA phosphate-phosphate bridges observed by Saenger et al (3), namely

Figure 5 : Hydrogen bonding contacts in a crystallographically determined B-type dodecamer.

Figure 6 : Phosphate bridging by water molecules in an A-DNA tetramer

O(1) of the 3'-phosphate being linked by a water molecule to either the O(1) or O(2) oxygens of the adjacent 5'-phosphate, are predicted by our calculations. However the O(2) to O(1) and O(1) to O(1) bridges proposed by these authors for Z-DNA are not found by us in the idealised Z-DNA structure.

Figure 7 : Phosphate group hydration in a B-DNA dodecamer.

This is due to the larger inter-phosphate separations in our structure and the presence of neighbouring apolar atoms which would block the approach of a water molecule. Our Z-DNA results show a state intermediate between A- and B-DNA; bridging of phosphates within a single strand does not take place, but the narrowness of the minor groove allows inter-strand phosphate bridging to take place. A peculiar feature of the Z-DNA grid point analysis is the total absence of bridges between the sugar (O1') oxygens and the base polar atoms. The multicontact method allows us to generalise the concept of economical DNA hydration, since many pairs of sites other than phosphate-phosphate are bridged. The multicontacts which we have listed include the bridging sites found by other authors (12-14). The results from the model B-DNA structure agree well with those of de Oliveira Neto (14) if our poly(dC-dG).poly(dC-dG) is compared with his poly(dC).poly(dG) and poly(dA-dT).poly(dA-dT) with poly(dA).poly(dT). The major bridges found by this author were N7(A)-N6(A), N7(G)-O6(G), N3(A)-O1', O2(T)-O1', N2(G)-O2(C)-O1' and phosphate bridges O1-O2 and O2-O5', all of which occur extensively in our results.

We also agree substantially with the bridging sites observed in experimental DNA structures. These have been reviewed by Saenger (12) and by Westhof (13). Typical base-base examples are N7(G)-O6(G), N7(A)-N6(A) and N2(G)-N3(G), in both A and B forms. In the A-DNA octamer (4), one-water bridges are between N3(G) and O1', O2(C) and O1', phosphate O1 and O1, or phosphate O1 and O2. The B-DNA dodecamer (9) has one-water bridges N3(G)-O1', O2(C)-O1', O4(T)-O4(T), N7(A)-N7(A), plus inter-strand bridges between O2(C) and O2(C), and between N3 and O2. A Z-form hexamer (15) had bridges between N2(G) and phosphate O1, N7(G) and O1', phosphate O1 and O2, and inter-strand between O2(C) and O2(C). With the exception of N7(G)-O1' in the Z-form, we have found all of these multicontact sites in our model structures.

Calculations based on experimental water positions in the two representative crystal structures do not exactly agree with the corresponding grid point predictions. This may be due to the inherent limitations in crystallographic determination of solvent positions or to differences between crystal and solution states(16). The complete absence of phosphate bridging waters in B-DNA is seen in both methods; however for A-DNA the proportion of single water-phosphate contacts is much higher for the water positions than for the grid points. The blockage of the minor groove in this structure (4), caused by adjacent molecules in the unit cell, may be a contributory factor. In conclusion, it appears that this geometric approach to bridging water molecule sites stabilizing nucleotide conformations is successful in

reproducing experimental data and in leading to a generalisation of the concept of economy of hydration.

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