Z-DNA Structure of a Modified DNA Hexamer at 1.4-Å Resolution: Aminohexyl-5′-d(pCpGp[br^{5}C]pGpCpG)

Yuch-Cheng Jean,‡ Yi-Gui Gao, and Andrew H.-J. Wang*

Biophysics Division and Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received September 3, 1992; Revised Manuscript Received October 29, 1992

ABSTRACT: Oligonucleotides with modification at the 5′-end have been used for various biochemical applications. As a first step to better assess the effects of those modifications on DNA conformation, we determined at 1.4-Å resolution the left-handed Z-DNA structure of a DNA hexamer, aminohexyl-5′-d(pCpGp[br^{5}C]pGpCpG), by X-ray diffraction analysis. This hexamer was crystallized in the monoclinic $C2$ (a = 51.13 Å, b = 18.44 Å, c = 34.67 Å, and β = 120.9°) space group. Its structure has been refined by the restrained least-squares refinement to a final R factor of 0.164 using 3727 [$>2.0\sigma(F)$] observed reflections. The overall conformation of the double helix resembles that of the canonical Z-DNA. The terminal 5′-phosphate groups of the dC residues adopt conformations ($\beta \sim 180°$ and $\gamma \sim 60°$) similar to phosphodiester's conformation of the internal dC residues. Two types of interhelical stackings are observed, one of which may serve as a model for a single-strand nick in the backbone of DNA double helix. A barium ion is found to bridge two side-by-side Z-DNA helices by coordinating to the O6 and N7 atoms of two guanines simultaneously. This "cross-linking" ability of barium ion may be a useful property in promoting the reversible aggregation of nucleic acids.

There have been great advances in our understanding on the structure of nucleic acids in recent years. In general, significant information regarding the sequence-dependent conformation of DNA has been accumulated through various biochemical and biophysical techniques (Rich et al., 1984). Similarly, the interactions between small molecular ligands (including antitumor drugs) and DNA have also been studied extensively (Wang, 1992). Thus far, almost all DNA oligonucleotides that have been studied crystallographically are molecules without any terminal phosphate groups. Only one example, the structure of a tetramer d(pApTpApT), is available (Viswamitra et al., 1978). The phosphate group at the 5′-end of nucleic acids plays essential roles in a variety of functions of DNA and RNA. For example, the ligation of a single-strand nick in a DNA double helix requires the presence of a 5′-phosphate (Kornberg & Baker, 1992). Transfer RNA requires a 5′-phosphate for its biological activity. It would be of interest to know what is the preferred conformation of the phosphate itself and whether the presence of a 5′-phosphate would have any effect on the conformation of the double helix near the 5′-terminal region.

Recently, a number of molecular probes such as intercalator dyes (fluorescein, rhodamine) have been attached to the 5′-phosphate group via an aminohexyl or other similar linkers (Helene, 1987; Thuong et al., 1989). Those modified nucleotides are particularly useful in their use as antisense DNAs, in the study of nucleic acid triplex, or for other applications (Helene, 1987; Thuong et al., 1989). For example, the aliphatic chain on the 5′-end of the oligonucleotide may enhance the resistance toward exonuclease activity. We are interested in knowing the influence of the hexyl linker on DNA conformation. This information may aid the design of better molecules for those applications. In this paper, we analyze the structure of a modified DNA hexamer, aminohexyl-5′-d(pCpGp[br^{5}C]pGpCpG), to address the aforementioned questions. We compare it with the canonical Z structures (magnesium and spermine forms) of the d(CG)$_3$ DNA hexamer (Wang et al., 1979; Gessner et al., 1989).

MATERIALS AND METHODS

The hexamer nucleotides containing an aminohexyl group attached at the 5′-phosphate of d(pCpGpCpGpCpG) were synthesized on an Applied Biosystems DNA synthesizer. Figure 1 shows the chemical structure of the aminohexyl-5′-modified 2′-deoxycytidine 5′-phosphate. Several 5-bromo- and 5-methyldeoxycytidine derivatives of the hexamer were also synthesized. A total of six different crystal forms were obtained (Table I). They were crystallized from a condition generally similar to those for other Z-DNA hexamers (Wang & Gao, 1991). The best result for the 5-bromo-dC derivative aminohexyl-5′-d(pCpGp[br^{5}C]pGpCpG) (abbreviated N-CG-[br^{5}C]GCG; other related hexamers are similarly abbreviated), whose structure was solved and is discussed in this paper, was obtained from a solution of 1.6 mM modified hexamer (single-strand concentration), 45 mM sodium cacodylate buffer at pH 6.0, 6.0 mM $BaCl_2$, 2 mM spermine tetrachloride, and 7% 2-methyl-2,4-pentanediol (2-MPD), equilibrated against 30% 2-MPD by the vapor diffusion technique. Other crystal forms could be obtained from similar conditions, but with small variations in parameters like pH or metal ions (using magnesium chloride or barium chloride). All crystal forms could be grown to the size of 0.2 × 0.3 × 0.5 mm.

The crystals of the hexamer N-CG[br^{5}C]GCG are in the monoclinic lattice with the space group $C2$ and have unit dimensions of a = 51.13 (2) Å, b = 18.44 (1) Å, c = 34.67 (2) Å, and β = 120.9 (3)°. One crystal was mounted in a thin-walled capillary tube and sealed with a droplet of the crystallization mother liquid for data collection. The diffraction data were collected on a Rigaku AFC-5R rotating-anode diffractometer, using an ω-scan mode at 25 °C to 1.4-Å resolution (2θ = 75°) with Cu Kα radiation (1.5418 Å with graphite monochromater) at a power of 50 kV and 180 mA.

* To whom correspondence should be addressed.
‡ Permanent address: Synchrotron Radiation Research Center, Hsin-Chu, Taiwan, ROC.

0006-2960/93/0432-381$04.00/0

Table I: Six Crystal Forms of Aminohexyl-5′-d(pCGCGCG) plus Its 5-Bromocytosine and 5-Methylcytosine Derivatives

crystal form	DNA	metal ion	unit cell dimensions (Å)			space group	resolution (Å)	*R* factor
			a	*b*	*c*			
I	N-CG[br^{5}C]GCG[a]	$BaCl_2$	51.13	18.44	34.67, β = 120.9°	*C*2	1.4	0.164
II	N-CG[br^{5}C]GCG	$MgCl_2$	25.80	25.80	38.00	$P3_212$[b]	1.4	
III	N-CG[m^{5}C]GCG	$BaCl_2$	18.23	33.60	43.93	$P2_12_12_1$	1.6	
IV	N-CG[m^{5}C]GCG	$MgCl_2$	18.01	18.01	43.63	$P6_5$	2.5	
V	N-CGCGCG	$MgCl_2$	18.58	18.59	73.49	$P3_212$[b]	1.4	
VI	N-CGCGCG	$BaCl_2$	52.98	21.79	32.24, β = 123.2°	*C*2	1.4	

[a] Abbreviations are used for various modified DNA hexamers of aminohexyl-5′-d(pCpGpCpGpCpG). [b] The space group may be $P3_212$ or $P3_221$.

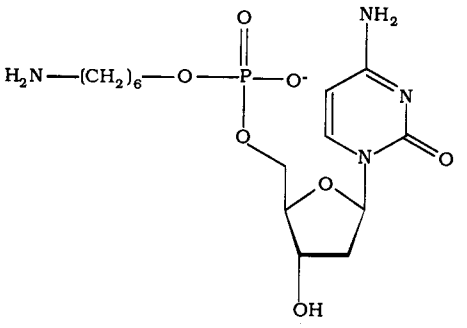

FIGURE 1: Molecular formula of the aminohexyl-5′-deoxycytidine monophosphate.

A total of 3727 (out of 4680 possible reflections to 1.4-Å resolution) unique reflections were considered to be observable at a $2.0\sigma(F)$ level above background, after Lorentz polarization, absorption, and decay corrections. They were used in the refinement.

Structure Determination. The structure was solved by considering the packing of the hexamers in the crystal lattice. The Patterson map of the *C*2 crystal clearly showed that the base pair stacking direction is along the diagonal of the unit cell *ac* plane. The length of this diagonal is 44.45 Å, which is nearly the same as that of the *c* axis of other Z-DNA orthorhombic or hexagonal crystals (Wang et al., 1979). This distance accommodates two hexamer Z-DNA duplexes. Along this diagonal, there are two independent 2-fold axes parallel to the *b* axis, one at the origin (0,0,0) and the other at (0.5,0.0,0.5). We surmised that the hexamers are stacked end-over-end along the diagonal and they are related to each other by the crystallographic 2-fold axes. The duplex molecule (without the aminohexyl linkers and the 5′-phosphates, but including the bromine atoms) was placed appropriately in the asymmetric unit, and it was rotated around the diagonal (which is assumed to coincide with the helix axis) with a 20° step to search for the correct solution. The program ULTIMA (Rabinovich & Shakked, 1984) was used for this rotation–translation search. The progress of the search was monitored by the crystallographic *R* factor using the data to 5-Å resolution. A clear minimum (with an *R* factor of 39%) was obtained with a resulting molecular orientation in which the molecular 2-fold axis of the hexamer lied nearly on the *ac* plane.

This preliminary model was then subjected to least-squares refinement using the Konnert–Hendrickson restrained refinement procedure (Hendrickson & Konnert, 1979; Westhof et al., 1985). After many cycles of refinement, the *R* factor was ~34% at 2.5-Å resolution. At that stage, $(2F_o - F_c)$ Fourier maps showed a very intense residual electron density, and this peak was assigned as a barium ion. Inclusion of the barium ion reduced the *R* factor to 28%. Additional $(2F_o - F_c)$ Fourier maps were used to locate the missing aminohexyl-5′-phosphate groups and solvent molecules, which were then included in the subsequent refinements. A total of 59 water molecules were located. The structure was refined to a final *R* factor of 16.4% with a root-mean-square deviation (RMSD) of bond lengths from the ideal value of 0.011 Å using 3727 reflections. The structure is well-refined at high resolution (1.4 Å) as illustrated by the clean difference Fourier map. An example is shown in Figure 2. The aminohexyl-DNA duplex contains 10 negative charges (12 negative charges from the phosphates and two positive charges from the linker amino groups) in the asymmetric unit. The single barium ion accounts for two additional positive charges, leaving eight negative charges to be balanced. However, no other metal (sodium) or spermine ions could be located despite the high-resolution structure. Presumably the remaining cations are disordered in the crystal lattice. The final atomic coordinates of the structure have been deposited in the Brookhaven Protein Databank. The structure determination of other crystal forms listed in Table I is in progress.

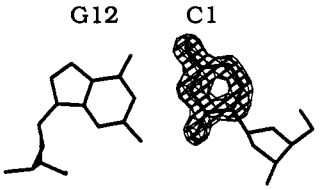

FIGURE 2: $(F_o - F_c)$ difference Fourier electron density map at 1.4-Å resolution showing the cytosine ring of the C1 residue.

RESULTS AND DISCUSSION

Molecular Structure. The overall DNA conformation of the modified hexamer is similar to the canonical left-handed Z-DNA structure observed in the d(CGCGCG) crystal (Wang et al., 1979; Gessner et al., 1989). The sugar–phosphate backbone follows a zig-zag path (Figure 3). The alternation of anti–syn conformation in the glycosyl torsion angles associated with the alternating dC–dG sequence of the molecule is clearly shown in this skeletal drawing.

The incorporation of 5′-phosphate residues into the molecule appears to change the backbone torsion angles and the sugar puckers relatively little (Table II). Very few torsion angles vary by more than 15° between the structures of N-CG[br^{5}C]-GCG and CGCGCG. The major difference occurs at the G2pC3 step where in N-CG[br^{5}C]GCG the phosphate linkage is in the Z_{II} conformation (α = 147° and β = −121° for C3 and ϵ = −177° and ζ = 82° for G2), whereas that in CGCGCG is in the Z_I conformation (α = 162° and β = 139° for C3 and ϵ = −120° and ζ = 65° for G2) (Wang et al., 1981). This difference may be due to the G2pC3 phosphate in N-CG-[br^{5}C]GCG crystal being involved in the packing interactions with the G8 deoxyribose of another hexamer duplex. Interestingly, the G4pC5 step in both hexamers is in the Z_{II} conformation. The observation of the frequent occurrence of the Z_{II} phosphate conformation suggests that the energetic

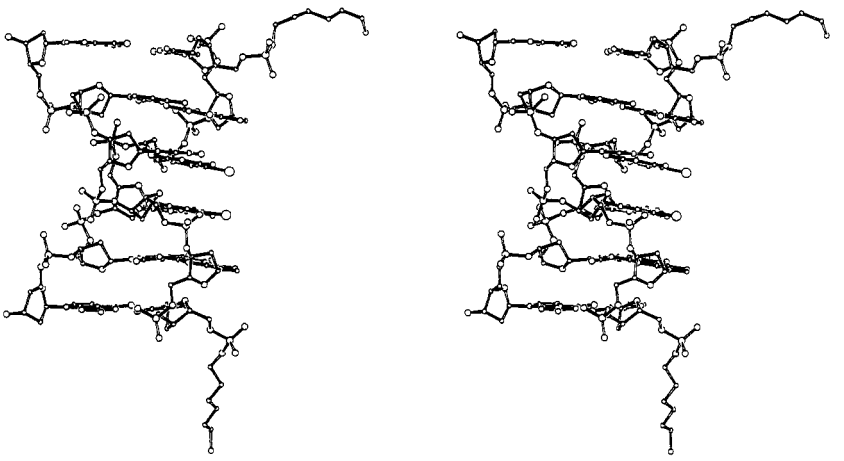

FIGURE 3: Stereoscopic drawing of the N–CG[br^{5}C]GCG Z-DNA duplex structure. The two aminohexyl groups can be seen to project in different directions.

Table II: Torsion Angles of the Sugar–Phosphate Backbone

		cytidine residues						
angle	form	C1	C3	C5	C7	C9	C11	mean
α	modified		162[a]	176[a]		−162[a]	−148	−148
	Mg		−147	166[a]		−162	−150	−149
β	modified		139[a]	146[a]		−128	−130	−129
	Mg		−121	160[a]	−173	−164[a]	−119	−120
γ	modified	69	56	47	61	51	59	58
	Mg	52	50	48	55	55	56	53
δ	modified	151	137	133	126	138	137	137
	Mg	145	148	142	139	140	142	143
ϵ	modified	−100	−96	−96	−93	−93	−96	−96
	Mg	−95	−100	−100	−92	−92	−101	−97
ζ	modified	77	65	70	72	73	64	69
	Mg	79	81	80	74	74	70	76
χ	modified	−160	−153	−159	−139	−160	−153	−153
	Mg	−150	−150	−152	−151	−154	−156	−152
P	modified	168	154	146	139	154	151	152
	Mg	154	145	151	157	153	151	152
sugar pucker	modified	C2′-endo	C2′-endo	C2′-endo	C1′-exo	C2′-endo	C2′-endo	C2′-endo
	Mg	C2′-endo	C2′-endo	C2′-endo	C2′-endo	C2′-endo	C2′-endo	C2′-endo
		guanosine residues						
angle	form	G2	G4	G6	G8	G10	G12	mean
α	modified	76	85	79	65	67	76	75
	Mg	62	64	77	67	64	84	64
β	modified	−174	−171	−173	−175	−169	−187	−175
	Mg	−172	−174	175	−171	−175	−177	−176
γ	modified	173	176	185	181	175	187	180
	Mg	178	180	−178	172	179	−177	179
δ	modified	95	98	143[b]	102	90	145[b]	96
	Mg	91	92	149[b]	101	96	149[b]	95
ϵ	modified	−177[a]	−156[a]		−136	−120		−128
	Mg	−120	−179[a]		−124	−116		−118
ζ	modified	82[a]	54[a]		−56[a]	−63		−60
	Mg	−65	69[a]		−25	−70		−68
χ	modified	56	51	77	65	60	67	63
	Mg	60	59	78	61	62	73	61
P	modified	27	53	161[b]	76	40	176[b]	39
	Mg	40	26	170[b]	36	35	162[b]	34
sugar pucker	modified	C3′-endo	C4′-exo	C2′-endo	O4′-endo	C4′-exo	C2′-endo	C3′-endo
	Mg	C4′-exo	C3′-endo	C2′-endo	C3′-endo	C3′-endo	C2′-endo	C3′-endo

[a] The labeled values have not been used for calculating the average torsional angles due to the Z_{II} or partial Z_{II} conformation (Wang et al., 1981). [b] The labeled values have not been used for calculating the average torsional angles due to end effects of the 3′-terminal guanosine nucleotide.

difference of those two conformers (Z_I and Z_{II}) is likely not very great.

The root-mean-square deviation (RMSD) between the two hexamers N-GC[br^5]GCG and CGCGCG, excluding the nonidentical atoms, is 0.731 Å. This consistency in the conformation of two hexamers crystallized in completely different crystal lattices points out the conformational invariance in Z-DNA. This is in contrast to the situation in B-DNA, which shows a much higher degree of conformational variability, depending on the nucleotide sequence as well as the crystal environment (Yanagi et al., 1991).

The conformations associated with two strands of the double helix are slightly different (see the Supplementary Material, Table 1S). In particular, the sugar puckers of the dG residues

Table III: Local Helix Parameters in Aminohexyl-5′-d(pCG[br^{5}C]GCG)[a]

base pair	helical twist angle (deg)		rise/residue (Å)		slide (Å)	θ_{roll} (deg)	θ_{tilt} (deg)	base pair inclination (deg)	propeller twist (deg)
C1–G12	−8.2		3.7		5.4	−5.4	−4.4	−7.7	0.5
G2–C11		−42.9		4.1	−1.1	−0.9	1.6	−3.3	−7.7
C3–G10	−9.1		3.7		5.5	−1.0	4.7	−4.8	−5.0
G4–C9		−47.8		3.5	−0.1	4.9	−1.9	−9.5	6.4
C5–G8	−14.2		3.7		5.4	2.9	−6.8	−7.6	6.1
G6–C7								−0.9	−0.5
av	−10.5	−45.4	3.7	3.8	3.0	0.1	−1.4	−5.6	0.0
SD	2.7	2.5	0.0	0.7	3.3	4.1	4.6	3.2	5.7
Z-DNA[b]									
Mg Form	−10.5	−50.0	3.8	3.7	2.9	−1.4	−0.1	−4.7	−0.6
Sp Form	−8.6	−50.8	3.7	3.7	3.1	0.9	0.0	−5.2	0.3

[a] These parameters were obtained from the program NEWHELIX(1991), provided by R. E. Dickerson and colleagues and are in accordance with EMBO workshop guidelines (Dickerson et al., 1989). [b] Values calculated from the coordinates of Gessner et al. (1989).

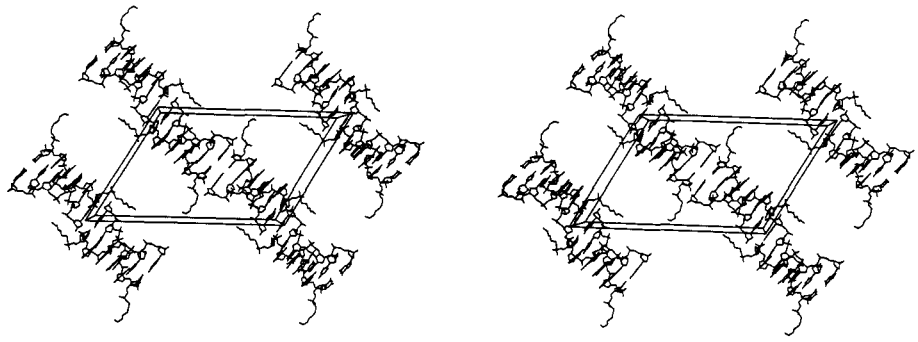

FIGURE 4: Crystal packing diagram of N-CG[br^{5}C]GCG Z-DNA molecules in the monoclinic *C*2 unit cell. The view is looking down the *b* axis. Along the diagonal of the *ac* plane, four molecules are aligned and stacked end-over-end, forming an infinite polymer.

show somewhat greater variations with the G4 and G8 residues adopting the C4′-exo and O4′-endo puckers (pseudorotation angles of 53° and 76°), respectively, in comparison to the C3′-endo pucker (an average pseudorotation value of 32°) found in the dG residues of the Mg form of d(CGCGCG) (Wang et al., 1979; Gessner et al., 1989).

The two 5′-phosphates adopt a very similar conformation, not unlike that associated with the internal dC residues. The torsion angles of β (av 180°) and γ (av 65°) of C1 and C7 are similar to the respective values of β = 160° (or −165°) and γ = 53° of the Z_{II} conformation of internal dC residues (Table 1S). The results prompt us to speculate that the free 5′-terminal phosphate in B-DNA may also adopt a preferred conformation similar to that of the B-DNA internal phosphodiester linkage. Both aminohexyl groups attached to the phosphate are in extended conformations. The atoms in those groups have high thermal factors, and their electron densities become weaker toward the amino end. It is likely those two groups have high degree of flexibility.

Despite of the rigidity of Z-DNA, some conformational variations were detected in this molecule. Inspection of the helical twist angles (Table III) shows that they are not as uniform as those seen in the CGCGCG structures. Both CpG and GpC steps are affected. The C5pG6 step (−14.2°) is slightly overwound, while both GpC steps (av −45.4°) are underwound (in the left-handed sense). The sum of the average helical twist angle per dinucleotide step is −55.9°, significantly lower than that (−60°) of a 12-fold Z-DNA helix. This variation in the stacking pattern are evident in Figure 1S (Supplementary Material). The G2p[br^5]C3 step has a particularly lower twist angle of −42.9°, which may be due to the movement of br^{5}C3 cytosine base to enhance the interaction of the bromine atom with guanine base.

The bases are nearly parallel to each other and roughly perpendicular to the helix axis with the base pair inclination ranging from −0.9° to −9.5°. The GC base pairs have propeller twist angles ranging from 6.4° to −7.7° (Table III). The relatively small variation is likely due to the local crystal environment. In contrast, AT base pairs in some B-DNA structures can have propeller twist angles as high as near −30° (Drew & Dickerson, 1981; Coll et al., 1987; Nelson et al., 1987).

As in other Z-DNA structures, the modified hexamer duplex has a narrow groove in which the water molecules are well organized into a spine (Wang et al., 1979; Gessner et al., 1989). The most prominent spine of hydration is consisted of the first-shell water molecules hydrogen-bonded to N2 of guanine and O2 of cytosine bases. The conservation of the hydration structure in Z-DNA crystals has been reviewed in detail recently (Schneider et al., 1992).

Stacking Interactions. Due to the nature of the packing arrangement (Figure 4), the hexamers have the appearance of an infinite Z-DNA polymer with its helical axis lying along the diagonal in the *ac* plane. In this C2 unit cell, columns of those pseudopolymers of Z-DNA run parallel to one another with an axis-to-axis distance of 25 Å within one layer of molecules. There are some direct intermolecular contact between helices. The strongest interactions are from the hydrogen bonds between the O1P oxygen of the terminal phosphate of C7 residue to the C1N4 (3.02 Å) and G6O3′ (2.56 Å) of symmetry-related helixes. An additional hydrogen bond is mediated through the NH_3 group of the aminohexyl tails. The amino group of the C7 residue is 2.61 Å away from the G2–O2P atom of the symmetry-related helix at $(1.5 - x, y - 0.5, 1.0 - z)$. Finally, there are strong interactions between layers of helices mediated by a hydrated barium ion as will be discussed later.

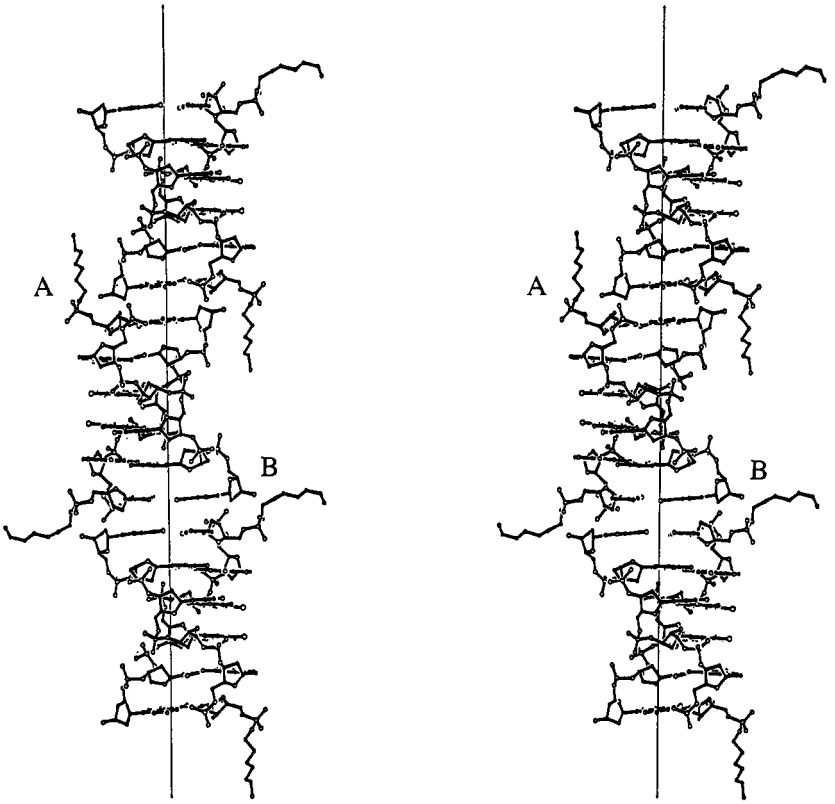

FIGURE 5: Stereoscopic drawing of three N-CG[br^{5}C]GCG hexamers stacked end-over-end along the helix axis. There are two types of interhelix stackings, denoted type A and type B in the diagram.

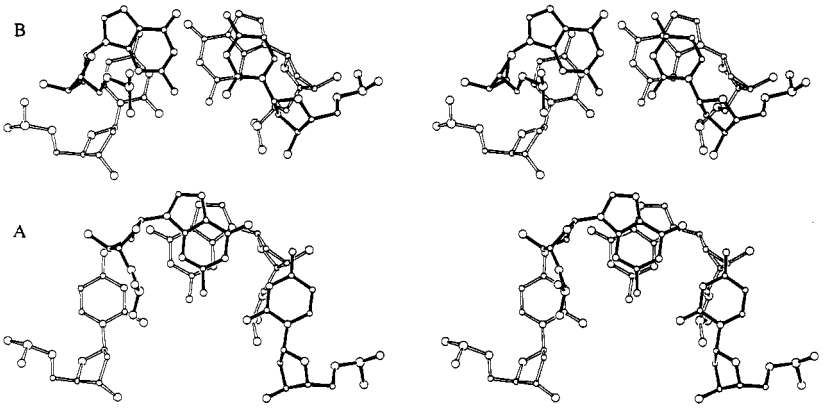

FIGURE 6: Diagram showing the type A and type B stacking interactions. Type B is very similar to the internal stacking pattern of the GpC step, while type A is a new type not seen in previous Z-DNA crystals.

Figure 5 shows that three hexamer duplexes are stacked along the helix axis in which two types of intermolecular stacking interactions (marked as type A and type B) are found. The disposition of the aminohexyl groups associated with these two types of interhelical junctions is different. Both aminohexyl groups adopt extended conformations. The aminohexyl group of C7 projects away from the helix, nearly perpendicular to it, into the solvent region at the type B junction. The aminohexyl group on C1 (near type A junction) runs along the helix direction, and it hugs closely the sugar phosphate backbone of C5pG6 of a symmetry-related duplex hexamer (at $0.5+x,-0.5+y,z$). Its amino group is near the phosphate group of G6 of the second hexamer but not directly hydrogen bonding to it. It is interesting to note that the flexibility of the aminohexyl group permits a dye molecule, attached at the amino end, to reach an intercalator site two or three base pairs away (Helene, 1987; Thuong et al., 1989). In the case of Z-DNA, both major and minor grooves are reachable as judged from Figure 5. We are synthesizing oligonucleotides with a dye molecule attached on the 5′-end to address the question of how a tethered dye molecule intercalates in B-DNA. In addition, we are synthesizing DNA oligomers with a longer linker such as spermine which can reduce the net charge of a spermine-attached oligonucleotide by four, rendering it more neutral.

These two types of intermolecular stacking interactions are shown in detail in Figure 6. Type B (Figure 6B) is very similar to the internal GpC stacking pattern in a Z-DNA helix. It is interesting to note that the terminal 5′-phosphate occupies nearly the same position as that of the internal GpC step. In fact, there is a very strong hydrogen bond (2.56 Å) between the O3′ hydroxyl of G6 and the O1P of C7* (* is at $1-x$, $y, 1-z$) as noted before. (An identical hydrogen bond related by a crystallographic 2-fold axis exists between the O3′

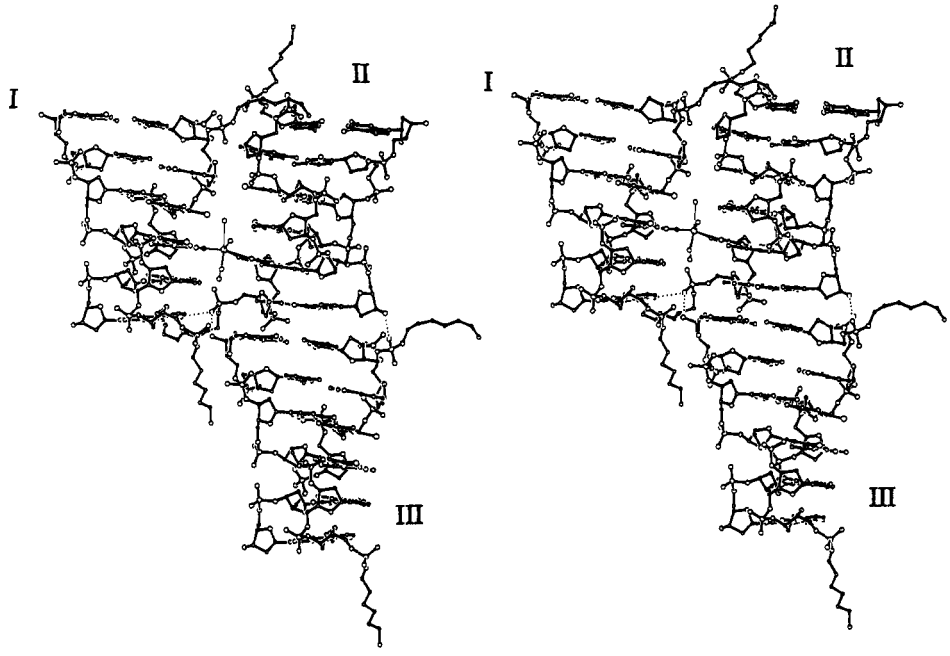

FIGURE 7: Detailed drawing showing the interactions between three Z-DNA hexamer helices (molecules I, II and III). They are at (x, y, z) for I, ($1.5 - x$, $y - 0.5$, $1.0 - z$) for II, and ($0.5 + x$, $-0.5 + y$, z) for III, respectively. A hydrated barium ion is found to locate in a cavity created by helices I and II. Several hydrogen bonds are shown as dotted lines.

hydroxyl of G12 and the O1P of C1.) This kind of hydrogen-bonding interactions is likely a result of the preferred conformation of the terminal phosphate and may exist in a nick location of DNA double helix. The close proximity between the 3′-hydroxyl and the 5′-phosphate of the succeeding nucleotide suggests that only a very small movement (less than 1 Å) of the participating atoms is required to form the phosphodiester bond. Presumably the ligase enzyme can achieve this easily. In the present case, if the sugar pucker of the G6 residue is changed from the C2′-endo type to the C3′-endo type (as seen in the internal G) and a small rotation is made about the P–O5′ bond of the C7* residue, the G6O3′ and the C7*O1P atoms will superimpose.

Many B-DNA decamer duplexes pack in the crystal using a similar type of end-over-end stackings (Yanagi et al., 1991). The O3′-hydroxyl of the 3′-end residue is often hydrogen bonded to the O5′-hydroxyl of the neighboring 5′-end residue. While no B-DNA molecule with a 5-phosphate has been crystallized, it would not be surprising that a similar situation exists in which the 3′-hydroxyl and the 5′-phosphate of the succeeding nucleotide are in close proximity. We also previously noted that in a nicked dodecamer duplex the base pair stack is completely continuous despite the missing phosphate (Aymami et al., 1990). Therefore, it is reasonable to envision that the 5′-phosphate at a nicked site can easily position itself close to the 3′-hydroxyl of the preceding nucleotide in B-DNA.

The type A intermolecular stacking pattern shown in Figure 6A has not been seen before. The left-handed helical twist angle between the two base pairs is nearly −80°. In addition, the base pairs slide against each other such that the two guanines have a large overlap. The C2′ of a dG residue lies 3.0 Å below the neighboring dC:dG base pair. It is interesting to note that both hydrogen atoms (H2′ and H2″) on the C2′ atom are pointing at the junction of the G and C bases, instead of underneath the aromatic ring of the base. This is in contrast to either the normal Z-DNA internal GpC stacking pattern, where the G and C have good base–base stacking interactions, or the internal Z-DNA CpG stacking pattern, where the sugar O4′ of the dC is in contact with the six-membered ring of the guanine base.

Environment around the Barium Ion. The intermolecular interactions along the *b* axis are mediated by barium ions located in a cavity created by the "major groove" surfaces from two adjacent hexamer helices (Figure 7). The top half of the cavity is occupied by four bromine atoms from two Z-helixes. The barium ion has eight coordination sites, four from two guanines and four from water molecules. The coordination distances range from 2.70 to 3.10 Å (listed in the Figure 7 legend). The detailed coordination geometry around the barium is shown in Figure 8. Two guanines, G10 and G8*, are nearly coplanar with a dihedral angles of 8°, and they are located on the same side of the coordination circle. The H8 hydrogens from the two guanines are 2.42 Å apart, indicating a van der Waals contact. A water molecule W2, which is slightly out of the best plane through the two guanines, supplies the fifth coordination site on the plane. The other three water molecules are significantly out of the plane made by the guanines.

The observation that a barium ion is capable of bringing two Z-DNA helices together by simultaneously coordinating to O6 and N7 positions of two guanines is interesting. Unlike some other metal ions, such as the transition metal ions (e.g., Zn^{2+}) or platinum(II) ion, the coordination of barium is completely reversible. The size of the barium ion allows it to have a high coordination number (ranging from 7 to 9) around the barium ion. The coordination distances around the barium ion (2.9 Å for Ba–N and 3.0 Å for Ba–O bonds) permit two Z-DNA helices be brought close to each other without steric hindrance. These properties seem to be responsible for our experience in crystallizing several DNA oligomers in the presence of barium ion. We have observed that even though in some cases barium ion was added in the crystallization dips, barium ion was not found in the crystal structure that had been determined. We believe that barium ion promotes the association of DNA molecules in solution and further facilitates their ordered arrangement prior to the formation of a crystalline array. However, it may or may not be

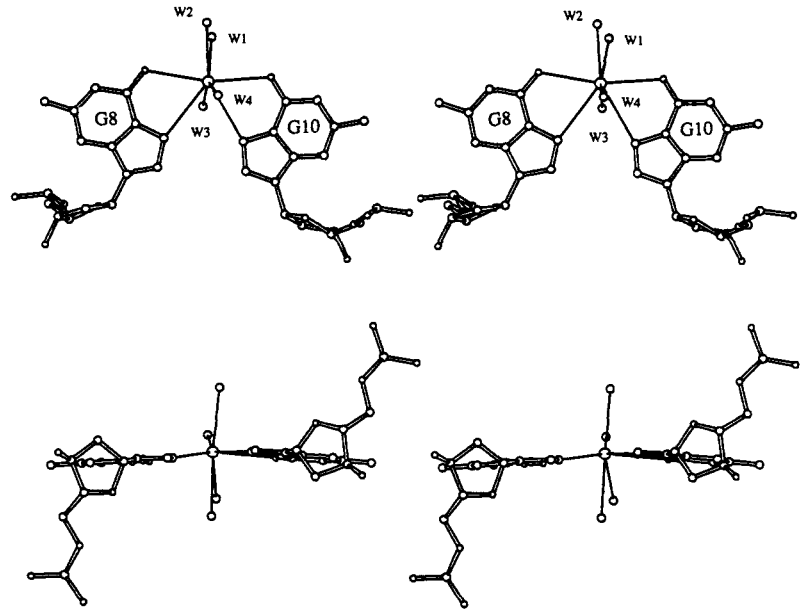

FIGURE 8: Detailed drawing showing the barium ion bridging two coplanar guanine bases. The barium ion has eight coordination sites, four of which are occupied by the O6 and N7 of guanines and the remaining four by water molecules (W1, W2, W3, and W4). The coordination distances between barium and various ligand sites are as follows: Ba–G10N7, 3.0 Å; Ba–G10O6, 2.80 Å; Ba–G8*N7, 3.10 Å; Ba–G8*O6, 2.80 Å; Ba–W1, 2.9 Å; Ba–W2, 3.0 Å; Ba–W3, 2.7 Å, and Ba–W4, 2.9 Å.

incorporated into the lattice orderly. Only ordered incorporation would result in a reasonable occupancy as in the present structure. A detailed analysis of the interactions between DNA and various ions, including barium, cobalt, and others, will be discussed elsewhere.

Crystal Polymorphism. We have been able to crystallize readily several modified hexamers related to N-CGCGCG (Table I). While only the structure of N-CG[br^{5}C]GCG has been solved and shown to be Z-DNA, the close relationship of the unit cell dimensions of other crystals in Table I to those of CGCGCG (Wang & Gao, 1991) suggests that their crystal structures are Z-DNA as well. The ease with which those molecules form Z-DNA crystals indicates that the aminohexyl modification and the terminal phosphate group at the 5'-end do not inhibit the alternating C–G sequence from adopting the Z-DNA conformation.

The multiple crystal forms of a simple DNA molecule is becoming more of a rule than an exception. We noted that there are at least five unique crystal forms (two $P2_12_12_1$, two $P6_5$, and one $C222_1$) for CGCGCG and CGCG (Wang & Gao, 1991). The present $P2_12_12_1$ form listed in Table I is apparently not isomorphous to either of the two $P2_12_12_1$ forms of CGCGCG, as the latter coordinates could not be used to refine the N-CGCGCG $P2_12_12_1$ structure. On the basis of the crystal structures that have been determined, it is clear that most of those crystals are formed by associating columns of Z-DNA molecules (stacked end-over-end) sideways. A small change in the molecule (e.g., methylation, araC substitution) or of the environment (e.g., pH, additives such as urea) would alter the relative orientation between columns of Z-DNA, resulting a different but related crystal lattice. It would be of interest to analyze the detailed intermolecular interactions when all the crystal structures become available.

ACKNOWLEDGMENT

This work was supported by grants from the NIH (GM-41612 and CA-52506) to A.H.J.W. We thank Ms. Yu-kun Li for her assistance in the purification of oligonucleotides. Y.C.J. acknowledges support from the Synchrotron Radiation Research Center (Taiwan, ROC).

SUPPLEMENTARY MATERIAL AVAILABLE

Table 1S listing the torsional angles and sugar puckers of the N-CG[br^{5}C]GCG hexamer and Figure 1S showing the stacking pattern of the three internal CpG steps and two GpC steps in the N-CG[br^{5}C]GCG hexamer (2 pages). Ordering information is given on any current masthead page.

REFERENCES

Aymami, J., Coll, M., van der Marel, G. A., van Boom, J. H., Rich, A., & Wang, A. H.-J. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2526–2530.

Coll, M., Frederick, C. A., Wang, A. H.-J., & Rich, A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8385–8389.

Dickerson, R. E., Bansal, M., Calladine, C. R., Diekman, S., Hunter, W. N., Kennard, O., von Kitzing, E., Lavery, R., Nelson, H. C. M., Olson, W. K., Saenger, W., Shakked, Z., Sklenar, H., Soumpasis, D. M., Tung., C.-S., Wang, A. H.-J., & Zhurkin, V. B. (1989) *Nucleic Acids Res. 15*, 247–265.

Drew, H. R., & Dickerson, R. E. (1981) *J. Mol. Biol. 151*, 535–556.

Gessner, R. G., Frederick, C. A., Quigley, G. J., Rich, A., & Wang, A. H.-J. (1989) *J. Biol. Chem. 264*, 7921–7935.

Helene, C. (1987) in *DNA–Ligand Interactions* (Gauschlbauer, W., & Saenger, W., Eds.) pp 127–140, Plenum Press, New York.

Hendrickson, W. A., & Konnert, J. (1979) in *Biomolecular Structure, Conformation, Function and Evolution* (Srinvasan, R., Ed.) pp 43–57, Pergamon Press, Oxford.

Kornberg, A., & Baker, T. (1992) *DNA Replication*, 2nd ed., W. H. Freeman and Co., New York.

Nelson, H. C. M., Finch, J. T., Luisi, B. F., & Klug, A. (1987) *Nature 330*, 221–226.

Rabinovich, D., & Shakked, Z. (1984) *Acta Crystallogr. A40*, 195–200.

Rich, A., Nordheim, A., & Wang, A. H.-J. (1984) *Annu. Rev. Biochem. 53*, 791–846.

Schneider, B., Cohen, D., & Berman, H. M. (1992) *Biopolymers 32*, 725–750.
Teng, M.-K., Liaw, Y.-C., van der Marel, G. A., van Boom, J. H., & Wang, A. H.-J. (1989) *Biochemistry 28*, 4923–4928.
Thuong, N. T., Asseline, U., & Montenay-Garestier, T. (1989) in *Oligodeoxynucleotides* (Cohen, J., Ed.) pp 25–51, CRC Press, Inc., Boca Raton, FL.
Viswamitra, M., Kennard, O., Jones, P. G., Sheldrick, G. M., Salisbury, S., Falvello, S., & Shakked, Z. (1978) *Nature 273*, 687–688.
Wang, A. H.-J. (1992) *Curr. Opin. Struct. Biol. 2*, 361–368.
Wang, A. H.-J., & Gao, Y.-G. (1990) *Methods (San Diego) 1*, 91–99.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L, van Boom, J. H., van der Marel, G. A., & 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.
Westhof, E., Dumas, P., & Moras, D. (1985) *J. Mol. Biol. 184*, 119–145.
Yanagi, K., Prive, G. G., & Dickerson, R. E. (1991) *J. Mol. Biol. 217*, 201–214.