Structural Variability and New Intermolecular Interactions of Z-DNA in Crystals of d(pCpGpCpGpCpG)

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ABSTRACT We have determined the single crystal x-ray structure of the synthetic DNA hexamer d(pCpGpCpGpCpG) in two different crystal forms. The hexamer pCGCGCG has the Z-DNA conformation and in both cases the asymmetric unit contains more than one Z-DNA duplex. Crystals belong to the space group $C222_1$ with $a = 69.73$, $b = 52.63$, and $c = 26.21$ Å, and to the space group $P2_1$ with $a = 49.87$, $b = 41.26$, $c = 21.91$ Å, and $\gamma = 97.12°$. Both crystals show new crystal packing modes. The molecules also show striking new features when compared with previously determined Z-DNA structures: 1) the bases in one duplex have a large inclination with respect to the helical axis, which alters the overall shape of the molecule. 2) Some cytosine nitrogens interact by hydrogen bonding with phosphates in neighbor molecules. Similar base-phosphate interactions had been previously detected in some B-DNA crystals. 3) Basepair stacking between the ends of neighbor molecules is variable and no helical continuity is maintained between contiguous hexamer duplexes.

INTRODUCTION

Since 1979, when the first paper describing the single crystal x-ray structure of a DNA duplex appeared (Wang et al., 1979), a large number of various DNA fragments in the A, B, and Z conformations have been studied by single crystal x-ray diffraction methods (reviewed by Dickerson, 1992; Wahl and Sundaralingam, 1995). Z-DNA is one of the best-studied double helices, since high-resolution single-crystal x-ray structures of Z-DNA fragments have been determined (Wang et al., 1979; Rich et al., 1984; Ho et al., 1985; Gessner et al., 1989, 1994). The variability of some of these structures was analyzed by Schneider et al. (1992). However, all Z-DNA fragments studied have almost the same packing arrangements: end-to-end stacked duplexes form pseudo-continuous Z-DNA helices, all oriented along the same direction in a manner that each one is tightly surrounded by six neighbors (Ban et al., 1996; Brennan et al., 1986; Coll et al., 1988; Drew et al., 1980; Egli et al., 1991; Fujii et al., 1985; Gessner et al., 1985, 1989; Ho et al., 1985; Kumar et al., 1992; Schroth et al., 1993; Wang et al., 1979, 1984). Such a packing mode could be a reason both for the high-resolution diffraction of Z-DNA crystals and for the structural rigidity (minor variations in conformation) of Z-DNA fragments in crystals. It is probable that other packing modes will provoke a structural variability. Unfortunately, obtaining different crystal forms of an oligonucleotide is not so simple. To overcome this, we have developed an experimental phase diagram technique for crystallizing double-helical oligonucleotides (Malinina et al., 1987a, b; Tereshko and Malinina, 1990a). We applied this technique to crystallization of the deoxyhexamer pCGCGCG, which has a 5′-end phosphate, and obtained six different crystal forms (Malinina et al., 1991). The presence of a 5′-end phosphate does not appear to have any significant influence on the conformation of the hexamer, as already found by Jean et al. (1993) in another hexamer. In fact, two of the crystal forms of pCGCGCG were isomorphous to previously solved Z-DNA crystals (Fujii et al., 1985; Wang et al., 1979). Another two were similar to each other and belonged to the space group $P2_1$, which has never been observed for Z-DNA. The two other forms had new unit cell parameters although they belonged to the space group $C222_1$, which had already been observed for the Z-DNA tetramer CGCG (Drew et al., 1980). For these two forms and for the best crystal form of $P2_1$ x-ray data have been collected.

The DNA molecule is quite flexible and packing forces do play a significant role in the structure of short DNA duplexes. Therefore, when one and the same DNA duplex is studied in different crystal packing arrangements, it gives an excellent opportunity to study its structural variability. For instance, when the structure of the A-DNA decamer GCGGGCCCGC was studied in two different crystal forms (Ramakrishnan and Sundaralingam, 1993), the authors concluded that the crystal environment dominated base sequence effects on DNA conformation. Other examples that demonstrate the conformational flexibility of DNA fragments in different crystal environments could also be mentioned both for A- (Fernandez et al., 1997; Jain and Sundaralingam, 1989) and B-DNA (Lipanov et al., 1993).

We have already reported the pCGCGCG structure in one crystal form (Strokopytov and Malinina, 1994). In this article we present the structure of the same fragment, pCGCGCG, in two other crystal forms. In all three cases crystals contain more than one duplex in the asymmetric unit and all hexamers form Z-DNA duplexes. We have discovered new crystal packing modes for Z-DNA and find

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that the Z-DNA structure is variable. This variability is essential for the unusual behavior that we found in another hexamer, CCGCGG (Malinina et al., 1994). In that case only the central tetramer forms a Z-DNA duplex. The ends interact in an intermolecular manner. The initial cytosine swings out and forms a Watson-Crick basepair with the terminal guanine of a symmetry-related molecule. In such a recombinationlike structure the basepairs of the central Z-DNA tetramer are inclined by 20°. In the pCGCGCG structures presented here we also find that Z-DNA can have such an inclination of basepairs. Other structural details of Z-DNA are discussed.

MATERIALS AND METHODS

Crystals and data collection

Synthesis and crystallization of the self-complementary deoxyhexanucleotide d(pCpGpCpGpCpG) have been described previously (Tereshko and Malinina, 1990b; Malinina et al., 1991). The x-ray data were collected at $T = 18°$ on an automatic four-circle diffractometer Syntex $P2_1$ using CuKα radiation ($\lambda = 1.5418$ Å). Intensities were corrected for absorption, LP-factor, and radiation damage. The unit cell dimensions and the characteristics of the data sets are presented in Table 1.

Structure solution and refinement

The structures were solved by molecular replacement with the help of the AMoRe program (Navaza, 1994). First, the structure of pCGCGCG in the orthorhombic crystal form (#1 in Table 1) was solved. It followed from the volume of the asymmetric unit that it should contain one and one-half duplex of pCGCGCG. It meant that at least one duplex should be located on a crystallographic dyad. Although we suspected that the hexamer would adopt the Z-conformation, we tried A-, B- and Z-DNA models. The calculation of the rotation function demonstrated that the best model was Z-DNA. Z1- and Z2-model coordinates were calculated by means of the NAHELIX program (Westhof et al., 1985) and the solution for the first molecule was obtained with the Z2-DNA model using the resolution regions 6–3.5 Å for the rotation function calculation; 15–5 Å for the translation function; and 8–2.5 Å for rigid-body refinement. The fact that the central dyad of this molecule coincided with one of the crystallographic dyads was additional evidence of the correctness of this solution. To obtain the solution for another molecule it was necessary to recalculate a rotation function using the resolution region 15–4.5 Å and then to calculate a translation function for resolution 15–7 Å, keeping the first molecule fixed. The final step was a rigid-body refinement of two molecules in the region 8–2.5 Å. The correlation coefficient was 57.6%, and the R-factor 46.8%.

After obtaining a solution we started refinement using the XPLOR program (Brünger, 1992). The entire data set was separated into a working set containing 90% and a reference set containing 10% of reflections randomly sampled throughout the resolution range. First, we applied a rigid-body refinement in the resolution region 8–3 Å with an increasing number of rigid groups in the model. At the beginning, each basepair was a group and at the end each nucleotide was represented as three rigid groups (base, sugar, and phosphate). The R-work/R-free decreased from 42.6/44.2% to 28.1/32.8%. The same strategy was then used in the resolution region 8–2.5 Å. R-factors changed from 30.4/34.8% to 28.2/34.1%. Next, we started molecular dynamics simulated annealing. For this procedure we included data between 8 Å and 2 Å, the molecule was "heated" up to 4000 K, and the final values of R-work/R-free were 27.6/32.0% (after refinement of atomic temperature factor, R-factors = 24.7/29.6%). After gradual addition of 42 water molecules into the map, positional refinement, refinement of atomic temperature factors, and using the XPLOR multiscaling procedure, dividing the data into 10 bins, the R-work/R-free decreased to 18.8/22.7% (8–2 Å). For a gradual addition of water molecules, we recalculated the difference Fo-Fc maps and the 2Fo-Fc maps. The refinement was terminated when the R-free value stopped to decrease with the addition of new water molecules. The final refinement parameters are presented in Table 1. An omit map for a DNA basepair is shown in Fig. 1

TABLE 1 Crystallographic data and refinement statistics

Crystal	#1	#2
Space group	$C222_1$	$P2_1$
Unit cell parameters		
a (Å)	69.73	49.87
b (Å)	52.63	41.26
c (Å)	26.21	21.91
γ (°)		97.12
Resolution (Å)	2.0	2.75
Asymmetric unit	1.5 duplexes 42 water molecules	3 duplexes 17 water molecules
Data collection statistics		
Unique reflections (*n*)	3232	2421
$R\sigma = \Sigma\sigma(I)/\Sigma I$	0.026	0.064
Completeness (%)	93.0	99.8
Final refinement parameters		
Resolution (Å)	8.0–2.0	8.0–2.75
Reflections $F > 2\sigma$ (F)	3171	2316
Observed/test data	2862/309	2097/219
Completeness (%)	83.9/9.1	90.0/9.4
R-work/R-free (%)*	18.8/22.7	19.9/23.8
R.m.s. deviation from ideal geometry of final model		
Bond lengths (Å)	0.011	0.007
Bond angles (°)	1.44	1.28
Improper angles for bases (°)	0.61	0.37

*Using the XPLOR multiscale procedure, dividing the dataset into 10 resolution shells.

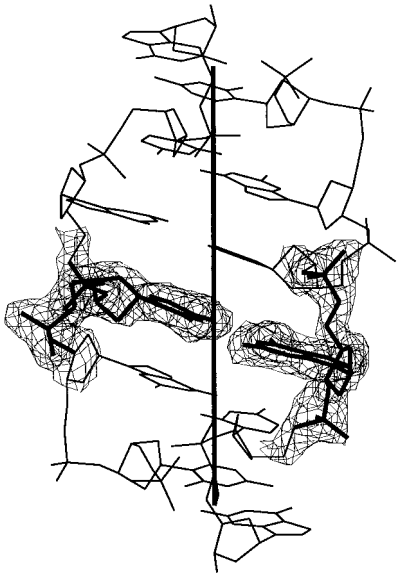

FIGURE 1 Omit map calculated at 1.0 σ level with 15–2.0-Å resolution and superimposed on appropriate DNA basepair (*thick lines*) in the orthorhombic crystal (#1) of pCGCGCG. Only 1 of 1.5 duplexes of the asymmetric unit is shown with its helical axis calculated with program CURVES.

as an example.

To reach a solution for the monoclinic crystal form (#2), we tried another model. It was the structure of one of the pCGCGCG duplexes from the orthorhombic crystal form #1 after a full cycle of refinement (see below). With such a model we obtained a solution for all three pCGCGCG duplexes in the asymmetric unit. The correlation coefficient was 68.8%, and the R-factor 37.2% for the resolution region 15–2.75 Å.

The same general strategy of refinement used for form #1 was applied to the monoclinic crystal form #2, which has three duplexes in the asymmetric unit. The only difference was that in this case we kept noncrystallographic restraints on structural differences between the six crystallographically independent pCGCGCG strands up to the moment when we began to insert water molecules into the map. At the end 17 water molecules had been inserted and the last cycles of positional refinement were done without noncrystallographic restraints. We terminated the addition of water molecules when R-free stopped decreasing. The refinement was concluded with an R-work/R-free 19.9/23.8% for a resolution range 8–2.75 Å (Table 1).

The previously solved and refined orthorhombic crystal form (Strokopytov and Malinina, 1994) has been rerefined. We started with another Z-DNA model (Wang et al., 1979; Nucleic Acid Database (NDB) entry ZDF001) and made rigid-body refinement with groups, as described above, and positional treatment. After the refinement of grouped temperature factors and the multiscaling procedure, the R-work/R-free were 18.2/24.5% (resolution 8–3 Å). These results were only used in the superposition, which will be presented below (Fig. 6 *A*).

Helical and conformational parameters have been calculated by means of the NEWHELIX93 program (R. E. Dickerson, personal communication, 1993). All helical axes in the figures have been calculated with the CURVES 5.1 program (Lavery and Sklenar, 1989). Coordinates and structure factors are available from the authors and will be deposited in the NDB.

RESULTS

A new crystal packing mode for Z-DNA

The original Z-DNA structure was determined from crystals of CGCGCG grown in the presence of spermine and magnesium ions (Wang et al., 1979). A second, isomorphous crystal structure of the same hexamer was obtained from crystals grown without spermine in the presence of high concentrations of magnesium ions (Gessner et al., 1985, 1989). The structure of CGCGCG in pure-spermine crystal forms was also reported (Egli et al., 1991; Bancroft et al., 1994). In all these cases the hexamer CGCGCG forms crystals demonstrating a similar packing arrangement. The Z-DNA duplexes are stacked one on the top of the other forming pseudocontinuous double helices oriented along the same direction in the crystal. However, as it follows from our results, pure-spermine (magnesium free) crystals of Z-DNA can show a quite different packing mode if a low concentration of spermine is used for crystallization.

In Fig. 2 the structural motifs of three end-to-end stacked Z-DNA duplexes in the orthorhombic (#1) and monoclinic (#2) crystals are shown. In the orthorhombic crystal form (Fig. 2 *A*) one of the crystallographic dyads is located in the center of this motif, so that two flanking duplexes, as well as two strands of the central duplex, are structurally identical. In the monoclinic crystal (Fig. 2 *B*) the three duplexes are not related to each other by any crystallographic symmetry.

The structural motif of three end-to-end stacked Z-DNA duplexes, being repeated by a translation, produces a column of Z-DNA in the crystal. Unlike all previously reported cases, this is not a column of a pseudocontinuous helix of DNA in the Z conformation. In each case the GC step between duplexes displays a different slide and helical twist, mostly right-handed, that results in G/G-stacking, described below in more detail. Besides, duplexes forming such a column are tilted differently with respect to its general axis (Fig. 2). As a result, the columns are curved.

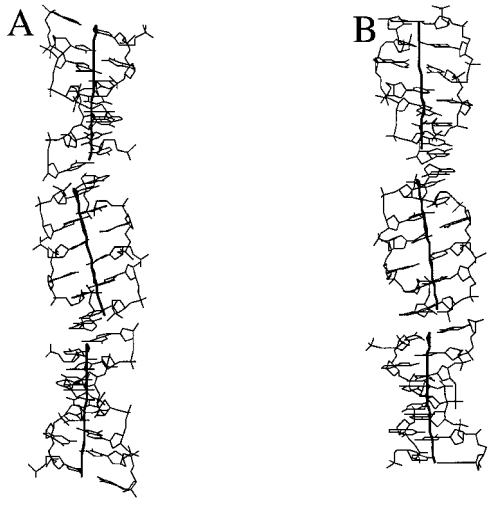

FIGURE 2 Structural motif of three end-to-end stacked Z-DNA duplexes (*A*) in the orthorhombic (#1) and (*B*) in the monoclinic (#2) crystals of pCGCGCG. Helical axes are shown for each duplex.

The curvature of the columns can be clearly seen in Fig. 3, where two neighboring three-molecule motifs from the orthorhombic crystal of pCGCGCG are shown. In the or-

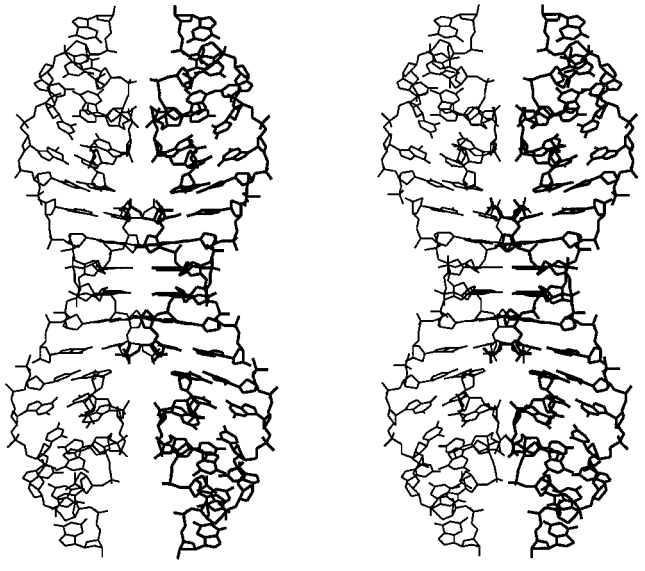

FIGURE 3 Stereo view of two neighboring three-molecule motifs in the orthorhombic crystal form of pCGCGCG. The columns are oriented along diagonals $(a+c)$ and $(a-c)$ of the unit cell and form an angle of 41° with each other. A view along the *c* axis is presented. The two central molecules are oriented face-to-face in the crystal.

thorhombic crystal Z-DNA columns are oriented along diagonals ($a+c$) or ($a-c$) of the unit cell. A view along the c axis is presented so that columns display equal tilt in opposite directions with respect to the plane of the figure. Two neighboring columns form an angle of 41° with each other.

In the monoclinic crystal, columns are oriented along diagonals ($a-b+c$) or ($a-b-c$); so the general arrangement is similar to that shown in Fig. 3, but neighboring columns now form a slightly smaller angle (35.4°).

New interactions between Z-DNA duplexes

Novel interactions between neighboring Z-DNA molecules can be seen in the packing arrangements described. One of the most striking interactions is shown in Fig. 4 for the case of the orthorhombic crystal. The central molecule of a three-molecules motif (shown in Fig. 2 *A*) forms six hydrogen bonds with a neighboring column. The two first basepairs of each flanking molecule of the neighbor column participate in this hydrogen bonding. They correspond to CpG steps, at which cytosines form an interstrand stack in Z-DNA (Wang et al., 1979). Such an arrangement allows that the N4 amino groups of both cytosines form hydrogen bonds with oxygen atoms O1P and O_2P of the same phosphate. Besides, one N7 guanine atom forms another hydrogen bond with the O3′H group of the central molecule, as it is also shown in Fig. 4. To make the explanation more clear, it can be said that the first CpG step of the flanking molecule recognizes the backbone of the last CpG step of the central molecule. This specific interaction is found at both ends of the central molecule due to the crystallographic twofold symmetry that can be easily appreciated in Fig. 4.

In the monoclinic crystal form the central (rather than the first) CpG step of only one flanking molecule is involved in a similar interaction. In addition, three single hydrogen bonds between the N4 amino group of other cytosines and the O_2P oxygen atoms of phosphates have also been found.

It should be noted that groove-backbone interactions have been previously described for B-DNA crystals with crossed structures (Timsit and Moras, 1991; Mayer-Jung et al., 1997). In these crystals the B-DNA backbone penetrates into the major groove of a neighbor. As a result, a groove-backbone hydrogen bonding is possible. As in our Z-DNA crystals, amino groups of both cytosines in a CpG step form hydrogen bonds with two oxygen atoms of the same phosphate group of a neighboring molecule. So, both in B- and Z-DNA a CpG step displays the ability to recognize a phosphate.

In Z-DNA the convex surface of the molecule makes the formation of such hydrogen bonds easier. Neither B- nor A-DNA has a convex surface, which is a characteristic of Z-DNA. As a result, active groups usually located in the major groove of the double helix (N4 amino groups of cytosines, O6 ketogroups, and N7 nitrogen atom of guanines), are exposed in Z-DNA. Such an arrangement allows them to interact easily with other molecules. Therefore, the interactions described above seem to be quite natural for Z-DNA. They might be very important for the eventual recognition and interactions of Z-DNA in vivo.

Face-to-face orientation of Z-DNA duplexes

In both crystals the Z-DNA convex surface provokes another kind of DNA-DNA interaction, previously observed in twinned Z-DNA duplexes (Malinina et al., 1994). One can see in Fig. 3 that convex surfaces of the two central molecules are oriented face-to-face. The figure corresponds to the orthorhombic crystal of pCGCGCG. However, such face-to-face structure is also present in another crystal. In the monoclinic crystal form the mutual orientation of two convex surfaces and the distance between them are very similar to what we found in the twinned structure. Such orientation should be stabilized by interactions between both surfaces. In fact, in the twinned Z-DNA structure we found a sodium ion that makes a bridge between the O6 guanine atoms of a twin. Besides, several water-water or water-Na^+ bridges connecting the two duplexes in the twin are also present (unpublished results). A similar interaction has also been found involving a barium ion in another hexamer (Jean et al., 1993). Unfortunately, the limited res-

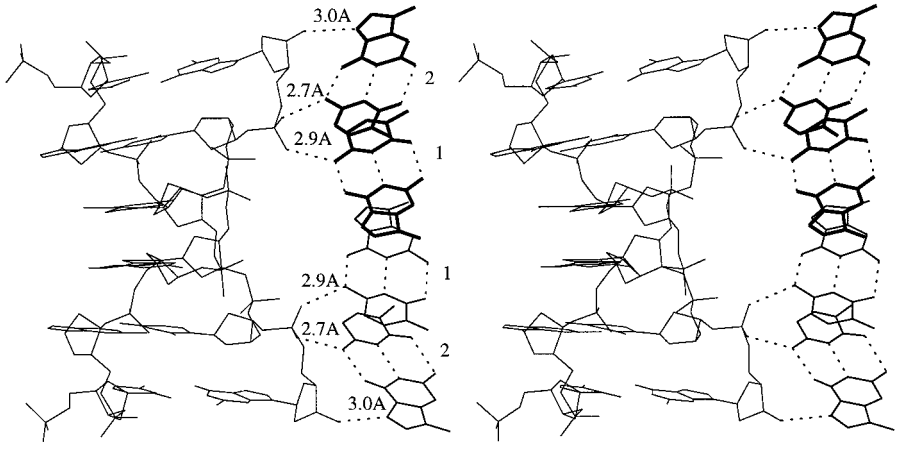

FIGURE 4 Hydrogen bonding between the phosphodiester backbone of the central molecule of a three-molecule motif and the base nitrogens in the convex surface of Z-DNA duplexes of a neighboring column in the orthorhombic crystal (#1) (see text for details). Basepairs 1 and 2 recognizing the phosphate group are shown without their phosphodiester backbone.

olution we have for the monoclinic crystal form does not allow localization of all solvent molecules in the unit cell. Therefore, we cannot determine the water and ionic bridges that may be present between two convex surfaces of Z-DNA, although it seems quite likely that such bridges should be present. In the orthorhombic crystal the mutual orientation of convex surfaces differs from that found in the twinned Z-DNA structure. Nevertheless, some water-water bridges between two molecules are evident.

Base-stacking interactions and helical parameters between end-to-end duplexes

The base-stacking pattern for all steps in the different duplexes present in all three crystal forms of pCGCGCG are similar to those found for other Z-DNA fragments: the GpC step exhibits intrastrand base-stacking between G and C, and in CpG steps cytosines form an interstrand stack (Wang et al., 1979). However, the GpC steps between duplexes show unusual base-stacking patterns (Fig. 5), because neither of the crystal forms produces pseudocontinuous double helices. Most of these intermolecular steps have a right-handed helical twist and slide with an opposite sign to that found in continuous Z-DNA helices (Table 2). These steps would correspond to GpC in continuous DNA, but the differences in slide and twist indicate a different arrangement with a variable guanine-guanine interstrand stacking in four cases. The other step has a large right-handed twist and slide of normal sign (Table 2). It shows an unusual base-stacking pattern (Fig. 5 *C*).

As was mentioned above, different duplexes are differently tilted in a column. The angle between the axes of consecutive duplexes is 6° and 12° in the orthorhombic crystal, and 19°, 16°, and 16° in the monoclinic one. Such a different inclination of duplexes combined with the unusual twist and slide of one duplex with respect to the next one results in kinking of the columns, which can be easily seen in Figs. 2 and 3.

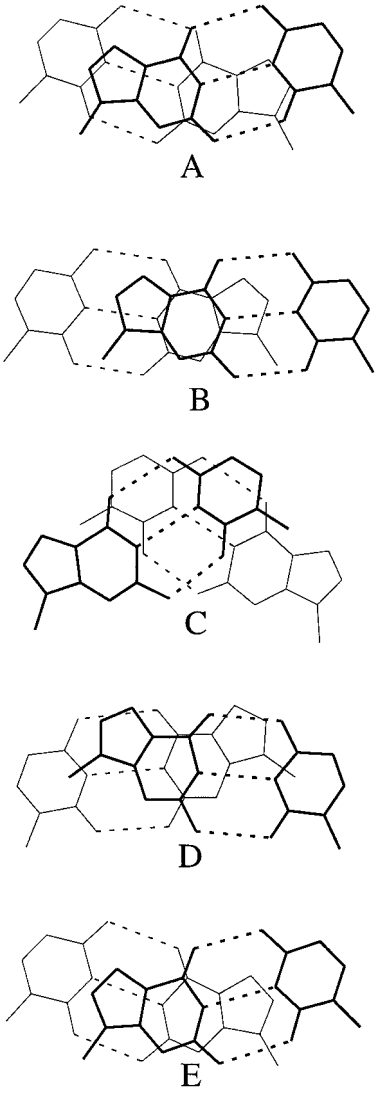

FIGURE 5 Stacking diagram illustrating the overlap of basepairs in the GpC step between consecutive duplexes. The upper basepair is shown in heavy lines. For details of the type of step see the text and Table 2. Base steps *A*, *C*, and *E* are right-handed, *D* is left-handed, and *B* shows no significant twist, only slide.

Structural differences between duplexes

The superposition of all pCGCGCG duplexes studied in this work with the Z1-DNA model of the same sequence is shown in Fig. 6 *A*, which clearly demonstrates that Z-DNA is not structurally rigid. The rerefined structure of Strokopytov and Malinina (1994) is also included in this figure. For describing the structural variability of pCGCGCG we consider below only crystal form #1, since it has a better resolution than the two other forms. Thus, Fig. 6 *B* displays the structural differences between the central and flanking molecules of this crystal form and the same Z1-DNA model. Both molecules are slightly underwound (there are 13 rather than 12 basepairs in a helical turn) and their basepairs are more inclined than in the ideal structure. The inclination of basepairs in the hexamer results in bending of the columns, which can be clearly seen in Fig. 3.

The helical parameters for these two molecules in comparison with the Z-DNA hexamer of the same sequence (Wang et al., 1979; NDB entry ZDF001) are presented in Fig. 7. It can be seen from Fig. 7 *A* that the central molecule is more underwound than the flanking one which is, in turn, more underwound than ZDF001. The mean inclination of basepairs are 13.1° and 19.2° for the central and flanking molecules, whereas ZDF001, which has the highest inclination of basepairs among all Z-DNA fragments studied up to now, exhibits a mean inclination of 8.9° (Fig. 7 *B*). In the case of the recombinationlike structure (Malinina et al., 1994) the basepairs of the central Z-DNA tetramer CGCG are inclined by ~21–24°. It follows from the pCGCGCG structures presented in this work that Z-DNA can easily take such a conformation.

TABLE 2 Twist and slide values for three end-to-end stacked Z-DNA duplexes CGCGCG

Crystal	#1		#2	
Step	Twist	Slide	Twist	Slide
1	−12.4	5.12	−7.2	4.72
2	−51.3	−0.91	−48.0	−0.40
3	−10.1	5.09	−14.4	5.07
4	−44.7	−1.07	−49.5	−0.39
5	−11.0	5.13	−10.5	4.83
6	14.3 (A)	2.05	48.7 (C)	−3.06
7	−12.4	5.28	−11.3	5.13
8	−46.3	−1.02	−50.1	0.01
9	−10.3	5.28	−12.6	5.07
10	−46.3	−1.02	−47.9	−0.26
11	−12.4	5.28	−10.6	5.26
12	14.3 (A)	2.05	−29.4 (D)	3.25
13	−11.0	5.13	−14.5	5.04
14	−44.7	−1.07	−51.4	−0.42
15	−10.1	5.09	−10.0	5.29
16	−51.3	−0.91	−46.5	−0.12
17	−12.4	5.12	−11.4	4.95
18	−2.2 (B)	3.54	16.5 (E)	2.5
CpG	−9.4	5.43		
GpC	−50.6	−1.14		

Steps 7–11 correspond to the central molecule in a column and 1–5 and 13–17 belong to its flanking molecules. Steps 6, 12, and 18 are located between consecutive duplexes in a continuous column. The variable values of these steps clearly show that the duplexes do not stack as a continuous helix. Odd steps correspond to CpG and even ones to GpC. Standard values for CpG and GpC steps of Z1-DNA are given at the bottom. Steps marked (A) to (E) are shown in Fig. 5.

The inclination of basepairs correlates with the rise values, so that a higher inclination corresponds to a higher rise for CpG and a lower one for GpC steps (Fig. 7 *C*). Besides, increasing the inclination results in decreasing the X-displacement of basepairs, the bases being displaced from the helical axis by 3 Å or more in the previously studied Z-DNA hexamers and only 1–1.5 Å in molecule 2 (Fig. 7 *D*), whose bases are inclined ~20°. The same effect could be noticed in the recombinationlike structure of CCGCGG (Malinina et al., 1994), in which basepairs of the central Z-DNA tetramer have an inclination of 21–24° and X-displacement of 0.5–1 Å.

DISCUSSION

For 15 years one of the objectives of DNA crystallography has been the study of the effect of base sequence on the fine structure of DNA. Investigations of this kind have been very much stimulated by the x-ray structure of the *trp*-repressor/operator complex (Otwinowski et al., 1988), where it was concluded that "the sequence seems to be recognized indirectly through its effects on the geometry of the phosphate backbone." However, the meaning of "fine DNA structure" is still unclear. If we assume that the structural variability is an intrinsic feature of the DNA molecule or any DNA fragment, then what do we have to consider as a fine DNA structure?

This question becomes especially important when we recall that DNA in vivo is always packed with itself or with proteins, giving compact structures or complexes essential for its biological functions. Therefore, it might be more important to study interactions of a DNA duplex with its neighbors than the fine DNA structure. It is also important to know how easily the structure of any DNA fragment can vary when its interaction with neighboring molecules changes. In order to answer all these questions it is helpful to study the structure of one and the same DNA fragment in different crystal environments, as it was mentioned in the Introduction.

Among all double-helical conformations, Z-DNA seems to be the most structurally rigid (Shakked, 1991). However, the conclusion about its structural rigidity can be a consequence of the similarity in the packing mode for Z-DNA fragments studied up to now. All of them had almost the same packing arrangements, although crystals belonged to different space groups. It should be noted that even small variations in the environment result in a conformational variability of the backbone, so that the phosphodiester conformation can be Z1, Z2 (Wang et al., 1979), or an intermediate one (Ban et al., 1996). However, it would be interesting to know whether some structural parameters of a Z-DNA duplex can vary when its contacts with neighbors differ.

In this work we have studied the single crystal x-ray structure of a Z-DNA hexamer pCGCGCG in new crystal packing modes, in which interactions of a duplex with its neighbors are different from previous cases. We have found that the alternating CG sequence can be underwound up to 13 basepairs per turn. We have also found that the mean inclination of basepairs is variable. Bases can be almost perpendicular to the helical axis (Egli et al., 1991; Bancroft et al., 1994) or can exhibit an inclination of 20° (this work, Fig. 7 *B*). Besides, basepairs can show different displacements from the helical axis than in previously reported CGCGCG structures (Fig. 7 *D*). All these changes of the Z-DNA structure can be seen in Fig. 6 *B*, where two of the pCGCGCG duplexes from this work are presented together with a Z-DNA model. However, it is not simple to visualize the variability of the helical twist for any step of Z-DNA because the difference between twist values of the CpG and GpC steps of the same molecule is always much bigger than twist variations of each individual step (Fig. 7 *A*). Therefore, we would like to emphasize the following. Although variations in the twist values of the GpC steps, for instance (Fig. 7 *A*), are smaller than the extremes found in some base steps in A- or B-DNA fragments, they are of the same magnitude as generally observed for most steps (Subirana and Faria, 1997; Gorin et al., 1995). In our case we find such variations in the same fragment of Z-DNA. Therefore, we conclude that the Z-DNA structure of this fragment varies depending on the crystal environment. It is often stated that Z-DNA helices display structural rigidity, but our work shows that

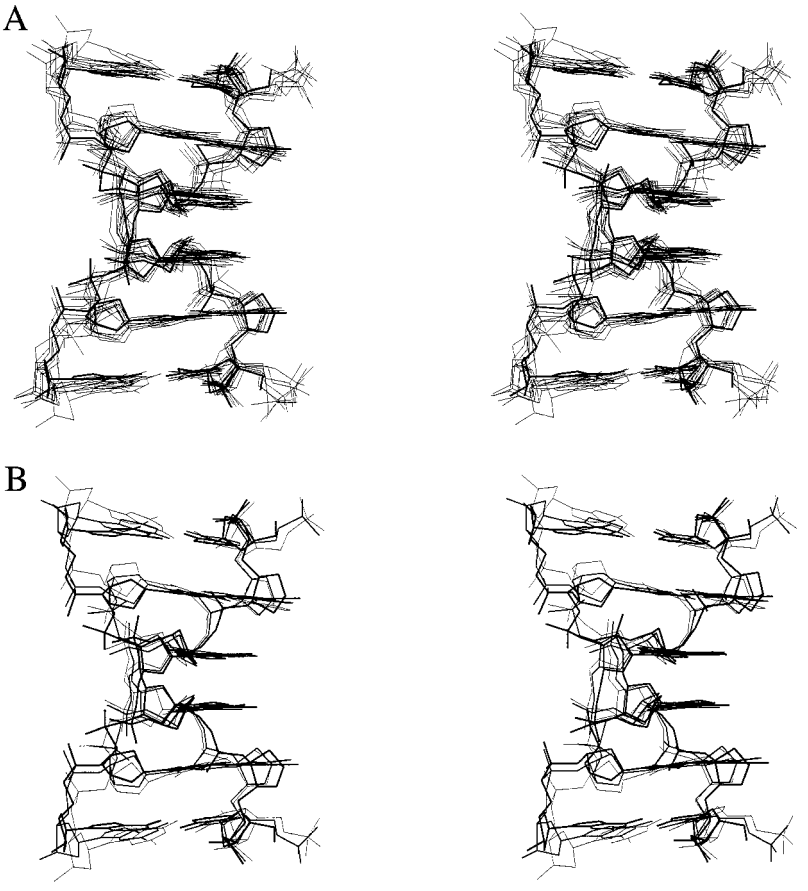

FIGURE 6 Stereo views showing a superposition of the pCGCGCG duplexes studied in this work (*thin lines*) with a Z1-DNA model of the same sequence (*thick lines*). The RMS atomic deviation upon superposition calculated for all atoms except for hydrogens varies from 0.724 to 1.178 Å when going from one duplex structure to another. (*A*) All duplexes from this work are presented. (*B*) Only the central and one flanking molecule from the orthorhombic crystal form of pCGCGCG are shown together with the Z1-DNA model.

this commonly held point of view should be changed: Z-DNA shows a range of variability similar to A- and B-DNAs.

As we stated above, the analysis of contacts between DNA molecules in a crystal might be important, since for understanding any biological process with a DNA participation we need to know the structure of a DNA/protein or a DNA/DNA complex. Sometimes a packing arrangement in DNA crystals gives a model for a DNA/DNA complex that could explain some features of the process. For instance, several models for the Holliday junction have been constructed on the bases of the crystal structure of some B-DNA fragments (reviewed by Goodsell et al., 1995). Another example of that kind is the model of possible DNA/DNA recombination complexes we developed on the basis of the recombinationlike structure of d(CCGCGG) (Malinina et al., 1994), and which might be useful for site-specific DNA recombination. In this model twinned B-DNA molecules have short central parts in the Z-conformation. These parts are oriented face-to-face and the exchange of strands between DNA molecules occurs at the flanking bases.

In this work we have analyzed the packing arrangements in two new crystal forms of Z-DNA and found face-to-face orientation of Z-DNA convex surfaces in both cases. A comparison of two molecules of one crystal form with the twinned duplexes of CCGCGG demonstrates the strong similarity in the mutual orientation of their convex surfaces and in the distance between two face-to-face duplexes. It means that such mutual orientation is quite natural for Z-DNA even if two duplexes do not exchange their strands as they do in the recombinationlike twin.

The presence of an unusual terminal basepair in the recombinationlike structure of CCGCGG causes the central tetramer to be distorted considerably from canonical Z-DNA (Malinina et al., 1994), with the main difference from the canonical form being a big inclination and small X-displacement of basepairs. Since in this work we also found such a big inclination/small X-displacement of basepairs in a standard DNA duplex, we conclude that such a structural variability is an intrinsic feature of Z-DNA. In other words, basepairs in Z-DNA can easily display the mean inclination of 20° with a mean X-displacement of 1–1.5 Å.

Since the biological function of Z-DNA is still unknown, the meaning of its structural features is not clear. However, it is natural to assume that the convex surface of Z-DNA exposes the bases so that they may interact with other molecules. Thus, two Z-DNA molecules can easily be placed at such a distance from each other that their face-to-face convex surfaces would interact through an ion bridge, as in the crystal of CCGCGG (Malinina et al., 1994). Another interaction that would be quite natural for Z-DNA is an interaction of its convex surface with phosphates of a neighboring molecule, as we have discovered in this work.

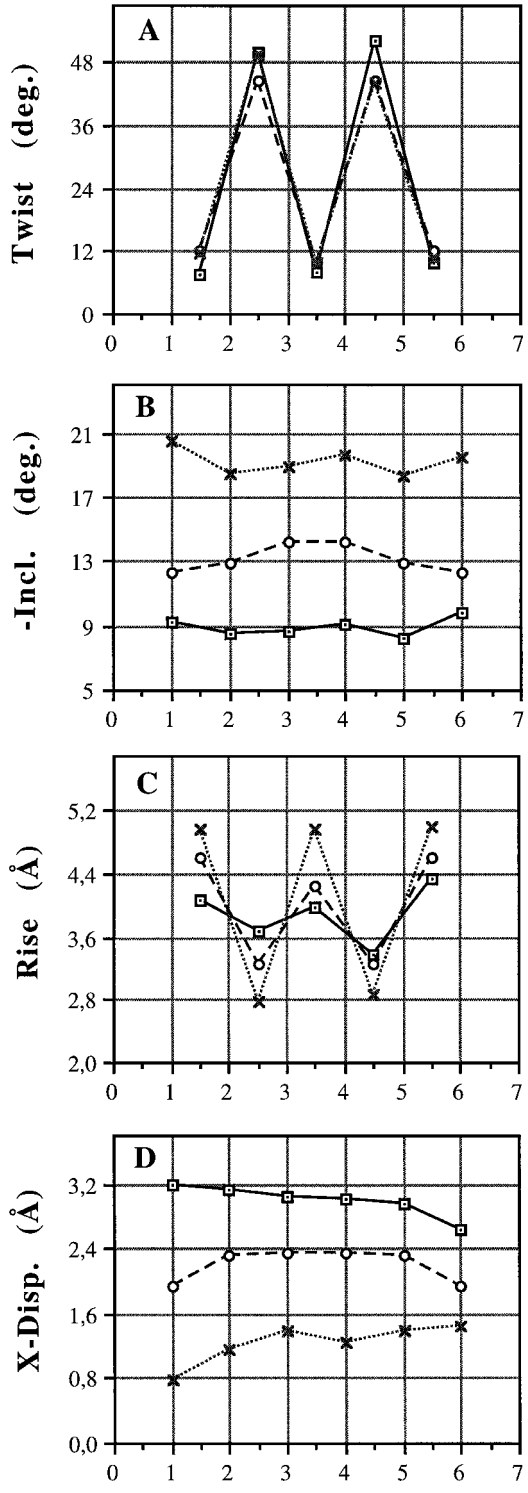

FIGURE 7 Helical twist (*A*), inclination of basepairs (*B*), rise (*C*), and X-displacement of basepairs from the helical axis (*D*) for the central (○) and flanking (×) duplexes of pCGCGCG in the orthorhombic crystal form #1. The values for another Z-DNA structure with the same sequence (⊡) are also shown (Wang et al., 1979; NDB entry ZDF001).

For the first time we observe examples of hydrogen bonds between the N4 amino group of cytosine and oxygen atoms of a phosphate group of a neighboring molecule of Z-DNA. Such hydrogen bonds determine specific interactions of a CpG step of one molecule with the sugar-phosphate backbone of a terminal CpG step of a neighboring Z-DNA molecule (Fig. 4). The absence of Mg^{2+} ions and the low values of the spermine/duplex ratio we used for crystallization of pCGCGCG may be instrumental in establishing such interactions. In the crystal structure of d[CGT(2-NH_2-A)CG] some of the bases in the convex surface of Z-DNA are also found to interact with the terminal basepair of neighbor molecules (Parkinson et al., 1995), although the phosphate groups are not involved in such interactions.

Finally, we would like to stress that our work shows that stacking interactions between neighbor duplexes can be quite variable (Fig. 7 and Table 2). The interactions found here are different among themselves and different from those present in standard Z-DNA crystals (Wang et al., 1979), in the d(CpG) dinucleotide that crystallizes with C/C stacking between neighbor molecules (Ramakrishnan and Viswamitra, 1988), and in a modified Z-DNA hexamer, which shows G/G stacking (Jean et al., 1993). These observations add weight to consider DNA as a very versatile molecule.

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