Crystal and Molecular Structure of a DNA Fragment Containing a 2-Aminoadenine Modification: The Relationship between Conformation, Packing, and Hydration in Z-DNA Hexamers†

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ABSTRACT: The crystal and molecular structure of d(CGUA′CG)$_2$ (where A′ is 2-aminoadenine) has been determined and refined to an *R* factor of 13.8% for data 8.0–1.3 Å. The structure is very similar to the original Z-DNA structures with the sequence d(CGCGCG)$_2$ [Gessner, R. V., Frederick, C. A., Quigley, G. J., Rich, A., & Wang, A. H.-J. (1989) *J. Biol. Chem. 264*, 7921] and shows that the substitution of 2-aminoadenine–uracil base pairs in the two central steps is consistent with Z-DNA formation. In addition, we show how waters mediating intermolecular interactions may help to explain the ZI–ZII conformational pattern found in many Z-DNA structures.

Since Z-DNA was first described (Wang et al., 1979; Drew & Dickerson, 1981), it has provided fertile territory for exploring a variety of biological and chemical issues that underlie the diversity and flexibility of nucleic acid structure. The roles of sequence and chemical environment are among the many issues that have been explored in attempts to understand this unusual and unexpected conformation.

A survey of the Z-DNA crystal structures (Table I) shows that, for hexamers, sequences containing alternating CG form Z-type conformations. When UA (Geierstanger et al., 1991), TA (Wang et al., 1984), or AT (Wang et al., 1985) are substituted in the central step and are accompanied by 5-methylated cytosines in the flanking regions, Z-DNA structures are also formed. The presence of 2-aminoadenine–thymine base pairs in the flanking regions is also consistent with Z-DNA formation (Coll et al., 1986). On the other hand, Raman spectroscopy measurements on crystalline d(CGTACG)$_2$ have shown that this sequence forms B-type structures (Peticolas et al., 1989). In the d(CGUA′CG)$_2$ structure reported here, we examine the question of whether Z-DNA forms when the central region contains 2-aminoadenine–uracil base pairs.

The hydration of Z-DNA structures has been the subject of considerable study (Zhou & Ho, 1990; Chevrier et al., 1986). The structure of d(CGUA′CG)$_2$ provides further opportunity to explore the interrelationships between conformation, packing, and water structure in Z-DNA hexamers.

EXPERIMENTAL PROCEDURES

Purified and lyophilized d(CGUA′CG)$_2$ (Gaffney et al., 1984) was dissolved in water to a concentration of approximately 6.0 mM. Crystals were grown at 4 °C by vapor diffusion of both sitting and hanging drops against a well consisting of water and 45–50% 2-methyl-2,4-pentanediol (MPD). Drops containing 1.8–2.0 mM DNA, 30 mM sodium cacodylate buffer (pH 7.0), 15 mM $MgCl_2$, 5 mM spermine tetrahydrochloride, and 20% MPD grew hexagonal rods in 2–4 weeks.

Table I: Structures of Z-DNA Hexamers Compared to d(CGUA′CG)$_2$[a]

structure description	rms	ZI/ZII	ref[b]	code
CGCGCG spermine/Mg^{2+}	0.12	4/5	1	ZDF001
CGCGCG Mg^{2+}	0.20	4/5	1	ZDF002
^{Me}CG^{Me}CG^{Me}CG	0.35	4/5	2	ZDFB03
^{Me}CGTA^{Me}CG	0.37	4/5	3	ZDFB06
^{Me}CGUA^{Me}CG/Cu^{2+}	0.41	4/5	4	ZDFB10
CA′CGTG	0.34	4/5	5	ZDFB11
CGC^{Me}GCG	0.33	4/5	6	ZDFB21
^{Me}CGUA^{Me}CG	0.43	4/5	7	ZDFB24
CGCGCG/Cu^{2+}	0.24	4/5 and 8/9	8	ZDF028
^{Br}CG^{Br}CG^{Br}CG 291 K	0.33	no	9	ZDFB04
^{Br}CG^{Br}CG^{Br}CG 310 K	0.34	no	9	ZDFB05
CGCG^{F}UG	0.50	no	10	ZDFB12
CGCGCG spermine	0.46	no[c]	11	ZDF029

[a] The column labeled "structure description" identifies a structure; "rms" lists root mean square deviations of a particular DNA structure with d(CGUA′CG)$_2$. All except the phosphate oxygen atoms were superimposed; "ZI/ZII" specifies the location of a ZII conformation in a sequence; "ref" is the reference to a structure listed in the next footnote; "code" is the Nucleic Acid Database (Berman et al. 1992) code name of a structure. [b] References: (1) ZDF001, ZDF002 (Gessner et al., 1989); (2) ZDFB03 (Fujii et al., 1982); (3) ZDFB06 (Wang et al., 1984); (4) ZDFB10 (Geierstanger et al., 1991); (5) ZDFB11 (Coll et al., 1986); (6) ZDFB21 (Ginell et al., 1990); (7) ZDFB24 (Zhou & Ho, 1990); (8) ZDF028 (Kagawa et al., 1991); (9) ZDFB04, ZDFB05 (Chevrier et al., 1986); (10) ZDFB12 (Coll et al., 1989); (11) ZDF029 (Egli et al., 1991). [c] A residual electron density at the backbone between residues 8 and 9 has been described as a partially occupied ZII conformation.

The crystal data were obtained at room temperature and showed that this sequence crystallizes in the identical space group and with cell dimensions similar to those found for other Z-DNA hexamers (Table II). X-ray intensity data were collected using $\omega/2\theta$ scans on an Enraf-Nonius CAD4 diffractometer. The intensities were corrected for Lorentz, polarization, absorption, and decay with the Enraf-Nonius program package MOLEN (Fair, 1990). A total of 13 324 unique reflections were collected to a resolution of 1.0 Å. Of those, 5790 were above $2\sigma(I)$. Analysis of the data statistics showed that the effective resolution was 1.3 Å since a significant drop was observed in the number of observed reflections above this resolution.

Although the crystal data of the structure reported here are similar to those of d(CGCGCG)$_2$ (Gessner et al., 1989), rotation and translation searches were conducted using

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Table II: Crystallographic Data for $d(CGUA'CG)_2$

Crystal Data	
contents of asymmetric unit	
molecules of d(CGUA'CG)	2
DNA formula	$C_{114}H_{140}O_{72}N_{48}P_{10}$
molecular weight of DNA	3644.4
space group	$P2_12_12_1$
unit cell	
a (Å)	17.944 (2)
b (Å)	31.282 (4)
c (Å)	44.70 (2)
$\alpha = \beta = \gamma = 90°$	
volume (Å^3)	25 092
Data Collection Statistics	
crystal size (mm)	0.19 × 0.90 × 0.15
temperature	22 °C
crystal mounting	capillary
data collection device	Enraf-Nonius CAD4
radiation	Cu Kα, graphite monochromator, rotating anode
unique data collected	13324
reflections $I > 2\sigma(I)$	5790
resolution limit (Å)	1.02
% reflections > $4\sigma(F)$	
up to 1.5 Å	83
1.5–1.3 Å	45
1.3–1.0 Å	17
Refinement Statistics	
resolution (Å)	8.0–1.3
reflections used $F_o > 4\sigma(F_o)$	4588
stoichiometric waters	77
water positions	84
R value[a]	13.8
wR value[b]	14.0[c]
residual electron densities	from +0.56 to −0.73 (e$^-$/Å^3)[d]
rms deviations and esd's	
sugar and base atom distances	0.017/0.030 Å
sugar and base atom angles	0.034/0.040 Å
planarity of bases	0.023/0.030 Å
average B factors (Å^2) of base atoms	5.8
backbone atoms	7.5
water molecules	28.0

[a] $R = \Sigma(F_o - F_c)/\Sigma(F_o)$. [b] $wR = [\Sigma w(F_o - F_c)^2/\Sigma w(F_o)^2]^{1/2}$. [c] $w = 1/[12.00 - 10.00(\sin\theta/\lambda - 0.16667)]$. [d] The number of electrons in the asymmetric unit was taken as number of electrons of the refined atoms.

MERLOT (Fitzgerald, 1988) in order to confirm that the crystal and molecular structures are similar. Rigid body refinement using CORELS (Sussman et al., 1977) was followed by restrained refinement using NUCLSQ (Westhof et al., 1985) and electron density fitting using FRODO (Jones, 1978) on an Evans and Sutherland PS390 graphics computer. At this point, the R factor was 22% for the data between 1.5 and 6.0 Å. Further refinement using data between 8.0 and 1.3 Å was conducted using SHELX76 (Sheldrick, 1976) and NUCLSQ with individual isotropic temperature factors.

Difference electron density maps calculated using PROTEIN (Steigemann, 1985) and displayed using FRODO revealed potential solvent molecules. Water molecules were modeled as oxygen atoms and fit into peaks no weaker than 3σ on the electron density maps. Eighty-four water molecule positions were located and refined. Fourteen of them were closer than 2.3 Å to one another and were considered disordered. The occupancies of most of the disordered waters refined to values close to 0.5, and two water molecules refined to a value of 0.75.

None of the water positions could be unequivocally described as a magnesium or sodium ion or a part of a spermine molecule. Some of the water molecules had six coordinating neighbors but with distances too long to be Mg^{2+}–H_2O interactions.

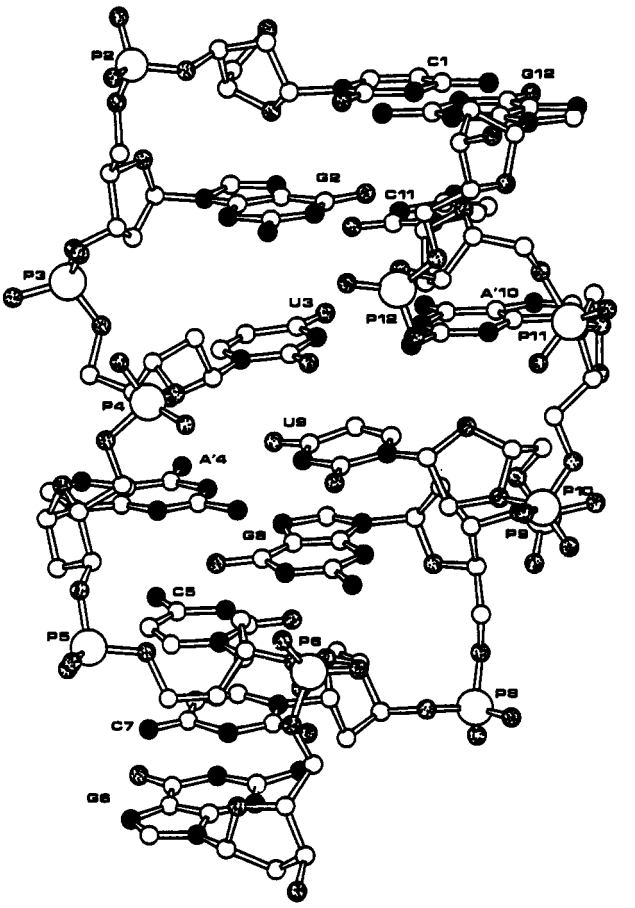

FIGURE 1: Conformation and numbering system for $d(CGUA'CG)_2$. The phosphate atoms are shown as large spheres, nitrogens are shaded, and the oxygens are stippled.

One solvent peak refined very close (2.16 Å) to O1 (P C9) and could be a metal cation. However, it was coordinated by only two other water molecules at distances of 3.1 and 3.3 Å and was, thus, refined as a water molecule.

The refinement converged at R = 13.8% using 4588 reflections stronger than $4\sigma(F_o)$ at resolution between 8.0 and 1.3 Å. Table II gives the final refinement statistics. Coordinates and structure factors have been deposited with the Brookhaven Protein Data Bank (Bernstein et al., 1977) (code 1D76).

RESULTS

The structure of $d(CGUA'CG)_2$ is in the Z-DNA conformation (Figure 1). The central uracil and 2-aminoadenines form base pairs that are oriented in the normal Watson–Crick fashion. The root mean square deviations between the $d(CGUA'CG)_2$ and other Z-DNA hexamers (Table I) show that this structure most closely resembles the magnesium and spermine forms of $d(CGCGCG)_2$ (Gessner et al., 1989).

The backbone torsion angles of this structure (Table III) are very similar to those found in the other Z-DNA structures. Figure 2 illustrates the conformation angles in this structure. From comparison with two standard Z-DNA structures (Gessner et al., 1989), it is clear that the substitution of the central CG dinucleotide with UA' in the hexanucleotide sequence CGCGCG does not affect the backbone torsion angles in any systematic way. In particular, this structure maintains the previously described ZI/ZII conformational mix (Wang et al., 1981), which is characterized by a difference in the conformations of the two strands in the duplex.

Table III: Conformation Angles in d(CGUA′CG)$_2$[a]

residue	α	β	γ	δ	ϵ	ζ	χ
C1	nd	nd	51	140	269	79	33
G2	69	190	175	86	240	295	250
U3	215	233	51	147	260	76	19
A′4	59	190	186	96	182	65	245
C5	166	166	46	143	267	76	33
G6	71	177	185	149	nd	nd	266
C7	nd	nd	53	139	269	81	29
G8	58	192	175	90	244	290	252
U9	217	226	57	143	272	74	20
A′10	62	180	185	99	245	296	249
C11	206	231	59	139	266	69	24
G12	83	184	184	147	nd	nd	262

[a] Angles are in degrees and defined as follows: α = O3′–P–O5′–C5′; β = P–O5′–C5′–C4′; γ = O5′–C5′–C4′–C3′; δ = C5′–C4′–C3′–O3′; ϵ = C4′–C3′–O3′–P; ζ = C3′–O3′–P–O5′ and χ = O4′–C1′–N1py/N9pu–C6py/C8pu. An entry "nd" in the table means that the angle is not defined for a particular residue.

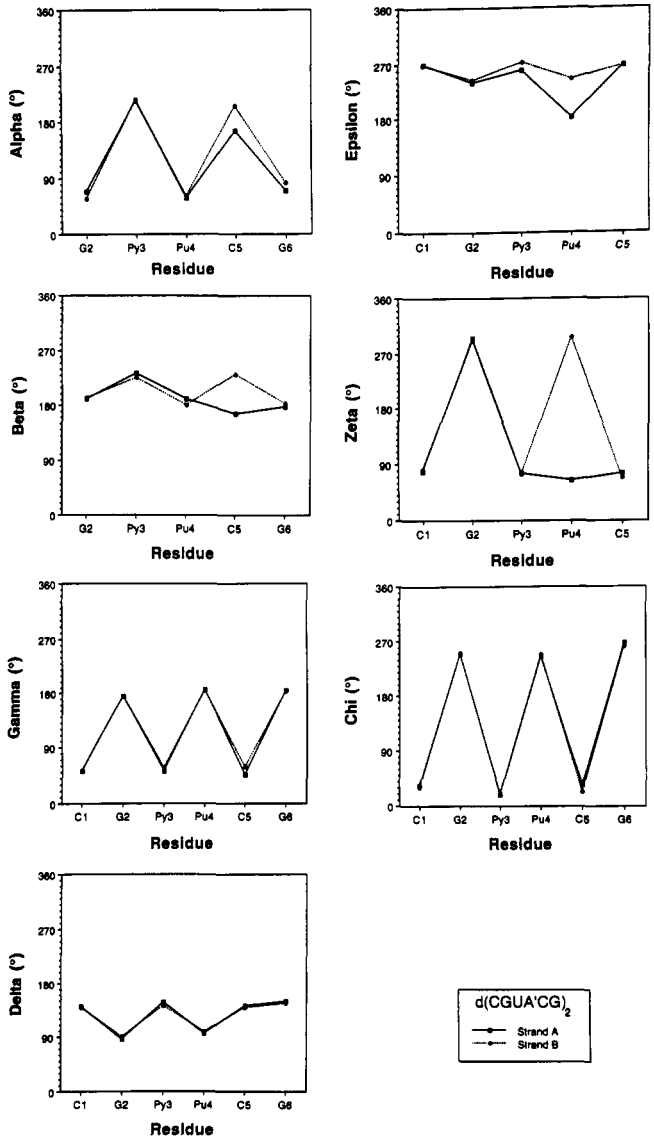

FIGURE 2: Comparison of torsion angles in the two strands of d(CGUA′CG)$_2$. The angles are defined in Table III.

In ZI, all pyrimidine steps have one set of torsion angle values, and the purine steps have another one. Only values of the δ torsion angle of the 3′ end residues G6 and G12 make an exception to this general rule. In the ZII conformation, the zig-zag pattern of torsion angles is broken. The difference between ZI and ZII is most pronounced in angles β and ζ as well as in α and ϵ (Figure 2).

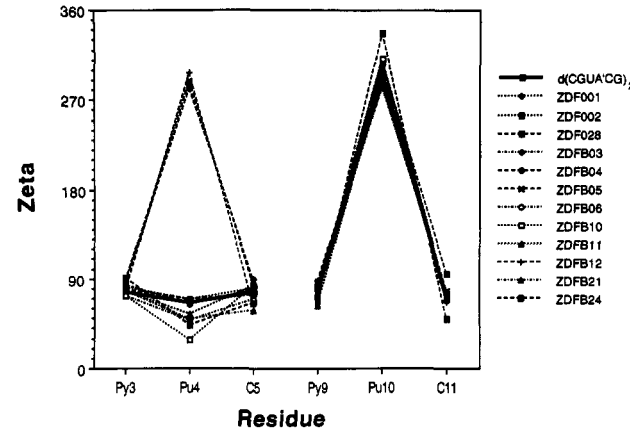

FIGURE 3: Comparison of values of the torsion ζ = C3′–O3′–P–O5′ for d(CGUA′CG)$_2$ and other Z-DNA hexamers (see Table I).

In d(CGUA′CG)$_2$ and several other structures (see Table I), there is just one stretch of the ZII conformation between residues 4 and 5 of one strand (strand A) making strands A and B conformationally different. The differences between the ZI and ZII conformations are observable only in the backbone. The bases from one strand can be superimposed very closely on the bases of the other strand.

Of all the angles, ζ = C3′–O3′–P–O5′ shows the largest differences between the ZI and ZII conformations. In ZI, ζ has a bimodal distribution with values in the + synclinal and − synclinal regions. It is always + synclinal for pyrimidine residues and usually − synclinal for purines. In the ZII conformation, it adopts a + synclinal value at a purine step.

The ZII conformation can be found in most Z-DNA hexanucleotides. Its location in Z-DNA hexamers is indicated in Table I. Figure 3 summarizes the behavior of the ζ angle at residues 3, 4, and 5 and 9, 10, and 11. Only four structures do not exhibit the ZII conformation in either strand: one of them is a cation-free d(CGCGCG)$_2$ structure cocrystallized only with spermine (Egli et al., 1991). This structure packs differently from other hexamers. Others are (^{Br}CG^{Br}CG^{Br}CG)$_2$ (Chevrier et al., 1986) with large bromine atoms and d(CGCG^{F}UG)$_2$ with the ^{F}U–G mismatch. For d(CGUA′CG)$_2$ and eight others, the ZII conformation is localized at the same position, between residues 4 and 5 of one strand. One structure, d(CGCGCG)$_2$ cocrystallized with Cu^{2+} (Kagawa et al., 1991), has the ZII conformation located on both strands in steps 4/5 and 8/9. A possible explanation for the conservation of the ZII location is given under Discussion.

The base pair morphology parameters (Dickerson et al., 1989) for d(CGUA′CG)$_2$ were calculated (Babcock & Olson, 1992), and they also follow the same general patterns observed in other Z-DNA structures (Figure 4). Substitution of UA′ at the central steps does not appear to affect these parameters substantially. One of the characteristics common to most structures is that buckle and twist alternate between high and low values along the sequence. Only in the case of the guanine O6 methylated structure d(CGC^{O6Me}GCG)$_2$ (Ginell et al., 1990) is this pattern for buckle broken. Perhaps not surprisingly, buckle shows a great variability at the ends of the sequences. Another pattern is seen in the graph of roll as a function of the sequence. Here, the structures with unmodified central TA and UA steps show distinctly lower values for the base step 3/4 than do other structures. The value for this step in the d(CGUA′CG)$_2$ structure is intermediate between the highest and lowest values.

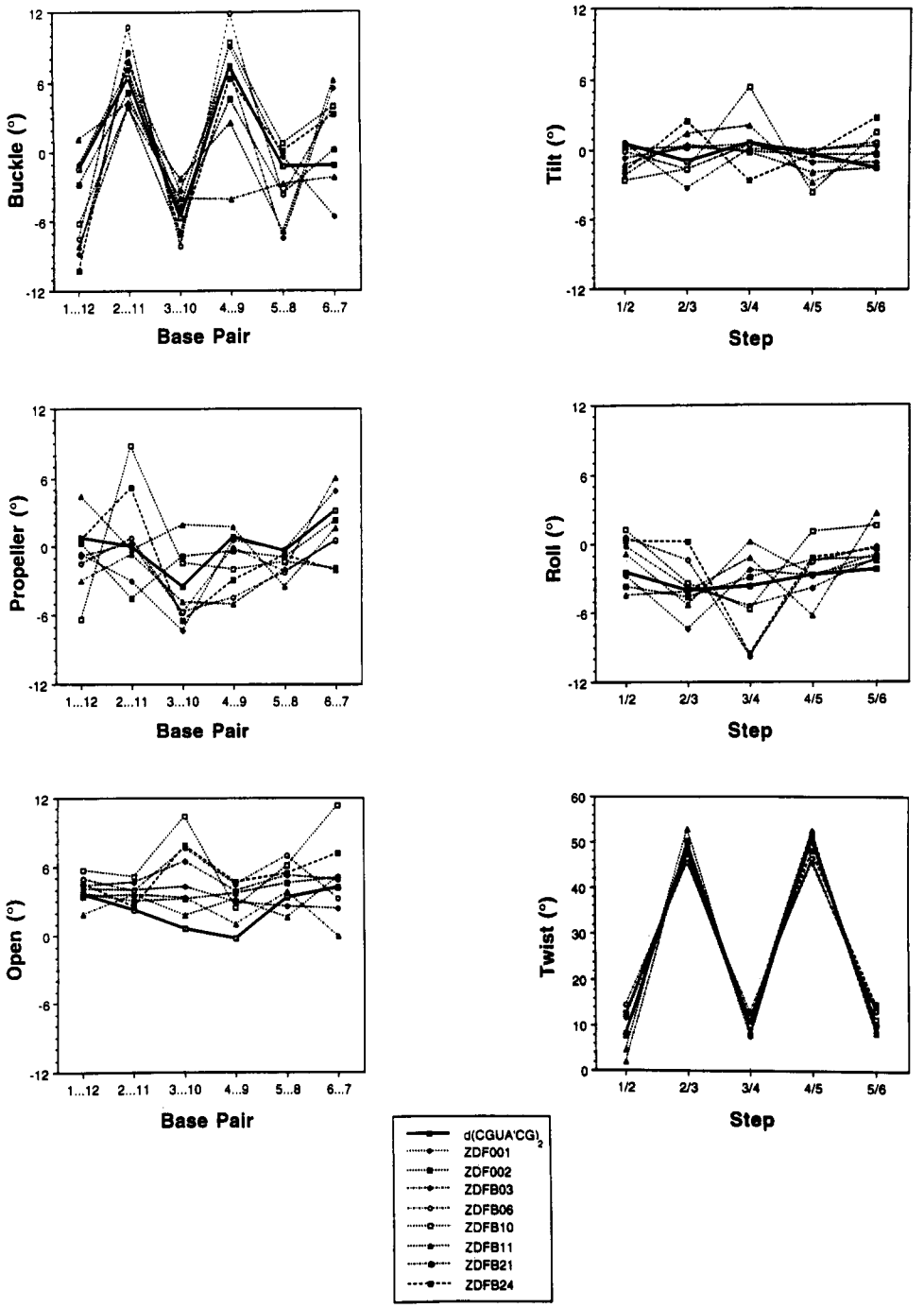

FIGURE 4: Comparison of base pair morphology among selected Z-DNA hexamers. Sequences and references for listed structures are given in Table I.

The hydrogen bonding and packing of DNA helices observed in this structure are the same as in other Z-DNA structures. That is, there is one hydrogen bond between the terminal O3′ (G12) and a symmetry-related O1 (P G2) (Figure 5). O3′ (G6) forms hydrogen bonds only to water molecules. Interestingly, although the interactions of the terminal O3′ atoms are different in both strands, the conformations are quite similar. Three structures, d(^{Me}CGTA^{Me}CG)$_2$ (Wang et al., 1984), d(CA′CGTG)$_2$ (Coll et al., 1986), and d(CGC^{O6Me}GCG)$_2$ (Ginell et al., 1990) also contain a hydrogen bond between O3′ (G6) and a symmetry-related O2 (P C9), but d(CGUA′CG)$_2$ does not show that hydrogen bond. These hydrogen-bonding contacts are the only DNA–DNA contacts not mediated by water molecules.

As has been observed for other Z-DNA structures, water molecules make extensive networks in this structure (Figure 6). There is a continuous spine of hydrogen-bonded water molecules interacting with DNA atoms and other waters along the center of the minor groove. There is one four-water and one three-water bridge between the phosphate groups across the minor groove. There is also a pentagon of water molecules attached to the base atoms of residues 2, 11, and 12 and to phosphate oxygens of residues 2 and 3. The pentagon is located in the more accessible area between two stacked double helices and the tightly constrained space of the minor groove where only limited branching of hydrogen bonds is possible.

Although the surface of the shallow major groove constrains the positions of water molecules much less than does the deep minor groove, most of its water molecules are still arranged into hydrogen-bonded networks. The largest network connects 10 major groove hydrophilic atoms from bases, sugars, and phosphates. This network is directly connected with the minor

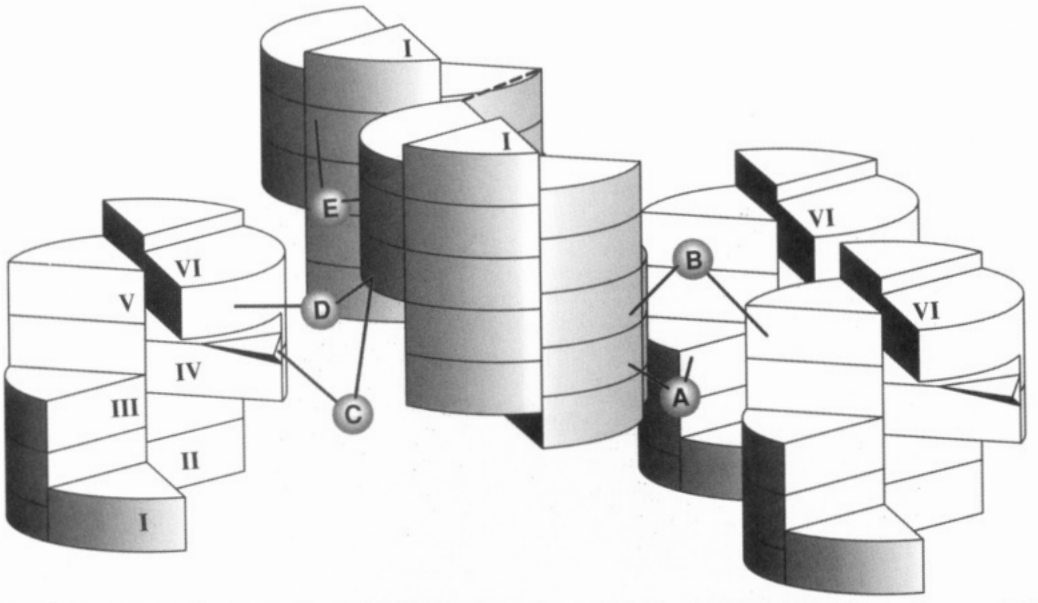

FIGURE 5: Schematic drawing showing the packing of Z-DNA hexamers in crystals. Base-paired nucleotides are drawn as half-cylinders with a step at the 5′ ends. The numbers I–VI refer to base-paired nucleotides: C1–G12 (I), G2–C11 (II), ..., G6–C7 (VI). The stretch of the ZII conformation between residues 4 and 5 of strand A is indicated by a pyramidal bulge. Only those water molecules are drawn which participate in intermolecular DNA–water–DNA interactions of residues 4/5 and 10/11. They are labeled A, B, C, D, and E, and their hydrogen bonds are listed in Table IV. Different backbone conformations at residues 4/5 (ZII) and 10/11 (ZI) are related to interactions with waters A, B, C, D, and E and are important for packing of Z-DNA hexamers. The DNA–DNA hydrogen bond between O1 (P G2) and O3′ (G12) is indicated by a dotted line.

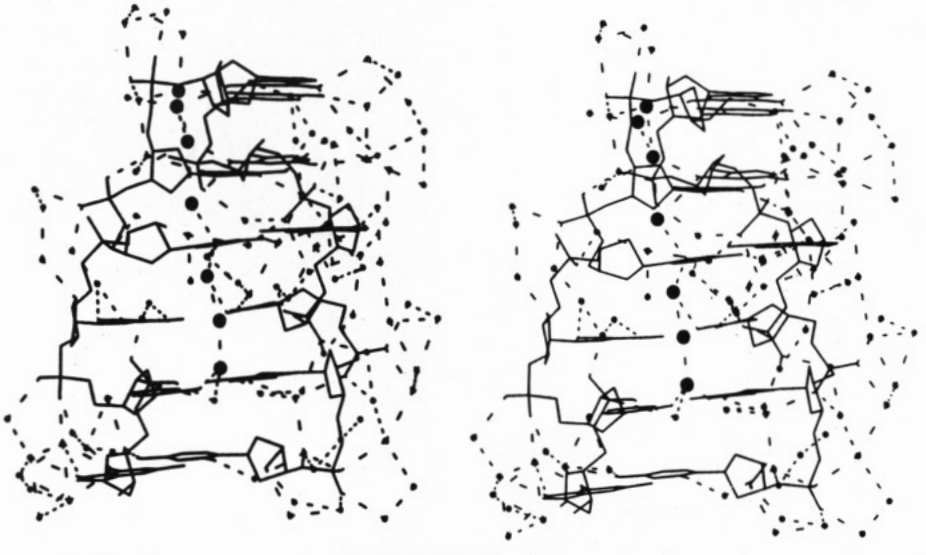

FIGURE 6: Stereodiagram showing the water networks of $d(CGUA'CG)_2$. Water molecules are drawn as spheres. The water molecules forming the spine of hydration in the minor groove are shown as larger spheres. Hydrogen bonds between water molecules and DNA atoms are indicated as dotted lines.

groove water spine via atoms O2 (C5), O2 (C7), and N2 (G8). Thus, a large part of the surface of the DNA molecule is covered by water. Another large major groove water network connecting six base and phosphate atoms was observed. Several two-water and three-water bridges connecting base and phosphate atoms were also present.

A comparison of the solvent positions in $d(CGUA'CG)_2$ with those found in the two standard Z-DNA structures, $d(CGCGCG)_2$ Mg^{2+}/spermine and $d(CGCGCG)_2$ Mg^{2+} (Gessner et al., 1989), reveals that the positions of many of the water molecules are similar. The $d(CGCGCG)_2$ Mg^{2+} spermine structure, as the one most similar to $d(CGUA'CG)_2$, has 62 water molecules within 1 Å from $d(CGUA'CG)_2$ waters. The magnesium form of $d(CGCGCG)_2$ has 33 such water molecules. Considering the numbers of refined solvent positions in these structures [84 waters in $d(CGUA'CG)_2$, 74 waters, one Mg^{2+}, and two spermines in $d(CGCGCG)_2$ Mg^{2+} spermine, and 84 waters and 4 Mg^{2+} in $d(CGCGCG)_2$ Mg^{2+}], it seems probable that the arrangement of water molecules around the DNA double helices of closely related conformations is conserved.

The hydration of the minor groove was compared qualitatively among several Z-DNA structures. The spine of waters in the center of the minor groove is found to be a strong structural feature. All of the first shell minor groove water molecules found in $d(CGUA'CG)_2$ were seen in $d(CGCGCG)_2$ Mg^{2+}/spermine at equivalent positions. In $d(CGCGCG)_2$ Mg^{2+} the water positions differ somewhat, but the minor groove spine of hydration is maintained. In $d(CA'CGTG)_2$ the positions of the water molecules are sufficiently different to give rise to a different net of hydrogen bonds. Neither of the thymines form hydrogen bonds with water. In $d(^{Me}CGUA^{Me}$-

Table IV: Inter- and Intramolecular Water Bridges Involved in Hydrogen Bonding of Residues 4/5 and 10/11 in $d(CGUA'CG)_2$[a]

i	j	k	sym[b]	D(i–j)	D(j–k)	angle i–j–k
		Intermolecular Bridges				
O1 (P C5)	A	O1(PA'10)	i	2.65	2.78	104
O4' (A'4)	B	O1 (P G8)	ii	2.77	2.64	100
O1 (P A'10)	C	O1 (P C5)	iii	2.78	2.65	104
O1 (P A'10)	D	O4' (G6)	iii	2.66	2.75	126
O1 (P C11)	E	N7 (G2)	iv	2.64	2.80	83
		Intramolecular Bridges				
O2 (P C5)	W	O6 (G6)	v	2.50	2.79	124
O2 (P C11)	W	O2 (PG12)	v	2.54	2.88	115
O2 (C11)	W	O2 (U3)	v	3.21	3.19	85

[a] The columns in the table titled *i*, *j*, and *k* are atoms involved in a bridge, "sym" refers to a symmetry operation of atom *k* in the bridge, "*D*(*i*–*j*)" and "*D*(*j*–*k*)" list distances (Å) between atoms in a bridge, and "angle *i*–*j*–*k*" is the angle of the hydrogen bond. Water molecules mediating the intermolecular contacts are labeled A, B, C, D, and E and are also shown in Figure 5 Those water molecules that make intramolecular bridges are labeled W. [b] Symmetry operations of atom *k*: (i) $-x + 1, y - 1/2, -z + 3/2$; (ii) $-x + 2, y - 1/2, -z + 3/2$; (iii) $-x + 1, y + 1/2, -z + 3/2$; (iv) $x - 1, y, z$; (v) x, y, z.

$CG)_2$, only discontinuous patches of water in the minor groove are present, and the pyrimidine O2 atoms interact with water molecules that lie in the base plane. In all other structures, water molecules hydrogen bonded to O2 atoms were found between the base planes.

In a previous study (Schneider et al., 1992), we analyzed the positions of water molecules around bases in all Z-DNA structures and found highly populated regions of the water arrangements. The water positions around the bases of $d(CGUA'CG)_2$ fall into the previously observed patterns.

DISCUSSION

This structure provides an excellent opportunity to reevaluate the relationships among packing, hydration, and conformation in Z-DNA structures. Comparison of the water structure in this crystal and in eight other Z-DNA hexamers with a stretch of the ZII conformation located only between residues 4 and 5 (Table I) reveals a number of similarities. The common DNA–water–DNA interactions may help to explain the persistence of the position of the ZII conformation between residues 4 and 5, and they indicate that the presence of the ZII conformation on only one strand may be related to packing of Z-DNA hexamers into the crystal lattice (Figure 5).

Although there are no DNA–DNA interactions in the vicinity of residues 4/5 and 10/11, the water environment around each strand is significantly different. The analysis of the water networks around residues 4/5 and 10/11 reveals some common DNA–water–DNA interactions (Table IV). In strand A, there is one intramolecular water bridge that connects a phosphate oxygen on P5 and a base heteroatom. Chain B has two intramolecular water bridges: one connects two phosphate oxygen atoms, and the other connects two base heteroatoms. Of the six other Z-DNA structures which have the ZII conformation only between residues 4 and 5 and for which water coordinates are available, three contain all of the intramolecular bridges and the other three contain some of the bridges. It is significant that the most highly refined structures contain all of the bridges.

Intramolecular water bridges where a water molecule binds to two atoms from the same double helix certainly help to stabilize both the ZI and ZII conformations. They do not, however, explain why two potentially equivalent strands crystallize differently and, more importantly, why the short stretch of symmetry-breaking ZII conformation always has the same location in the nucleotide chain.

Of more relevance to understanding crystal packing are the DNA–water–DNA contacts that bridge two symmetry-related double helices at residues 4/5 and 10/11 (Table IV and Figure 5). One contact is made by the ZII stretch, two by the ZI stretch, and one bridge couples the two duplexes between the phosphate groups 5 and 10. Of the six other structures analyzed, four contain *all* the bridges, one contains four bridges and one contains two. The last of these structures appears to be incompletely refined.

Thus, we conclude that the water bridges described here (Figure 5 and Table IV) are related to the differences of backbone conformations in the Z-DNA hexamers and that they play a key role in the packing of Z-DNA crystals. The water bridges specifically recognize and bind other duplexes "labeled" by the ZII stretch between residues 4 and 5. Cooperative formation of the ZII stretch and the bridges may initialize crystallization. Therefore, the distortion of a part of one strand into the ZII conformation and subsequent loss of 2-fold symmetry between the strands facilitate a unique packing of Z-DNA molecules into the crystal structure. The proposed mechanism of crystallization could help to rationalize experimental conditions of Z-DNA crystallization discussed by Ho et al. (1991).

The prevalence of the ZI conformation in most Z-DNA backbones suggests that this conformation is energetically more favorable than ZII. The extent and position of the distortion into the ZII conformation is a compromise between the energy cost of the backbone twist, the gain in intermolecular interaction, and entropical changes during the crystallization.

If we assume that the ZI conformation is energetically more favorable, we suggest that the advantage of better recognition between duplexes in terms of both interaction energy and entropy is greater than the energy loss related to the backbone deformation in the majority of Z-DNA structures. The existence of three structures which pack in the same way as most Z-DNA hexamers but either do not have a ZII conformation in either strand [$(^{Br}CG^{Br}CG^{Br}CG)_2$ 291 and 310 K] (Chevrier et al., 1986) or have it in both strands [$(CGCGCG)_2$ Cu^{2+}] (Kagawa et al., 1991) proves, however, that flexibility of packing interactions is great and that nature is not committed to a single solution even in simple cases.

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REFERENCES

Babcock, M. S., & Olson, W. K. (1992) in *Computation of Biomolecular Structures: Achievements, Problems, and Perspectives*, Springer-Verlag, Heidelberg.

Berman, H. M., Olson, W. K., Beveridge, D. L., Westbrook, J., Gelbin, A., Demeny, T., & Hsieh, S.-H. (1992) The Nucleic Acid Database: A Comprehensive Database of Three-Dimensional Structures of Nucleic Acids, *Biophys. J.* (in press).

Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T., & Tasumi, M. (1977) *J. Mol. Biol. 112*, 535–542.

Chevrier, B., Dock, A. C., Hartmann, B., Leng, M., Moras, D., Thuong, M. T., & Westhof, E. J. (1986) *J. Mol. Biol. 188*, 707–719.

Coll, M., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H., & Rich, A. (1986) *J. Biomol. Struct. Dyn. 4*, 157–172.
Coll, M., Saal, D., Frederick, C. A., Aymami, J., Rich, A., & Wang, A. H.-J. (1989) *Nucleic Acids Res. 17*, 911–923.
Dickerson, R. E., Bansal, M., Calladine, C. R., Diekmann, S., Hunter, W. N., Kennard, O., von Kitzing, E., Lavery, R., Nelson, H. C. M., Olson, W., Saenger, W., Shakked, Z., Sklenar, H., Soumpasis, D. M., Tung, C.-S., Wang, A. H.-J., & Zhurkin, V. B. (1989) *EMBO J. 8*, 1–4.
Drew, H. R., & Dickerson, R. E. (1981) *J. Mol. Biol. 152*, 723–736.
Egli, M., Williams, L. D., Gao, Q., & Rich, A. (1991) *Biochemistry 30*, 11388–11402.
Fair, C. K. (1990) *MOLEN: An interactive structure solution procedure*, Enraf-Nonius, Delft, Netherlands.
Fitzgerald, P. M. D. (1988) *J. Appl. Crystallogr. 21*, 273–278.
Fujii, S., Wang, A. H.-J., van der Marel, G., van Boom, J. H., & Rich, A. (1982) *Nucleic Acids Res. 10*, 7879–7892.
Gaffney, B. L., Marky, L. A., & Jones, R. A. (1984) *Tetrahedron 40*, 3–13.
Geierstanger, B. H., Kagawa, T. F., Chen, S.-L., Quigley, G. J., & Ho, P. S. (1991) *J. Biol. Chem. 266*, 20185–20191.
Gessner, R. V., Frederick, C. A., Quigley, G. J., Rich, A., & Wang, A. H.-J. (1989) *J. Biol. Chem. 264*, 7921–7935.
Ginell, S. L., Kuzmich, S., Jones, R. A., & Berman, H. M. (1990) *Biochemistry 29*, 10461–10465.
Ho, P. S., Kagawa, T. F., Tseng, K., Schroth, G. P., & Zhou, G. (1991) *Science 254*, 1003–1006.
Jones, T. A. (1978) *J. Appl. Crystallogr. 11*, 268–272.
Kagawa, T. F., Geierstanger, B. H., Wang, A. H.-J., & Ho, P. S. (1991) *J. Biol. Chem. 266*, 20175–20184.
Peticolas, W. L., Patapoff, T. W., Wang, Y., & Thomas, G. A. (1989) *Ber. Bunsenges. Phys. Chem. 93*, 295–300.
Schneider, B., Cohen, D., & Berman, H. M. (1992) *Biopolymers 32*, 725–750.
Sheldrick, G. M. (1976) *SHELX76: A program for crystal structure determination*, University of Cambridge, England.
Steigemann, W. (1985) *PROTEIN*; Max-Planck Institut fuer Biochemie, 8033 Martinsried bei Munchen, Germany.
Sussman, J. L., Holbrook, S. R., Church, G. M., & Kim, S.-H. (1977) *Acta Crystallogr. A33*, 800–804.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature 282*, 680–686.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., van der Marel, G. A., van Boom, J. H., & Rich, A. (1981) *Science 211*, 171–176.
Wang, A. H.-J., Hakoshima, T., van der Marel, G., van Boom, J. H., & Rich, A. (1984) *Cell 37*, 321–331.
Wang, A. H.-J., Gessner, R. V., van der Marel, G. A., van Boom, J. H., & Rich, A. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3611–3615.
Westhof, E., Dumas, P., & Moras, D. (1985) *J. Mol. Biol. 184*, 119–145.
Zhou, G. W., & Ho, P. S. (1990) *Biochemistry 29*, 7229–7236.