Crystal Structure of d(GCGCGCG) with 5'-Overhang G Residues

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ABSTRACT   The crystal structure of the DNA heptamer d(GCGCGCG) has been solved at 1.65 Å resolution by the molecular replacement method and refined to an R-value of 0.184 for 3598 reflections. The heptamer forms a Z-DNA d(CGCGCG)$_2$ with 5'-overhang G residues instead of an A-DNA d(GCGCGC)$_2$ with 3'-overhang G residues. The overhang G residues from parallel strands of two adjacent duplexes form a *trans* reverse Hoogsteen G · G basepair that stacks on the six Z-DNA basepairs to produce a pseudocontinuous helix. The reverse Hoogsteen G · G basepair is unusual in that the displacement of one G base relative to the other allows them to participate in a bifurcated (G1)N2 · · · N7(G8) and an enhanced (G8)C8—H · · · O6(G1) hydrogen bond, in addition to the two usual hydrogen bonds. The 5'-overhang G residues are *anti* and C2'-*endo* while the 3'-terminal G residues are *syn* and C2'-*endo*. The conformations of both G residues are different from the *syn*/C3'-*endo* for the guanosine in a standard Z-DNA. The two cobalt hexammine ions bind to the phosphate groups in both GpC and CpG steps in Z_I and Z_{II} conformations. The water structure motif is similar to the other Z-DNA structures.
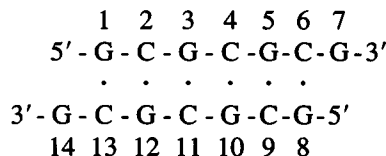
INTRODUCTION

More than 20 years ago it was discovered that alternating poly-d(C-G) duplexes could adopt an unusual conformation that was thought to be left-handed (Pohl and Jovin, 1972). Soon afterward single-crystal x-ray crystallography conclusively proved the existence of the left-handed duplex, termed Z-DNA (Wang et al., 1979). Since then there was a search for the biological significance of Z-DNA and its function in vivo. It was shown that the formation of Z-DNA could be induced by negative supercoiling in covalently closed circular DNA (Gruskin and Rich, 1993; Lucomski and Wells, 1994) and by *anti*-Z-DNA antibodies bound to certain DNA stretches (Pietrasanta et al., 1994). Recently, the discovery of a Z-DNA-binding protein has been announced, implying that Z-DNA may play a role in certain promoter sequences (Zhang et al., 1992). Besides G · C basepairs, A · T (Fujii et al., 1985; Brennan et al., 1986), G · ^{Br}U (Brown et al., 1986), G · T (Ho et al., 1985), and C · I (Kumar et al., 1992) basepairs have been found in the Z-form. A · T basepairs are known to destabilize the Z-DNA duplex (Wang et al., 1984) and this might explain why longer stretches of alternating A · T basepairs favor the B-form (Yoon et al., 1988). Aside from the above sequences, a few other oligomers that deviate from the regular d(C-G)$_n$ scheme have been found to form the left-handed structures, including the nonalternating hexamer d(C-CGCGG) (Malinina et al., 1994) and others, incorporating ribose or arabinose sugar units (Teng et al., 1989).

We have been interested in the factors governing the interconversion of left- and right-handed nucleic acid du-

plexes. In this respect, we have so far concentrated on the nature of the 5'-terminal nucleotide (Ban et al., 1996) and base modifications like m^{5}C and Br^{5}C (Tippin and Sundaralingam, unpublished results). While for polymers the identity of the 5'-nucleotide (G or C) is unimportant, short alternating oligomers with a 5'-purine start crystallize as A-DNA (Jain et al., 1987; Bingman et al., 1992; Mooers et al., 1995) as found in solution (Quadrifoglio et al., 1984). Interestingly, it seems that the tendency of the 5'-purine start can be partially overcome by increasing the length of the alternating GC step to 10 nucleotides (Ban et al., 1996). It is known that methylation of C-residues at position 5 confers additional stability on the Z-conformation (Fujii et al., 1982). Therefore, it came as a surprise that the Z-DNA decamer d(GC)$_5$ (Ban et al., 1996) converted to the A-form upon methylation of one or several C's (Tippin and Sundaralingam, unpublished results). Apparently there is only a narrow energetic margin that transforms A- to Z-DNA, and vice versa.

To further investigate the relative stability of the A-, B-, and Z-forms, we were interested in selecting a nucleic acid molecule that can form either a right- or left-handed structure with a 3'- or 5'-overhang, respectively. The self-complementary heptamer d(GCGCGCG) was therefore designed for these conformational studies. The central duplex portion of the molecule can contain six alternating deoxynucleotides starting with either a purine that may adopt the right-handed A- (Jain et al., 1987; Bingman et al., 1992; Mooers et al., 1995) or B-form DNA (Cruse et al., 1986) (Scheme 1), or a pyrimidine that may adopt the left-handed Z-form DNA (Wang et al., 1979) (Scheme 2).
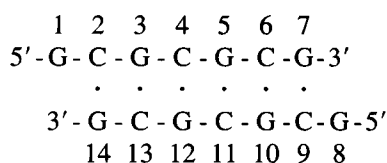
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              1   2   3   4   5   6   7
       5' - G - C - G - C - G - C - G - 3'
             ·   ·   ·   ·   ·   ·
       3' - G - C - G - C - G - C - G - 5'
             14  13  12  11  10  9   8
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(Scheme 1)

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         1   2   3   4   5   6   7
5'-G - C - G - C - G - C -G-3'
         .   .   .   .   .   .
    3'- G - C - G - C - G -C- G -5'
        14  13  12  11  10  9   8
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(Scheme 2)

This represents the first study of a DNA sequence that can intrinsically have three different conformations (A-, B-, or Z-form). In this paper we will describe the outcome of this conformational competition.

MATERIALS AND METHODS

Synthesis, crystallization, and data collection

The DNA heptamer d(GCGCGCG) was synthesized by the phosphoramidite method using an in-house Applied Biosystem DNA synthesizer 381 (Foster City, CA). The DNA was cleaved from the solid support using 5 ml ammonium hydroxide (30% NH_3 in water) and was deprotected in the same solution at 55°C overnight. The extract was lyophilized and then precipitated using 100% ethanol in the presence of 2.5 M ammonium acetate at −25°C. The preparation was cycled through ethanol precipitation and lyophilization until white fluffy material was obtained, which dissolved readily in water. A stock solution of 2 mM single-stranded heptamer was prepared in distilled water and used for crystallization without further purification. The crystallization was carried out by the hanging-drop vapor diffusion method at room temperature. The best crystals were obtained with 1 mM DNA (single-stranded concentration), in the presence of 20 mM sodium cacodylate buffer (pH 7.0), and 0.5 mM spermine tetrachloride, equilibrated against a reservoir of 0.2 ml of 10% 2-propanol. Crystals appeared after one day and continued to grow for about a week; the crystals were light amber in color. A crystal with dimensions 0.2 mm × 0.2 mm × 0.3 mm was mounted in a thin-walled glass capillary tube with some mother liquor at one end, and the capillary sealed with wax. The crystal belongs to the orthorhombic space group $P2_12_12_1$ with unit cell constants a = 20.41 Å, b = 29.65 Å, and c = 51.86 Å and with one duplex in the asymmetric unit. The intensity data were collected at room temperature using our R-axis IIc imaging plate and graphite monochromated CuKα x-ray beam. The crystal-to-detector distance was 65 mm. Thirty-five frames with a framewidth of 2° and an exposure time of 20 min per frame yielded 9304 reflections with 3813 independent reflections up to 1.65 Å resolution [$F^2/\sigma(F^2) > 5.0$] with an R_{merge} of 6.6% and 92.3% completeness. The frames were processed using the data processing software for Rigaku R-axis IIc.

Structure solution and refinement

The structure was solved by the molecular replacement method. Rotation searches with A-DNA (Mooers et al., 1995), B-DNA (Chen et al., 1994), and Z-DNA (Wang et al., 1979) hexamer duplexes gave best results for Z-DNA. The translation search with the best orientation using 591 reflections in the 8.0–3.0 Å range with $F > 10\sigma(F)$ gave the solution with an R factor of 48.5%. A rigid body refinement using the X-PLOR program package (Brunger, 1990) dropped the R-value to 40.2%. The data were extended to 2.0 Å resolution, and positional and overall B-factor refinement resulted in an R-value of 36.7%. The model was then annealed by heating the system to 4000 K and slowly cooling to room temperature with 0.5-fs sampling intervals, dropping the R-value to 28.6%. Positional and individual B-factor refinement, extending the resolution to 1.65 Å with 3598 reflections [$F > 2\sigma(F)$], raised the R-value to 30.3%. The omit difference ($F_{obs} - F_{cal}$) map clearly showed the electron density for the omitted residues and was also used to position the 5'-overhang G residues on the two strands, which formed reverse Hoogsteen basepairs (Fig. 1).

FIGURE 1   The (F_o-F_c) electron density map showing the reverse Hoogsteen G · G basepair between the 5'-overhang residues.

Further positional and B-factor refinement dropped the R-value to 27.8%. The difference ($F_{obs} - F_{cal}$) map showed two octahedral clusters of density (>13σ) which were interpreted as cobalt hexammine ions. Including the two cobalt hexammine ions in further refinement lowered the R-value to 24.0%. Water molecules were included that had nearly spherical densities (>3σ) in the difference ($F_{obs} - F_{cal}$) map. In the first round, 34 water molecules were located and included in the refinement, dropping the R-value to 20.4%. Using the next difference ($F_{obs} - F_{cal}$) map an additional 23 water molecules could be located, and further refinement lowered the final R-value to 18.4%. Although spermine was used in the crystallization trials, no ordered polyamine could be located in the density maps. The final model contains 284 nucleic acid atoms, 2 cobalt hexammine ions, and 57 water molecules. The crystallographic refinement parameters are listed in Table 1. The atomic coordinates have been deposited with the Nucleic Acid Database (Berman et al., 1992; ID #ZDG057).

RESULTS

Overall structure

The heptamer d(GCGCGCG) crystallized in the Z-DNA conformation with a duplex portion formed between the six

TABLE 1   Crystal data and refinement parameters

Space Group	$P2_12_12_1$
Unit cell dimensions (Å)	
a	20.41
b	29.65
c	51.86
Asymmetric unit	1 duplex
Volume/bp (Å³)	1120
Resolution range (Å)	8.0–1.65
Number of reflections used [$F > 2.0\sigma(F)$]	3598
Final R-Value (%)	18.4
Final model	
Nucleic acid atoms	286
Cobalt hexammine ions	2
Water molecules	57
Average Thermal Parameters (Å²)	
Nucleic acid atoms	18.7
Cobalt hexammine ions	14.1
Water molecules	40.3
Parameter File	param_nd.dna
RMS deviation from ideal geometry	
Bond lengths	0.020 Å
Bond angles	3.2°
Dihedral angles	31.8°
"Improper" angles	1.6°

self-complementary residues 2–7/9–14 and the 5'-G1 and G8 as overhangs (Fig. 2). The molecular structure and crystal packing of the heptamer are shown in Fig. 3. The helices stack head-to-tail pseudocontinuously along the c-axis. G1 stacks within the strand while G8 loops out to basepair with G1 of an adjacent duplex. Thus the two overhang G's of the reference duplex basepair with two different adjacent duplexes. The G · G basepair formed is reverse Hoogsteen from parallel strands of adjacent duplexes. The reverse Hoogsteen basepair stacks on the central Z-DNA duplex portion with six Watson-Crick C · G basepairs.

Nucleoside and backbone conformation

Table 2 lists the sugar-phosphate backbone and glycosidic torsion angles for the heptamer structure. It is seen that the nucleosides in the central part of the sequence (C2–C6/C9–C13) have the same sugar pucker and glycosidic conformation as in regular Z-DNA, namely, *anti*/C2'-*endo* for C and *syn*/C3'-*endo* for G (Wang et al., 1984). The overhang residues G1 and G8 are both in the *anti* glycosidic conformation and adopt a C2'-*endo* sugar pucker as in B-DNA. The 3'-terminal residues G7 and G14 are intermediate between Z- and B-DNA, having the C2'-*endo* sugar pucker as in B-DNA and *syn* glycosidic conformation as in Z-DNA. The conformations of G7 and G14 are the same as those of the 3'-terminal G's in the hexamer Z-DNA d(CGCGCG) (Wang et al., 1979).

FIGURE 3   (a) Stereo view showing the structure of d(GCGCGCG) with each strand bound to a cobalt hexammine ion. Strand 1 is shown in darker lines including residues 1 to 7, while strand 2, in lighter lines, includes 8 to 14. G1 stacks in the duplex and G8 swings out of the helix. (b) Stereo view showing the 5'-overhang G's in adjacent molecules involved in the reverse Hoogsteen G · G basepairing and head-to-tail crystal packing along the c-axis. (c) Crystal packing viewed down the c-axis showing the entry of the overhang G8 into the adjacent duplex.
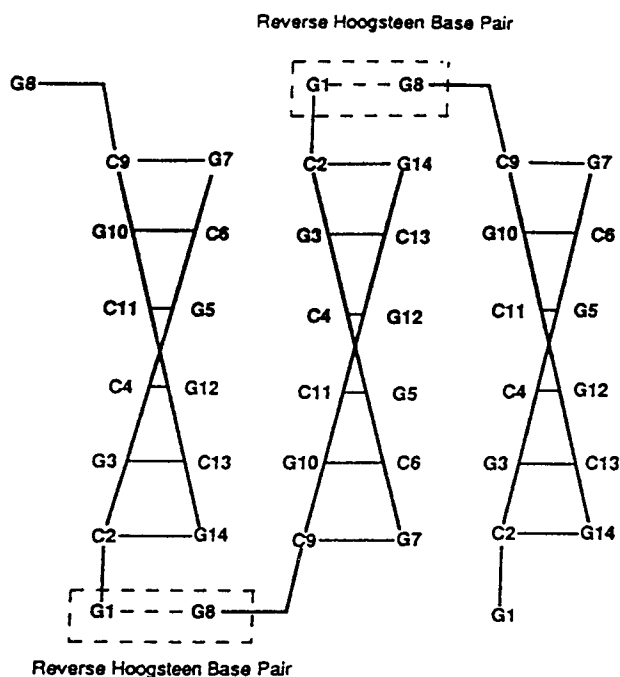
FIGURE 2   Schematic diagram of the basepairing in the heptamer structure. Besides the Watson-Crick C · G basepairs, the reverse Hoogsteen G · G basepairs are shown at the termini.
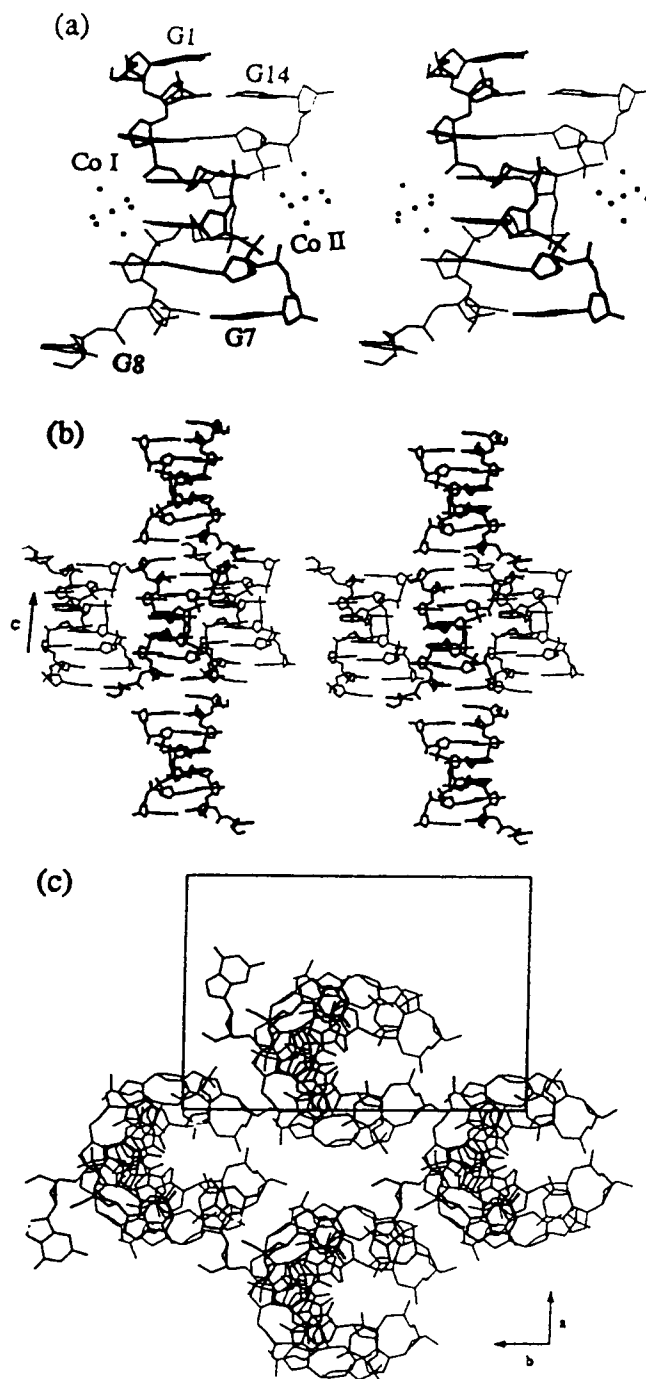
The novel G · G basepair

The overhang bases G1 and G8 form a *trans* reverse Hoogsteen basepair (Fig. 4 a). The hydrogen atoms generated by

TABLE 2 Nucleoside conformation and backbone torsion angles for d(GCGCGCG)

	α	β	γ	δ	ε	ζ	χ	P
Strand 1								
G1	—	—	59	162	−87	151	123 (anti)	181 (C2'-endo)
C2	−157	170	56	139	265	73	−164 (anti)	146 (C2'-endo)
G3	82	−168	168	84	−179	73	52 (syn)	33 (C3'-endo)
C4	160	158	57	143	−92	72	−145 (anti)	149 (C2'-endo)
G5	61	−169	−179	100	−122	−50	65 (syn)	8 (C3'-endo)
C6	−176	142	67	129	−101	73	−156 (anti)	139 (C1'-exo)
G7	79	−175	−177	142	—	—	73 (syn)	152 (C2'-endo)
Strand 2								
G8	—	—	54	156	−64	162	−92 (anti)	168 (C2'-endo)
C9	−61	−115	84	142	−95	75	−150 (anti)	172 (C2'-endo)
G10	79	−175	172	92	−171	53	58 (syn)	31 (C3'-endo)
C11	177	168	40	143	−78	64	−143 (anti)	161 (C2'-endo)
G12	71	−173	175	90	−116	−70	65 (syn)	29 (C3'-endo)
C13	−147	−126	53	138	−92	67	−150 (anti)	154 (C2'-endo)
G14	76	−178	−170	141	—	—	69 (syn)	163 (C2'-endo)

The backbone torsion angles as defined by IUPAC-IUB (1983) are O3'-P-α-O5'-β-C5'-γ-C4'-δ-C3'-ε-O3'-ζ-P-O5'.

X-PLOR are shown in Fig. 4, a and b. Besides the two standard hydrogen bonds (G1)N1—H $\cdots$ N7(G8) (1.72 Å) and (G1)N2—H $\cdots$ O6(G8) (2.05 Å), a bifurcated hydrogen bond (G1)N2—H $\cdots$ N7(G8) (2.39 Å) and a "forced" weak C—H $\cdots$ O hydrogen bond interaction (Jeffrey and Saenger, 1991) (G8)C8—H $\cdots$ O6(G1) (2.52 Å and with an angle of 138.1° between C—H—O) are formed. The base-pair is also stabilized by interaction with water molecules. G1 is hydrated by three water molecules at N3, O6, C8, and N7. Atoms N1 and O6 of G8 are hydrogen-bonded to one water molecule while N3 and N2 to two and three water molecules, respectively. These water molecules form a water string bridged by N2, spanning the Watson-Crick sites of G8 (Fig. 4 d).

Stacking of G · G basepair

Fig. 5 shows the base stacking of the overhang G · G basepair with the adjacent C · G basepairs in the duplex portion. The orientation angle between the overlapping G7 and G1 is ~30° (Fig. 5 a), while that between G8 and G14 is ~90° (Fig. 5 b). In both cases, the N4 group of cytosine stays inside the five- or six-membered aromatic ring of guanosine, respectively. It is obvious that the presence of this overhang basepair greatly increases the stacking interaction between the two adjacent duplexes, thus stabilizing the crystal structure.

Cobalt hexammine ion binding

Two ordered cobalt hexammine ions, referred to as Co I and II, bind to strand I and strand II, respectively (Fig. 3 a). The details of the coordination of cobalt hexammine ions to the heptamer are shown in Fig. 6. Table 3 gives the distance of these interactions. Co I binds to phosphate groups P4, P5, P14, and base G5 from two adjacent duplexes. P4 is in Z_{II} conformation while the other two phosphates are in Z_I. Co II binds to phosphate groups P4 (the same phosphate bound

by Co I), P11, P13, P14, and base G12 from three adjacent duplexes. P4 and P11 are in Z_{II} conformation while the others are in Z_I. In the present structure cobalt hexammine ions bind phosphates not only in GpC steps (P4, P11, P13) but also in CpG steps (P5, P14).

Hydration of the structure

The present heptamer structure reveals the common water structure motifs for the Z-DNA hexamer d(CGCGCG) (Gessner et al., 1994). Ten water molecules in the minor groove constitute the two different motifs: 1) the water bridge between O2 keto groups of cytosine from alternating strands, and 2) the water bridge between N2 amino groups of guanosine and phosphate groups (Fig. 7 a). The first motif is intact while the second is disrupted near the end of the duplex. In the major groove surface, eight water molecules constitute two water structure motifs: 1) the water bridge connecting the N4 amino group of cytosine in alternating strands, and 2) the water bridge linking the O6 keto group of guanine in alternating strands (Fig. 7 b). The second motif is disrupted at the binding site of Co I, where two regular water bridges are missing and the central bridge is connected to an ammonia.

DISCUSSION

Flexibility of overhang bases

The less constrained overhang bases are conformationally more flexible. For instance, the DNA sequences of d(GC-GAATTCG) (Meervelt et al., 1995) and d(GGCCAAT-TGG) (Vlieghe et al., 1996) with one or two overhang G's formed the major groove triplets. When the present sequence d(GCGCGCG) was designed, both overhang G's were expected to stay in the helix, producing an infinite duplex along the helix axis (Wahl et al., 1996) or to form a (C · G)*G triplet by interacting with the terminal C · G
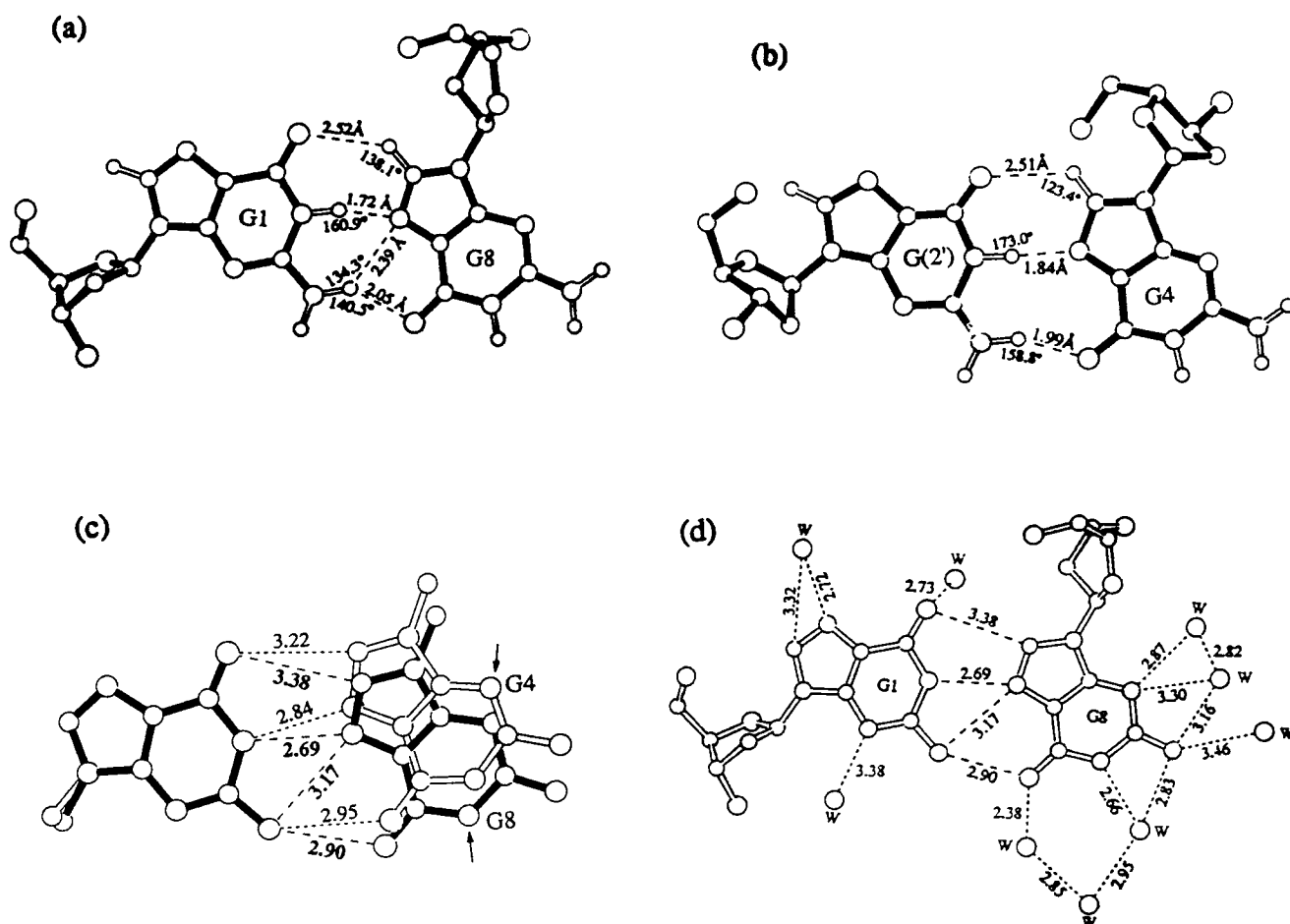
FIGURE 4   (a) The reverse Hoogsteen G · G basepair formed by 5'-overhang G1 and G8 in d(GCGCGCG) showing the CH · · · O and bifurcated hydrogen bonding interactions. The hydrogen atoms are generated by X-PLOR. The bond lengths and bond angles of the connected hydrogen atoms are as follows: C8—H: 1.05 Å; N7—C8—H: 123.0°; N9—C8—H: 123.0°; N1—H: 1.01 Å; C2—C1—H: 118.0°; C6—C1—H: 115.2°; N2—H: 1.01 Å; C2—N2—H1: 120.0°; C2—N2—H2: 120.0°; H1—N2—H2: 120.0°. (b) The "standard" reverse Hoogsteen G · G basepair G(2') · G4 in the cyclic diguanylic acid. The hydrogen atoms are generated by X-PLOR. The bond lengths and bond angles of the connected hydrogen atoms are the same as those in (a). (c) Superposition of G1 and G(2') of the basepairs of (a) and (b) to compare the positions of G8 and G4 in the respective basepairs. The distance between N2 of G(2') and N7 of G4 is 3.69 Å, indicating that the standard G · G basepair in (b) is not a strong bifurcated hydrogen bond. All distances are in Å. (d) Hydration of the reverse Hoogsteen G · G basepair. The Watson-Crick sites of N1 and O6 are involved in hydrogen bonding to water molecules. All distances are in Å.

basepair in the major groove (Meervelt et al., 1995; Vlieghe et al., 1996) or in the minor groove (Ramakrishnan and Sundaralingam, 1993). However, the conformations of the overhang G's are different. G1 stacks within the duplex while G8 loops out to form a reverse Hoogsteen basepair with G1 of an adjacent duplex (Fig. 3). This is the first observation of a reverse Hoogsteen G · G basepair stacked with a hexamer Z-DNA helix. The present conformation for the overhang bases adds yet another motif, in addition to the observed minor/major groove triplets. This reflects the polymorphism of overhang bases.

Z-DNA is normally characterized by a number of strongly alternating structural features that are in phase with the base-sequence alternation. Guanosine usually adopts the C3'-endo sugar pucker and a syn conformation about the glycosidic bond, while cytidine is usually in the C2'-endo pucker and anti glycosidic conformation. The present struc-

ture can be explained by the conformational flexibility of the overhang G's, which are less restricted than the other residues in the helix. The overhang G's adopt the anti/C2'-endo conformations, which deviate from the syn/C3'-endo pattern observed in the Z-DNA duplex. A similar precedent is seen in d(CCGCGG) (Malinina et al., 1994), where the central tetramer d(CGCG) forms the Z-DNA duplex with the 5'-terminal C1 pushed away from the duplex and G6 is stacked within the Z-DNA duplex. The major difference in the latter is that C1 and G6 are complementary bases.

G · G basepairing

G · G basepairs exist in DNA telomeric quadruplex and in (purine · pyrimidine)*purine   DNA   triplet   structures. G-quartets with G · G Hoogsteen basepairs are found in
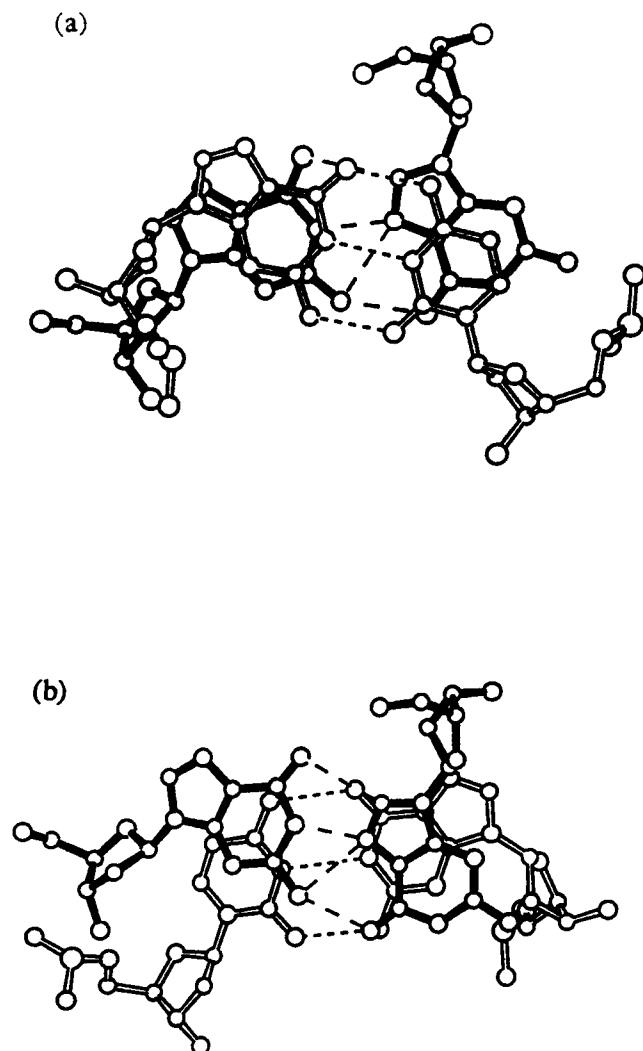
(a)

(a)

(b)

FIGURE 5 The base stacking of d(GCGCGCG). (a) Stacking between basepair G7 · C9 (*light bonds*) and G1 · G8 (*dark bonds*). (b) Base stacking between basepair G1 · G8 (*dark bonds*) and C2 · G14 (*light bonds*).
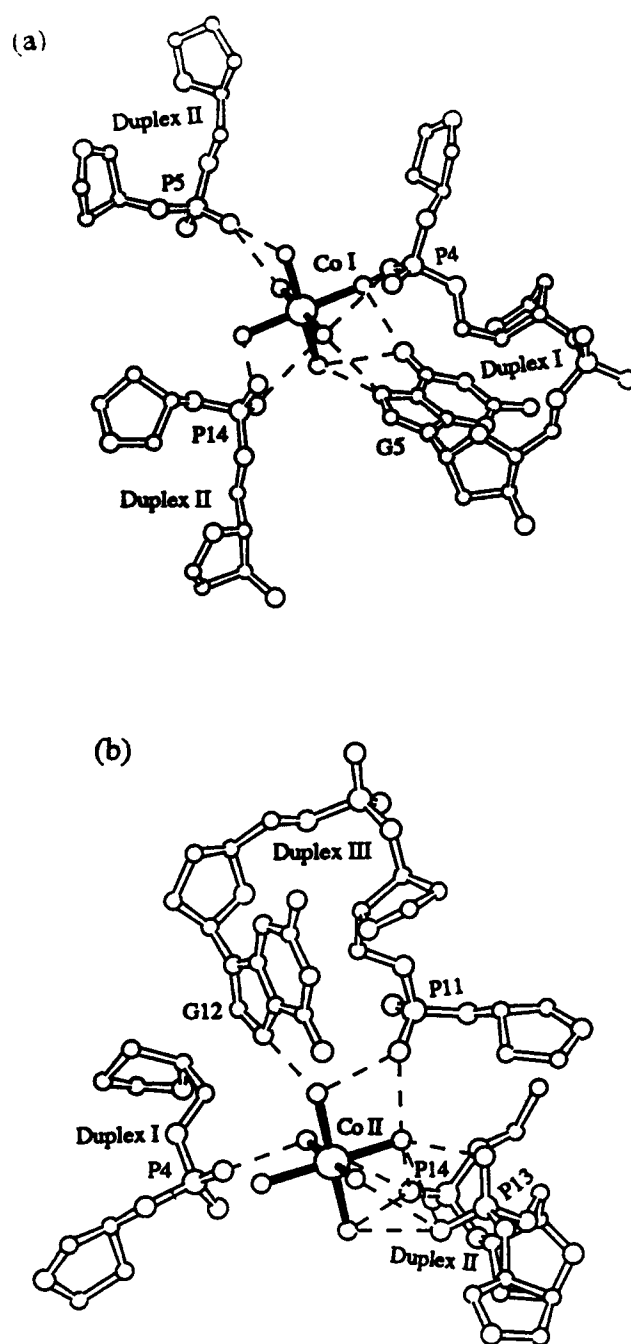
(b)

FIGURE 6 Cobalt hexammine binding to d(GCGCGCG). (a) Co I binds to two symmetry-related adjacent duplexes. (b) Co II binds to three symmetry-related adjacent duplexes.

parallel stranded structures with G(*anti*) · G(*anti*) glycosidic conformation (Laughlan et al., 1994) and antiparallel stranded structures with G(*anti*) · G(*syn*) glycosidic conformation (Kang et al., 1992). G(anti) · G(anti) Hoogsteen basepairing is also displayed in the crystal structures of the 5'-overhang B-DNA of d(GCGAATTCG) (Meervelt et al., 1995) and the B-DNA duplex d(CGCGAATTGGCG)$_2$ (Skelly et al., 1993). Both G(*anti*) · G(*anti*) Hoogsteen and reverse Hoogsteen basepairs have been observed in the crystal structure of the B-DNA d(GGCCAATTGG) (Vlieghe et al., 1996), in which the 5'-overhang G's invade the major groove of a symmetry-related duplex to form a (C · G)*G base triplet with the terminal C · G basepair. All these basepairs involve hydrogen-bonding of the O6 and N7 atoms of one G residue to the N1 and N2 atoms of the other G residue.

Fig. 4, a and b show a comparison of the conformations and hydrogen bonding of the observed reverse Hoogsteen

G · G basepair with that observed in the high-resolution (0.9 Å) structure of cyclic diguanylic acid (Egli et al., 1990), which may be regarded as the "standard." Besides the expected hydrogen bonds between N1(G1) to N7(G8) and N2(G1) to O6(G8), the shifting of the G bases relative to each other in the present structure has resulted in a new bifurcated hydrogen bond between N2(G1) and N7(G8) and enhancing the C—H · · · O interaction (Fig. 4 c). Other examples of standard reverse Hoogsteen G · G basepairs are

TABLE 3 Distance between atoms of cobalt hexammine and DNA duplex

CoHex atom	DNA atom	Distance (Å)
N1 (Co I)	14O1P (II)	2.80
N2 (Co I)	O6 (G5) (I)	2.72
N2 (Co I)	4O2P (I)	2.72
N3 (Co I)	4O2P (I)	3.06
N3 (Co I)	N7 (G5) (I)	2.80
N3 (Co I)	14O1P (II)	3.16
N4 (Co I)	5O2P (II)	2.80
N5 (Co I)	O6 (G5) (I)	2.80
N5 (Co I)	N7 (G5) (I)	3.27
N6 (Co I)	5O2P (II)	3.09
N1 (Co II)	11O2P (III)	2.70
N1 (Co II)	13O1P (II)	3.36
N1 (Co II)	13O2P (II)	3.32
N1 (Co II)	14O2P (II)	2.66
N3 (Co II)	N7 (G12) (III)	2.85
N3 (Co II)	11O2P (III)	2.65
N4 (Co II)	13O1P (II)	3.35
N4 (Co II)	14O2P (II)	2.91
N5 (Co II)	4O1P (I)	2.73
N5 (Co II)	14O2P (II)	3.45
N6 (Co II)	13O1P (II)	3.04

I, II and III refer to the number of duplex in Fig. 6.

found in the (C · G)*G base triplet in the B-DNA d(GGC-CAATTGG) (Vlieghe et al., 1996), and the low-resolution structures of yeast tRNA^{Phe} (Schimmel et al., 1979).

Cobalt hexammine ion binding

Cobalt hexammine ions have been regarded as very effective in stabilizing the left-handed Z-DNA helix (Behe and Felsenfeld, 1981; Brennan et al., 1986). Gessner et al. (1985) found that two cobalt hexammine ions interact with guanine and phosphates in GpC steps and the phosphates are in Z_{II} conformation. In the present structure, both Z_I and Z_{II} conformations for the phosphates are observed when cobalt hexammine ions bind. It is noted that when cobalt hexammine ion binds to both a phosphate and a nearby G residue, the phosphate is in Z_{II} conformation otherwise the phosphate is in Z_I conformation.

Conformations of overhangs

It has been shown that the T_m for $\{d[(GC)_3TT]\}_2$ (54.2 ± 0.5°C) is essentially the same as that for $\{d[TT(CG)_3]\}_2$ (54.4 ± 0.5°C) (Senior et al., 1988). Based on this result, the structure of Scheme 1, $\{d[(GC)_3G~]\}_2$, and Scheme 2, $\{d[G(CG)_3]\}_2$, should have similar thermal stability. In other words, these two different conformations are expected to coexist in the solution. The crystallization of the former may lead to the A- or B-duplex conformation with 3'-overhang G's, while the crystallization of the latter may lead to the Z-duplex with 5'-overhang G's. Fig. 8 shows the circular dichroism (CD) spectra for the present heptamer d(GCGCGCG) and hexamer d(CGCGCG) in both 2.5 M
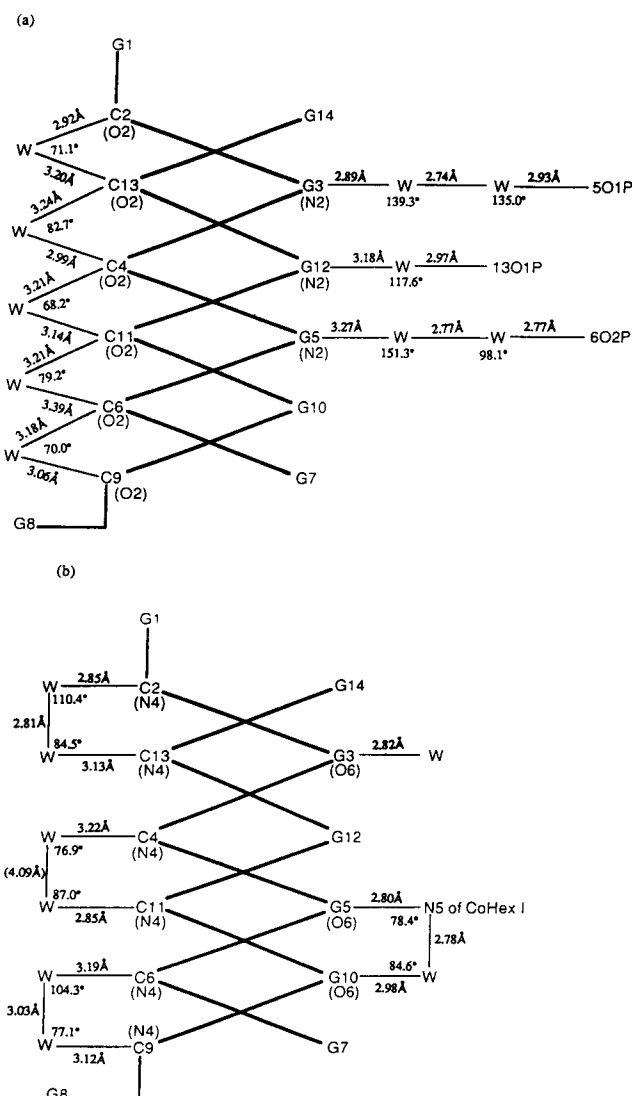
FIGURE 7 Hydration of the heptamer structure d(GCGCGCG). (a) Water structure in the minor groove. The backbones of the heptamer are shown in dark lines as zig-zag chains, while hydrogen bonds are shown as light lines. (b) Water structure in the major groove.

and 5 M NaCl solutions. The spectra indicate clearly that the present heptamer adopts the B-form in both high and low salt. In contrast, the hexamer undergoes B-Z transition from low salt to high salt. The crystalline state of the heptamer d(GCGCGCG) adopts the left-handed Z-form with 5'-over-hangs even in low salt crystallization conditions. However, in solution it seems that dehydrating the heptamer with 5 M NaCl does not result in the B-Z transition. The packing interactions and the hydration in the crystal may influence the outcome.
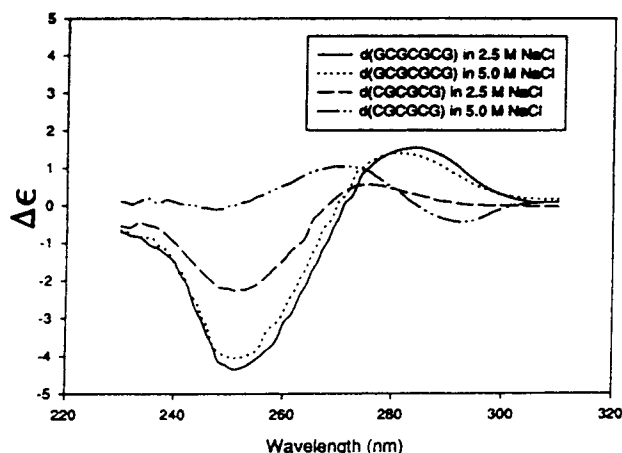
FIGURE 8 CD spectra of the present heptamer d(GCGCGCG) and hexamer d(CGCGCG). The initial crystallization conditions were added with 2.5 M and 5 M NaCl, respectively. The spectra were measured using a JASCO J-500A spectropolarimeter, with sensitivity of 0.1, time constant of 16 s, and step size of 1.0 nm at room temperature.

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