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The tetramer d(CpGpCpG) crystallizes as a left-handed double helix

(x-ray diffraction/sugar pucker/base stacking/conformational changes)

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ABSTRACT **The structure of the tetramer d(CpGpCpG) has been solved by x-ray analysis in two different crystal forms with and without spermine cations. The molecules crystallize in hexagonal unit cells and they form a left-handed double helix of Z-DNA similar to that previously reported for the hexamer d(CpGpCpGpCpG). In the crystal lattice the molecules stack together to form a virtually continuous left-handed double helix in which every fourth phosphate group is missing. The stacking of bases upon each other is similar to that seen in the hexamer. However, the base pairs have a slightly different orientation in that the cytosine residues are slightly removed from the axis of the molecule compared to the position they occupy in the hexamer. The structures are similar in two crystal forms with and without spermine cations.**

Most of our earlier knowledge of the organization of the DNA double helix came from fiber x-ray diffraction studies. These studies had the advantage that diffraction data are relatively easy to obtain but they had the substantial disadvantage that the data are limited in resolution and consequently the finer structural details are lost. It has been apparent for at least 7 years that fragments of nucleic acid double helices can be seen in a single crystal diffraction analysis which, in principle, can lead to atomic resolution data (1, 2). Our own initial efforts with DNA fragments yielded a small dideoxynucleotide monophosphate which formed a double helix organized around an intercalator (3). This stimulated us to try larger fragments of DNA. Over 2 years ago we decided to investigate all of the self-complementary DNA tetramers containing guanine and cytosine residues. All four of these were synthesized and three of them yielded crystals. One of the crystals, with the sequence d(CpGpCpG), diffracted quite well. A short time later, Drew *et al.* (4) reported that a number of different crystals of this tetramer could be obtained by varying the salt concentration.

After our initial attempts to solve this tetramer structure failed, we decided to extend the investigation to the hexamer d(CpGpCpGpCpG). It yielded crystals that diffracted to atomic resolution; solution of the structure revealed an unusual left-handed double helix which we termed Z-DNA, due to the zigag character of the sugar phosphate backbone (5). Recently, Drew *et al.* (6) solved the structure of the high-salt orthorhombic crystal form of the tetramer d(CpGpCpG) which forms a fragment of a similar left-handed double helix.

Here we report on the solution of two hexagonal crystal forms of the same tetramer which also crystallize as left-handed Z-DNA. Because of the difference in crystallization conditions, the orthorhombic and hexagonal forms of the tetramer differ slightly in the geometry of the molecule.

At the present time, seven different crystals containing fragments of left-handed Z-DNA have been solved, including both hexamers and tetramers (Table 1). In addition, a fiber diffraction pattern of poly(dG-dC) has yielded a pattern consistent with a polymeric version of Z-DNA (7). In all of the crystals we find some slight modifications in the basic geometry of the left-handed helix. These variations can be seen because of the relatively high resolution of the crystal diffraction data. This emphasizes the fact that there is configurational variability in the left-handed double helix, a variability which no doubt will eventually be found when right-handed double-helical DNA is studied by these same methods. A major biological feature of double-helical DNA involves its interactions with small molecules and ions as well as with proteins, and all of these are likely to induce variations in conformation. Many of these configurational variations are sequence-dependent and therefore reflect the informational content of these long molecules.

EXPERIMENTAL METHODS

The ammonium salt of the deoxy tetramer d(CpGpCpG), designated $d(CG)_2$, was prepared by modification of the recently developed phosphotriester method (8, 9). Two different crystal forms were obtained by using the vapor diffusion method with 5% isopropanol as the precipitating agent. Both crystal forms were obtained from solutions containing 30 mM sodium cacodylate buffer (pH 7.0), 15 mM $MgCl_2$, and 2 mM $d(CG)_2$. One solution also contained 10 mM spermine tetrachloride. The crystals grew in the form of hexagonal rods measuring up to 0.5 × 0.5 × 2.0 mm. They were mounted in sealed glass capillaries with a droplet of mother liquor, and three-dimensional data were collected to a resolution of 1.3 Å by using a Picker diffractometer, although the data beyond 1.5 Å were only weakly represented. Crystallographic data for the spermine and nonspermine crystal forms are given in Table 1 together with the other known crystal forms of Z-DNA.

Our initial survey of the diffraction patterns suggested that the space group was $P6_222$ (or 6_422). However, further analysis suggested that the reflection 003 is actually a Renninger or ghost reflection due to multiple scattering rather than a true reflection. Recognition of this suggested a space group of $P6_522$ (or $P6_122$). Later we recognized that the 2-fold symmetry of the diffraction pattern is only approximate and that the real symmetry was $P6_5$ (or $P6_1$). The R value $(\Sigma|I - \langle I\rangle|/\Sigma\langle I\rangle)$ between pseudo-symmetry related reflections was 9.07% for the spermine form and 11.86% for the nonspermine form. The R value relating the two different crystal data sets is 17.65%.

When the data were collected more than 2 years ago we thought that this molecule might have one of the known right-handed DNA forms. We tried to solve the structure by using the method of molecular replacement and assuming a number of search molecules (A-DNA, B-DNA, C-DNA, D-DNA). Finally we became convinced that the method was not

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Abbreviation: $d(CG)_2$, d(CpGpCpG).

Table 1. Crystals of Z-DNA

Oligomer	Crystallization conditions	Space group	*a*, Å	*b*, Å	*c*, Å	Volume/ base pair, Å^3	Resolution, Å	R value	Ref.
$d(CG)_3$	10 mM $MgCl_2$ 7 mM spermine Cl_4	$P2_12_12_1$	17.88	31.55	44.58	1047	0.9	0.13	5
$d(CG)_3$	10 mM $MgCl_2$ 7 mM spermidine Cl_4	$P2_12_12_1$	17.96	31.19	44.73	1044	1.3	0.17	10
$d(CG)_3$	10 mM $BaCl_2$ 7 mM spermine Cl_4	$P2_12_12_1$	17.94	31.54	44.68	1053	1.1	0.18	10
$d(CG)_3$	10 mM $MgCl_2$	$P2_12_12_1$	17.98	30.94	44.81	1039	1.8	0.13	10
$d(CG)_2$	0.2 mM $MgCl_2$	$C222_1$	19.50	31.27	64.67	1232	1.5	0.20	6
$d(CG)_2$	15 mM $MgCl_2$ 10 mM spermine Cl_4	$P6_5$	31.25	31.25	44.06	1035	1.5	0.19	This work
$d(CG)_2$	15 mM $MgCl_2$	$P6_5$	30.92	30.92	43.29	996	1.5	0.21	This work

working or that somehow the structure was quite different from what we had anticipated. Accordingly, we concentrated on the crystals of the hexamer $d(CG)_3$, for which the structure was eventually solved by using the method of multiple isomorphous replacement (5). We had attempted to use this method with the tetramer but were unable to get derivatives. Solution of the hexamer's strikingly unusual structure and the clear similarity of the cell dimensions between the tetramer and hexamer suggested that we should again attempt the method of molecular replacement using a trial model of left-handed Z-DNA.

In carrying out the molecular replacement search, three independent variables had to be used. The hexagonal lattice has sites with 6-fold and 3-fold screw symmetry (11). Thus, we had to vary the relative angular rotation of the two types of molecules in the 3-fold and 6-fold screw symmetry sites as well as the translation of one of these molecules relative to the other along the Z axis. A replacement or R search for the spermine form was carried out using 2.5-Å data in two stages. First, the R value was calculated for only the molecules at the 3-fold screw symmetry sites. The R value showed a minimum of 41% as the model was rotated. These molecules were fixed at the positions indicated by the minimum, and the molecules at the 6-fold screw symmetry sites were rotated and translated. The R value again showed one distinct minimum, at 36%. This provided us with a starting model for refinement by using the Konnert–Hendrickson refinement procedures (12). Solvent molecules were introduced from difference Fourier and sum function Fourier ($2F_O - F_C$) maps. At the present time the spermine form has been refined to an R value of 19.3% at 1.5 Å. In addition to the three chains of the tetramer the asymmetric unit also contains one spermine molecule and 85 water molecules. The refined model of the spermine crystal was used as the starting model for the nonspermine form. The data for the nonspermine crystal have been refined to an R value of 21.0% at 1.5 Å, and the unit cell contains 79 water molecules. At this level of refinement no additional anions have been identified, and no cations have been seen.

Fig. 1 shows a projection down the *c* axis, comparing the tetramer and hexamer crystal; heavy lines delineate the two unit cells. There are two types of molecules in the hexagonal unit cell: those at the corners of the diamond-shaped figure, and those that are internal. The molecules at the corners are required to have 6-fold screw axes and the molecules inside have 3-fold screw axes. The *c* axis of 44.6 Å is such that it will just accomodate a helix with 12 base pairs per turn, as observed in the hexamer crystal (5). Thus, the symmetry of the internal molecules with 3-fold screw axes matched precisely what one would anticipate with three segments of tetramer forming a left-handed helix with 12 base pairs per turn of the helix. The molecules at the corners with 6-fold screw axes presented a slight problem. The asymmetric unit for these molecules ideally would not be a tetramer but rather would be a dimer repeated

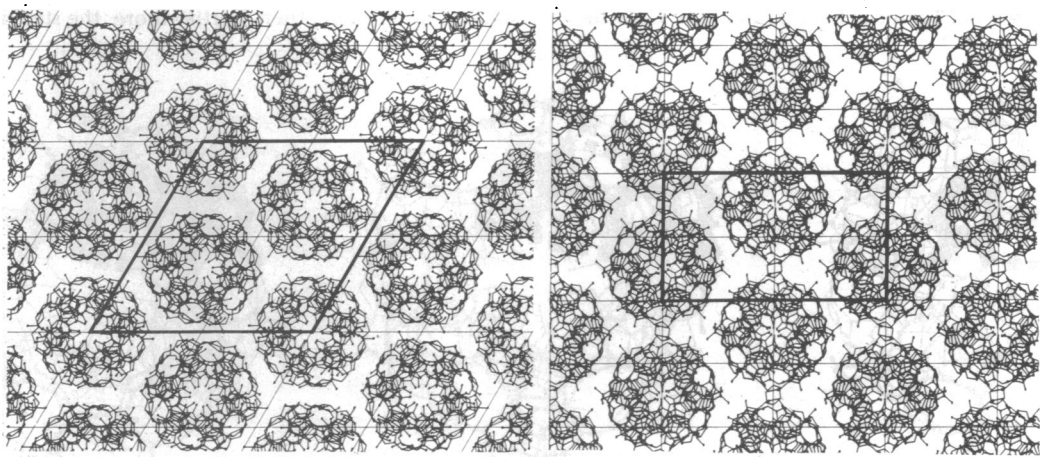

FIG. 1. Diagram of molecular packing of Z-DNA molecules as viewed down the helix axis. (*Left*) Packing of the hexamer $d(CG)_3$ is shown in the outlined orthorhombic unit cell. (*Right*) Closely related packing of the tetramer $d(CG)_2$ is shown in a hexagonal unit cell. The horizontal axes in both the orthorhombic and hexagonal unit cells are close to 31.5 Å. The hexagonal unit cell is somewhat larger than the orthorhombic and it can been seen to contain two different kinds of molecules: those found at the corners of the diamond-shaped unit cell and the two that are internal. Those molecules found at the corners of the unit cell have 6-fold screw axis symmetry whereas the internal molecules have 3-fold screw axis symmetry. The molecules in the orthorhombic hexamer crystal have 2-fold screw symmetry. Only the oligonucleotides are plotted; spermine ions and solvent molecules are omitted. It is interesting to note that all of the molecules in both lattices are completely sheathed in water, with no direct contacts between the oligonucleotides. The apparent contact between the hexamer nucleotides does not actually exist because the two parts of the molecule that appear to be in contact are actually separated by half the unit cell.

around a 6-fold screw axis in order to make a helix with 12 base pairs per turn. Because the molecules in the crystals are tetramers rather than dimers, this analysis suggested that the molecules on the 6-fold sites have a two-position disorder along the *c* axis.

In the internal 3-fold screw sites, the four tetramer molecules form a complete turn of the helix. However, the tetramer is a tetranucleoside triphosphate; every fourth phosphate group is missing. In the 6-fold symmetry sites it is as if every other phosphate is missing, rather than every fourth phosphate. The molecules on the 6-fold sites are organized so that the absent phosphate is partially found in two different sites. The half-occupancy phosphate is not clearly visualized and therefore its geometry is not discussed. The tetramer contains two sequences of CpG and one of GpC; the half-weight phosphate is of the GpC type. Overall, solution of the structure reveals two similar but distinct types of helical molecules: those made up of complete tetramers inside the unit cell and those containing dinucleotides of CpG connected by half-weight phosphates located at the corners of the unit cell (Fig. 1).

RESULTS

Fig. 2 shows a stereo side view of the packing of four duplex molecules of the tetramer along the 3-fold screw axis. This view of the molecule is similar to the stereo view of the hexamer (5)—i.e., a left-handed helix with a zigzag organization of the ribose phosphate backbone. The molecules are stacked along the *c* axis in such a manner that there appears to be a virtually continuous double helix with every fourth phosphate group missing. As with the hexamer, there is one deep groove in the molecule which is analogous to the minor groove of B-DNA (5). The guanine residues are in the *syn* conformation; cytosine is *anti*. The G·C base pairs are located on the outer wall of the molecule, and what corresponded to the concave major groove of B-DNA now forms a convex outer surface of the left-handed Z-DNA.

We have examined the overlapping or stacking of bases in this helix and the results are virtually identical to those reported for the hexamer (5). There are two types of base stacking: the stacking of CpG and of GpC sequences. As in Z-DNA, the CpG sequences are sheared so that the C residues of one base pair are stacked over the C residues of the adjoining pair, whereas the G residues are stacked over the O1′ of the deoxyribose ring. There is little rotation between the base pairs in the CpG sequence. In the GpC sequences, on the other hand, there is a significant rotation—nearly −60°—and the base pairs are stacked upon each other. Furthermore, the stacking of mole-

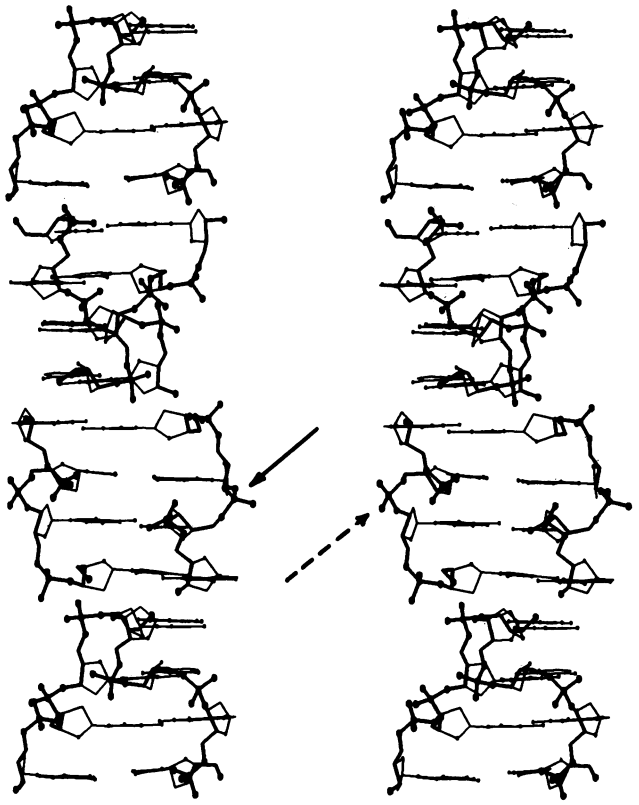

FIG. 2. Stereodiagram showing three units of the tetramer $d(CG)_2$ as they are found stacked along the *c* axis of the crystal. They form a continuous double helix in which every fourth phosphate group is missing. Careful inspection of the central GpC phosphates reveals that the phosphate group is found in different orientations on the two sides of the helix. One phosphate (solid arrow) is rotated toward the groove in the helix, and the other phosphate (broken arrow) is rotated away from the groove. These two conformations have been called Z_I- and Z_{II}-DNA, respectively (10). The organization of successive molecules in the crystal lattice is quite regular, and the stacking of the end bases is the same as that found in the center of the molecules. The stereodiagram shown here is that found for the molecules sitting on the 3-fold screw axes inside the unit cell.

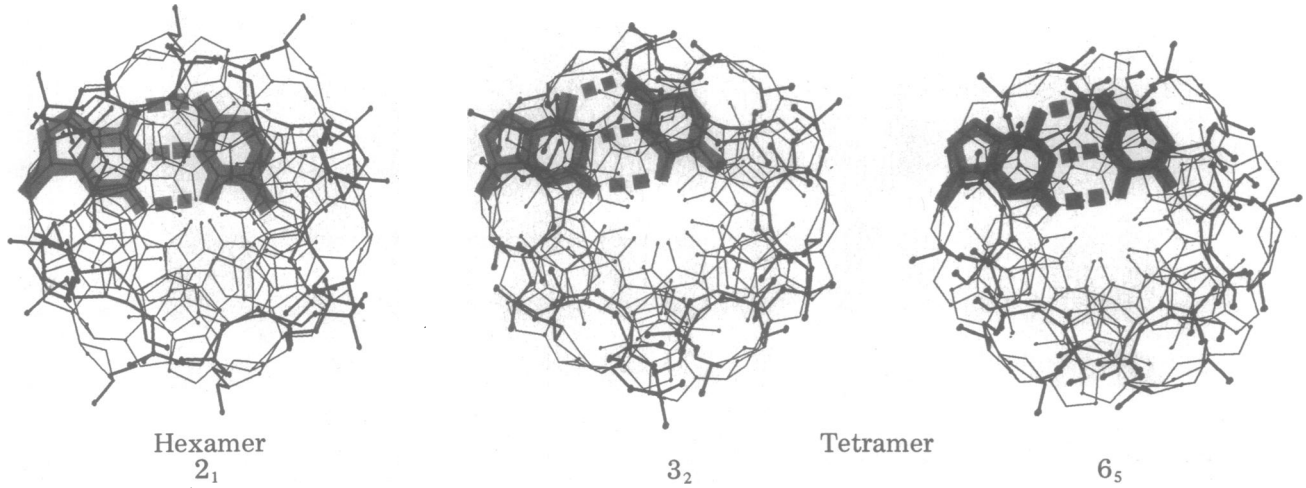

FIG. 3. A view down the helix axis comparing the hexamer, which has a 2-fold screw axis (2_1) and the two different types of tetramers found on the 3-fold (3_2) and 6-fold (6_5) screw axes. The molecules are quite similar to each other. One C·G base pair has been emphasized by shading to facilitate comparison of the structures. The three structures are similar to each other but the cytosines of the tetramers are located somewhat further away from the center than in the hexamer crystal. This accounts for the somewhat larger hole in the center of those molecules than in the hexamer.

cules along the *c* axis reveals the same base stacking as that found inside the molecule. It is interesting that the orthorhombic tetramer crystal does not use this same end-to-end stacking (6). These differences appear to be associated with different ions found in the two types of crystals.

For comparative purposes, Fig. 3 shows the end view of the molecules found in the tetramer 3-fold and 6-fold screw positions together with the spermine hexamer crystal. One G·C base pair is emphasized by shading in each of these lattices. There is overall similarity between the hexamer and tetramer molecules even though there are also some interesting differences. Both of these crystals contain 12 base pairs per turn of the helix, and they both have diameters near 18 Å, in contrast to the slightly larger diameter (20 Å) found in B-DNA. There are minor differences in the projection of the molecules in the 3-fold and 6-fold positions but the similarities between them are much more striking than the minor differences. The differences arise from the fact that these are independent molecules—i.e., they are not constrained to be identical by any lattice symmetry. What is remarkable about these helical molecules is the extent to which they are similar to each other, even though each of the strands is independent. This is a consequence of the regularity of their internal interactions rather than being superimposed by lattice constraints. There are some differences between the Z-DNA in the tetramer and the Z-DNA in the hexamer. The imidazole ring of guanine is found in the periphery of the molecule in both cases but the cytosine residues in the hexamer crystals are located closer to the center of the axis than is the case of the cytosine residues in the tetramer crystals. These are located slightly away from the axis and are responsible for producing the larger hole seen there. However, these differences are not associated with significant differences in the stacking of the bases as mentioned above.

Fig. 4 shows two van der Waals side views of the packing of the tetramer along the *c* axis. A heavy line goes from phosphate to phosphate to facilitate following the backbone. The similarity of these views with those published for the hexamer is quite striking (5). The zigzag array of phosphate groups form the edge of the groove. The groove actually extends to the axis of the molecule.

The results from the spermine-free crystal are similar to those presented for the spermine form of the crystal. The spermine-free crystals diffracted to approximately 1.4 Å resolution instead of 1.3 Å. Furthermore, the *c* axis is 0.8 Å shorter in the crystal without spermine; this is associated with a slightly tighter packing of the bases in the entire structure along the *c* axis. Thus, the molecules appear to pack somewhat more tightly in the absence of spermine. This change in the *c* axis is also associated with a slight modification of the solvent packing.

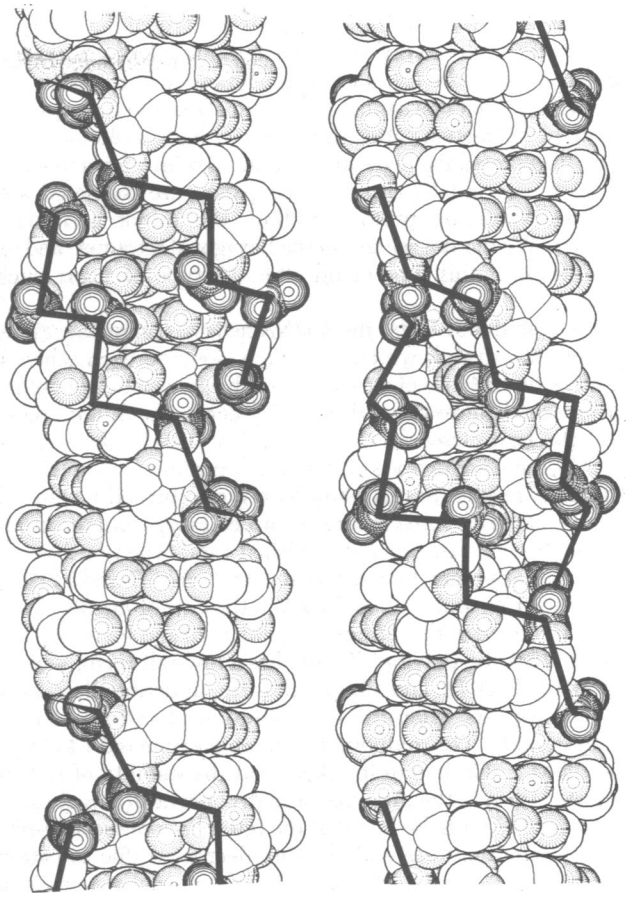

FIG. 4. van der Waals diagram showing two views of the packing of the tetramer $d(CG)_2$ in the crystal lattice. The molecules are organized in the form of a regular helix. A heavy black line drawn from phosphate to phosphate illustrates the zigzag conformation of the backbone. The line goes from phosphorus atom to phosphorus atom except for those places between molecules where it goes to the positions the phosphorus atoms would occupy if it were a continuous double helix. In the view on the left, part of the depth of the groove can be seen. The groove actually extends to the axis of the molecule. The convex outer portion of the molecule consists largely of what would correspond to the major groove portion of the guanine and cytosine base pairs in B-DNA. Hydrogen atoms are not included in the diagram, and the two views are drawn 90° apart.

DISCUSSION

A detailed analysis of the hexamer $d(CG)_3$ structures revealed that two different positions were found for the phosphate group in the GpC sequence (10). The phosphates of most GpC sequences were oriented so that they faced the groove, but in some cases the phosphate of GpC was rotated away from the helical groove so as to enhance the zigzag appearance of the backbone. This rotation was frequently associated with the presence of a magnesium ion coordinated to the imidazole N7 of a neighboring guanine. When the phosphate group was rotated away from the groove, one of its oxygens formed a tight hydrogen bond to one of the water molecules in the magnesium hydration shell. These two conformations have been called Z_I and Z_{II}, respectively; Z_{II} is the conformation rotated away from the groove. In the four reported crystal structures of $d(CG)_3$, all of them have some representation of Z_{II} although the majority of the GpC conformations were of the Z_I type (10). In the present structure two GpC sequences are found, both associated with the helices at the 3-fold screw positions. The helices in the 6-fold screw positions have effectively lost the phosphate in GpC due to the disorder. In the 3-fold screw position, we find one phosphate rotated toward the center of the groove in the Z_I position and the other rotated outward away from this in the Z_{II} position (Fig. 2). In the high-salt orthorhombic crystal structure of $d(CG)_2$, two GpC conformations are seen and they appear to be similar to the Z_I and Z_{II} orientations (6). The same situation seems to be found in the spermine-free crystal form of the tetramer. Thus, it appears that both Z_I and Z_{II} conformations of GpC phosphate groups may be found in all seven of the crystal structures reported.

In the hexamer, the cytosines had the C2′-*endo* sugar ring pucker which is normally found in B-DNA, whereas the guanine residues had the less common C3′-*endo* pucker. In the tetramer crystals, differences in pucker were found although we are less certain of the details. The hexamer crystal structure analysis was highly refined with a resolution of 0.9 Å; thus every atom was visualized. In the tetramer structure, only the 1.5-Å data have been used and the level of refinement is not nearly as great. Thus, we cannot be as certain about the pucker; however, it appears to us that the cytosine residues are of the

C2′-*endo* class whereas the guanine residues are of the C3′-*endo* class. In the high-salt orthorhombic tetramer all of the residues appear to be in the C2′-*endo* class; however, it has been emphasized (6) that one cannot be certain about the sugar pucker when the diffraction pattern is not of very high resolution. Drew *et al.* interpreted the high-salt tetramer to be in a form in which chloride ions, usually at partial occupancy, are associated with hydrogen-bonding protons in both the minor and major grooves. We did not see this in the hexagonal tetramer, and ion complexes of this type could alter the type of sugar puckering.

Compared to B-DNA, the Z-DNA helix has phosphate groups in opposite strands which are much closer to each other. In B-DNA the distance of closest approach between two phosphate groups on different strands is 11.5 Å across the minor groove, and the next closest distance is 12.7 Å (13). In the hexamer $d(CG)_3$ there are two sets of distances, depending upon whether the GpC residue has its phosphate group turned toward the groove in the Z_I conformation or turned away from the groove in the Z_{II} conformation (unpublished data). The two distances of closest approach between two strands are 7.7 and 11.6 Å in the case of Z_I, and 8.6 and 13.5 Å in the case of Z_{II}. Similar results are found in the tetramer crystal. Pohl and Jovin (14) have shown that the polymer dG-dC has a low-salt form, which is probably B-DNA, and a high-salt form, undoubtedly Z-DNA, which appears when the concentration of cations increases considerably. The midpoint for the conversion is 0.7 M $MgCl_2$ or 2.5 M NaCl. It is quite likely that the stability of the left-handed form in the presence of high concentrations of cations is associated with the fact that the interchain phosphate-phosphate repulsions are considerably decreased due to cationic screening. At low ionic strength it is the phosphate-phosphate repulsion that stabilizes the molecule in the right-handed B-DNA form. Alterations in the hydration of the molecule may play a significant role in this conversion (6); however, it is difficult to evaluate the role of hydration until we know more about the organization of water molecules around both Z-DNA and B-DNA molecules. At present we know a great deal about the manner in which water molecules are organized around Z-DNA molecules, and these results will be presented elsewhere. However, nothing is known about the organization of water molecules around B-DNA and this information will not be forthcoming until we have atomic resolution single crystals of DNA in the right-handed B conformation.

One of the remarkable features of the tetramer and hexamer crystals is that they all were obtained from low-salt solutions. The total concentration of ions in the crystallization mixture was such that the stable form of the alternating dG-dC copolymer was right-handed B-DNA; nonetheless, the crystals that emerged all were the left-handed Z-DNA. This is undoubtedly associated with the fact that an equilibrium must exist between right-handed and left-handed molecules in solution. It is likely that more stable packing is found for the formation of crystals involving the left-handed Z-DNA and, as these crystals grow, the equilibrium shifts until eventually all molecules are converted to the left-handed form even though the solution conditions favored the right-handed form. The difference in crystallization may reflect the fact that the Z-DNA helix appears to be more rigid than B-DNA and therefore small fragments of Z-DNA are more readily crystallizable than the more flexible fragments of B-DNA.

In the hexamer crystal all of the cations could be seen, including magnesium and spermine ions (5). In the tetramer crystals we can see one sperimine ion, but no other cation is identifiable. The spermine ion is organized in the groove of one molecule where it can interact with phosphate groups and water molecules. Failure to see the other cations is undoubtedly related to the fact that the data are not at atomic resolution but are only useful down to a limit of 1.5 Å. In our attempts to solve the structure of this molecule we tried to soak in heavy atom cations, but none of them produced systematic changes in the diffraction pattern. This may reflect the fact that the cations are somewhat disordered in the structure. Part of this may be associated with the disorder found around the 6-fold screw axis positions, but it may also be associated with the fact that the crystals do not pack as well with the tetramer as with the hexamer.

Seven different crystal structures of left-handed Z-DNA have now been solved containing either hexamers or tetramers of alternating deoxyguanosine and deoxycytidine residues. In these structures the polynucleotide chains are crystallographically independent. Therefore, we now have a great deal of information about the manner in which these sequences form left-handed helical molecules. It is interesting that small variations in conformations are seen in all of these structures, which suggests that there is a family of left-handed DNA molecules. Furthermore, the particular conformation appears to reflect differences due to the presence of cations such as magnesium or spermine or, in the biological system, perhaps the presence of positive charges on protein molecules. Because these conformational changes are sequence-dependent, this is another mode for expressing the biological information that is present in the nucleotide sequence of the double helix. These structural details thus provide a window into the manner in which nucleic acids may function in biological systems.

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1. Rosenberg, J. M., Seeman, N. C., Kim, J. J. P., Suddath, F. L., Nichols, H. B. & Rich, A. (1973) *Nature (London)* **243**, 150–154.
2. Day, R. O., Seeman, N. C., Rosenberg, J. M. & Rich, A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 849–853.
3. Wang, A. H.-J., Nathans, J., van der Marel, G., van Boom, J. H. & Rich, A. (1978) *Nature (London)* **276**, 471–474.
4. Drew, H. R., Dickerson, R. G. & Itakura, K. (1978) *J. Mol. Biol.* **125**, 535–543.
5. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979) *Nature (London)* **282**, 680–686.
6. Drew, H. R., Takano, T., Tanaka, S., Itakura, A. & Dickerson, R. E., *Nature (London)* in press.
7. Arnott, S., Chandrasekaran, R., Birdsall, P. L., Leslie, A. G. W. & Ratliff, R. L. (1980) *Nature (London)* **283**, 743–745.
8. de Rooij, J. F. M. (1979) Dissertation (Leiden University, Leiden, The Netherlands).
9. Arentzen, R., van Boeckel, C. A. A., van der Marel, G. & van Boom, J. H. (1979) *Synthesis* **137**, p. 137.
10. Wang, A. H. J., Quigley, G. J., Kolpak, F. J., van der Marel, G., van Boom, J. H. & Rich, A., *Science*, in press.
11. Henry, N. F. M. & Lonsdale, K., eds. (1962) *International Tables for X-Ray Crystallography* (Hynoch, Birmingham, England), 3rd ed., Vol. 3, p. 277.
12. Hendrickson, W. A. & Konnert, J. (1979) in *Biomolecular Structure, Conformation, Function and Evolution*, ed. Srinivasan, R. (Pergammon, Oxford), Vol. 1, pp. 43–57.
13. Arnott, S., Smith, P. J. C. & Chandrasekaran, R. (1976) in *CRC Handbook of Biochemistry and Molecular Biology*, ed. Fasman, G. D. (CRC, Cleveland, OH), Vol. 2.
14. Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375–396.