The Molecular Structure of the Left-handed Z-DNA Double Helix at 1.0-Å Atomic Resolution

GEOMETRY, CONFORMATION, AND IONIC INTERACTIONS OF d(CGCGCG)*

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The idea of DNA polymorphism is now a generally accepted phenomenon. What was once thought to be a stable and rather uniform molecule has been shown to exist in many different conformations. To date the most extreme differences are seen in Z-DNA. This was originally demonstrated from a single crystal x-ray diffraction study of the hexanucleoside pentaphosphate d(CpGpCpGpCpG) (abbreviated d(CGGCG)G or simply d(CGCGG)) (1). This crystal form, with both magnesium and spermine as cations, diffracted to 0.9-Å resolution. That structure and the subsequent studies dealing with macromolecular DNA showed that DNA can undergo a reversible conformational change between the right-handed B-DNA form and left-handed Z-DNA. As reviewed elsewhere (2), this conformational equilibrium is influenced by negative supercoiling, changes in the ionic environment, and the presence of Z-DNA interacting proteins. Since the original structures of the hexamer d(CGGCGG) (1) and the tetramers d(CCGG) (3, 4), oligonucleotides have been crystallized in the Z-DNA conformation which include fragments containing AT base pairs (5–7), nonalternating purine-pyrimidine sequences (8), and wobble guanine-thymine base pairs (9, 10). In addition, the specific interactions of the helix with transition metal ion complexes have also been investigated at atomic resolution (11, 12).

We also discovered that the d(CGGCGG) DNA hexamer can be crystallized purely in the presence of magnesium chloride and cacodylate buffer. Like the spermine form of d(CGGCGG), the crystals of this second form also diffract x-rays to better than 1.0-Å resolution. This high resolution crystal structure provides us with the rare opportunity to examine fine details of the geometry and conformation of a macromolecular nucleic acid fragment. In both structures, a hexamer double helix is found in the asymmetric subunit, and therefore each of the 6 base pairs is crystallographically distinct. Up to the present time, no other nucleic acid double helix longer than 2 base pairs has been studied at such high resolution. In the present comparison, we discuss both d(CGGCGG) crystal structures in order to describe fully the manner in which these molecules interact with solvent and positively charged cations. We also consider how the detailed geometry of these structures compares to what is generally known about the geometry of DNA.

EXPERIMENTAL PROCEDURES

In this study of the detailed geometry and conformation of the DNA hexamer d(CGGCGG), the two forms will be referred to as the spermine and magnesium forms, respectively. The three dimensional x-ray diffraction data for the spermine form of the crystal of d(CGGCGG) were collected and analyzed as described previously (1). In this crystal form, 240 nonhydrogen atoms of the DNA hexamer molecule were located in the asymmetric unit of the crystal lattice in addition to two spermine ions, one hydrated magnesium ion, and 68 solvent water molecules.

Initially the structure was refined with isotropic temperature factors using the Konnert-Hendrickson constrained least squares procedure (13) to a residual factor (R factor) of 14%. This was followed by a full matrix least squares individual atom refinement (14) using anisotropic temperature factors. The positions of all 240 atoms in the DNA molecule and virtually all of the solvent molecules were well refined, resulting in reasonable anisotropic thermal factors. The same was true of only one of the two spermine molecules in the lattice,
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suggesting that there may be some disorder associated with the second spermine molecule. Three complete cycles of full matrix refinement at 0.9 Å were performed in this analysis, some results of which are represented in Fig. 1. The details of the molecular motions associated with this structure have been analyzed previously (15).

The magnesium form of the same hexamer was crystallized out of a solution containing 1.4 mM DNA (single strand concentration), 20 mM sodium cacodylate, pH 7.0, and 120 mM MgCl₂, using vapor diffusion against a reservoir of 10% 2-methyl-2,4-pentanediol. Two crystals of approximately 0.7 × 0.3 × 0.3 mm were used for the data collection on a Nicolet P3 diffractometer using the ω scan method. The two data sets ranging from 10 to 1.2 Å (8,319 reflections) and from 1.5 to 1.0 Å resolution (9,505 reflections) were scaled together by minimizing the root mean square deviations between all \( F_o > 2\sigma(F_o) \) in the overlap region (1.2-1.5 Å). Using the 2,400 overlapping \( F_o > 2\sigma(F_o) \) reflections, an R value of 13.5% was obtained between the two data sets in this high resolution range. Since the crystals were isomorphic with those of the spermine form (space group P2₁2₁2₁, cell dimensions \( a = 18.01 \) Å, \( b = 31.03 \) Å, \( c = 44.80 \) Å) the atomic coordinates of the spermine form were used as a starting model for the initial low resolution refinement.

Eventually, all 10,893 unique reflections with \( F_o > 2\sigma(F_o) \) were included for the high resolution structure refinement. Eighty-eight solvent molecules were located during the course of this iterative refinement. The regular octahedral arrangement of some solvent molecules with intermolecular distances in the range of 2.0 Å (instead of 2.8 Å as in regular hydrogen bonds between water molecules) led to the identification of four magnesium hexahydrate complexes. These ion complexes as well as those water molecules which are associated with the first hydration shell of the DNA are in general well refined. However, they still do not account for all the counterions required to neutralize the negative charges of the 10 phosphate groups. It is possible that the missing cation is delocalized within the less ordered solvent channel, a region which is more distant from the DNA and may thus contain multiple possible water structures. Altogether 275 cycles of refinement were computed with increasing relaxation of the constraints to yield a final R factor of 17.5%. The final root mean square deviation of bond lengths from their ideal values was less than 0.04 Å. The final atomic coordinates of both crystal forms have been deposited in the Brookhaven Protein Databank.

RESULTS

Overall View—A representative portion of the final \( 2F_o - F_c \) electron density map of the magnesium form of d(CCGCGG) is shown in Fig. 2. This fraction of the map contains the last base pair of one hexamer and the first one of the next molecule along the c axis. These two base pairs exhibit the same stacking as the GpC dinucleotide repeats within one molecule; the only difference is the missing phosphodiester linkage. The generation of a continuous helix is made possible for two reasons. First the helix axis coincides with a crystallographic 2-fold screw axis parallel to \( c = 0.75, b = 0.5, a = 0.75 \) and second the helical twist for one dinucleotide repeat in Z-DNA is \(-60°\), leading to the required \(-180°\) hexanucleotide. This packing imitates a DNA molecule of almost infinite length extending along the c axis throughout the crystal, and thus end effects are almost negligible when

![Fig. 1. Stereo thermal ellipsoid plot of the anisotropically refined spermine form of d(CCGCGG) using the program ORTEP (35). The ellipsoids represent a 50% probability. Two DNA hexamers are displayed stacked upon each other in an end-to-end fashion as they occur in the crystal lattice, displaying one full helical turn. The numbering scheme for the individual bases is shown at the right.](image)
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FIG. 2. Stereo projection of a $2F_o - F_i$ electron density map. Map ranges from 37.33 Å (3/12 c) to 41.07 Å (11/12 c). Half-unit length along both the a axis (vertical) and the b axis (horizontal) are indicated with solid lines. The refined model in the center of the map is drawn with thick solid bonds and the symmetry-related molecules with thinner lines. The nucleotide sequence number for the main molecule is indicated in the diagram. In addition, two hydrated magnesium ions are indicated as C and D. The coordinative bonds of these hexahydrated complexes are also shown as solid lines. The letter W, within electron density contours, indicates the refined position of water molecules. (Since the model is drawn further than the electron density map, the outermost parts seem not to be fully enclosed in electron density.)

FIG. 3. Stereo van der Waals diagram of the spermine form of d(CGCGCG). Two DNA molecules are shown in the same orientation as in Fig. 1. The deep groove is shaded. The positions of the phosphorous atoms (including those which are missing at the end of the hexamers) are connected by a black zigzag line, emphasizing the dinucleotide repeat in the backbone of Z-DNA. Carbon atoms are displayed with concentric circles, nitrogen and phosphorous atoms are stippled, and oxygen atoms are drawn with broken lines. The blank hydrogens atoms have been generated by assuming standard conformations.

compared to other forms of crystal packing. In fact, the missing phosphate between the O-3' of guanosine G-6 and the O-5' of cytidine C-1 is even mimicked by a water molecule connecting both hydroxyl groups via hydrogen bonds. On the opposite strand a similar hydrogen bonding is not possible since the terminal 3'-OH of guanosine G-12 is directly hydrogen-bonded to the nearby phosphate group (P-12) of a symmetry-related parallel DNA molecule. The ribose pucker for this terminal deoxyguanosine residue is C-2'-endo instead of C-3'-endo as seen for the internal dG residues (see Table III). In this plot (Fig. 2) part of the ionic structure around the DNA is also seen, showing the highly regular coordination geometry of some magnesium ion complexes. The ion complexes clustered around the DNA also form hydrogen bonds to symmetry-related DNA molecules, thereby stabilizing the lattice. Furthermore, the typical hydrogen bonding of a row of water molecules within the deep groove connecting the O-2 keto groups of successive cytosine residues is clearly
visible. The details of the solvent arrangement in the different Z-DNA crystal structures will be described elsewhere.

Fig. 3 is a stereo van der Waals diagram of the spermine form of the d(CGCGCG) molecule with the deep helical (minor) groove shaded. As described previously (1), the residues along one chain alternate with all cytidines in the anti conformation and all guanosines in the syn conformation leading to a dinucleotide repeat in the helix and a zigzag appearance of the backbone and hence the name Z-DNA. As we suggested earlier (8), Z-DNA may be defined in terms of this alternation of syn and anti conformations. This type of representation also emphasizes the elongated shape of Z-DNA and the flatness of what formally constitutes its major groove. On the whole, the molecule has the appearance of a long cylinder with the outer walls formed by the G-C base pairs and with the imidazole rings of guanine protruding onto this outer surface.

Bond Angles and Distances—As a result of the high resolution at which these structures have been determined, it is possible to calculate accurate interatomic distances and angles for comparison with data obtained from small molecule crystal structures. Fig. 4 summarizes the averages of the bond distances and angles for the G-C base pairs, including the three hydrogen bonds between the bases. Table I lists all the individual hydrogen bond lengths and bond angles of the 6 base pairs as well as their mean values and the corresponding standard deviations (σ). Of the three hydrogen bonds involved in a Watson-Crick G-C base pair, two outer ones are between an amino and a keto group, while the inner one is formed between two imino groups. In an unconstrained base pair one

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Table I

<table>
<thead>
<tr>
<th>Angle between the N-glycosyl bond and the C-1'-C-1' axis of the magnesium and the spermine (Sp) form.</th>
</tr>
</thead>
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<tr>
<td><strong>Angle</strong></td>
</tr>
<tr>
<td>Asx - G-N9</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Asx - C-N1</td>
</tr>
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<td></td>
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Table II

**Bond lengths of the magnesium and the spermine (Sp) forms**

<table>
<thead>
<tr>
<th>Bond</th>
<th>Form</th>
<th>C7-C8</th>
<th>C8-C9</th>
<th>C9-C10</th>
<th>C10-C11</th>
<th>C11-C12</th>
<th>Mean</th>
<th>σ</th>
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<tr>
<td>C7-C8</td>
<td>Mg</td>
<td>1.24</td>
<td>1.26</td>
<td>1.24</td>
<td>1.26</td>
<td>1.26</td>
<td>1.26</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>Sp</td>
<td>1.24</td>
<td>1.26</td>
<td>1.24</td>
<td>1.26</td>
<td>1.26</td>
<td>1.26</td>
<td>1.26</td>
</tr>
</tbody>
</table>

**Backbone Conformation**—The conformation of the polynucleotide backbone can be described by the torsion angles along the sugar phosphate chain, χ through χ, the torsion angles within the ribose ring, ψ, through ψ, and the torsion angle of the glycosyl bond, χ. This is illustrated diagrammatically in Fig. 5A, which displays the three nucleotides, G-2, C-3 and G-4. Fig. 5B shows the actual backbone conformation of the nucleotides G-4 to G-6, using the common notation of torsion angles with the symbols +sc (synclinal+, gauche+), -sc (synclinal-, gauche-), and ap (antiperiplanar, trans) (an IUPAC convention).

Two conformations are found for the GpC phosphate groups in Z-DNA (20). The common one, Zs, is illustrated for the phosphate linking residues G-2 and C-3 in Fig. 5A. For this conformation the χ torsion angle of G-2 is in the -sc conformation while the α angle of C-3 is in the ap conformation. Fig. 5B shows the less common Zl conformation between the residues G-4 and C-5. Here the phosphate group is rotated downward relative to the Zs conformation in Fig. 5A, corresponding to a change in the preceding χ and χ torsion angles from -sc/-sc to ap/+sc, respectively. In this case the Zl conformation is associated with the presence of a magnesium ion that is coordinated to the N-7 of the neighboring residue G-6 (labeled as magnesium C in Figs. 7 and 8). One of its coordinating water molecules is hydrogen-bonded to a phosphate oxygen stabilizing the Zl conformation. This specific interaction of an ion complex is found in both the magnesium and the spermine form as well as in other published Z-DNA crystal structures (5, 11).
Table IIc lists the mean nucleotide torsion angles for both structures with separate entries for the syn and anti conformation nucleotides. It is interesting to note that the root mean square standard deviations for all of the torsion angles are fairly small (1-6°), indicative of a highly regular structure. The regularity of the nucleotide backbone and sugar conformations may be a reflection of the considerable rigidity of the Z-DNA structure. The deoxyribose rings of the cytidine residues have an average pseudorotation angle of 152° (Mg+2 form) and 153° (spermine form), typical of a regular C-2'-endo conformation. Consistent with this fact, the δ torsion angles associated with the deoxycytidines are 143° and 146° for the magnesium and spermine forms, respectively. For the deoxyguanosine residues on the other hand, omitting the terminal residues whose conformations are effected by hydrogen bonding interactions with neighboring chains, the average pseudorotation angles are 34° and 30° for the magnesium and spermine forms, respectively, with corresponding average δ angles of 95° and 98°. Contrary to previous suggestions (21), these fall well within the pseudorotation angle range assigned to the C-3'-endo ribose conformation (22).

The torsion angle δ is not very useful for assessing the ribose conformation since the relative magnitude of all rotation angles within the tetrahydrofuran ring determines the ribose pucker. In Z-DNA, the ribose ring of the guanosine residues is found to be relatively flat when compared to the mean Δmax angle of 39° reported in Ref. 22 for single nucleo-
Molecular Structure of Z-DNA at High Resolution

![Diagram of Molecular Structure of Z-DNA](image)

Fig. 5. Backbone conformation of the magnesium form of d(CGCGCG). In A the regular $Z_I$ conformation of the GpC phosphodiester bond is displayed. B shows the less common $Z_{II}$ conformation, which is only observed in crystal structures when stabilized by specific ionic interactions. Here the transition is caused by the magnesium ion complex marked C in Fig. 2. The torsion angles are indexed and classified according to the IUPAC-IUB convention (36). Oxygen atoms were diagonally striped; nitrogens are stippled, and the magnesium atom is shown with lighter stippling.

TABLE IIIa
Torsion angles of the ribose phosphate backbone for the magnesium and the spermine (Sp) forms

<table>
<thead>
<tr>
<th>Oxygen Residue</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
<th>ε</th>
<th>ζ</th>
<th>θ</th>
<th>Ψ</th>
<th>χ</th>
<th>ψ</th>
<th>Ψmax</th>
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<tr>
<td>α Mg</td>
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<td>166°</td>
<td>-185°</td>
<td>-190°</td>
<td>-97°</td>
<td>-97°</td>
<td>-97°</td>
<td>-30°</td>
<td>-20°</td>
<td>-72°</td>
<td>-34°</td>
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<tr>
<td>β Mg</td>
<td>-123°</td>
<td>166°</td>
<td>-190°</td>
<td>-185°</td>
<td>-97°</td>
<td>-97°</td>
<td>-97°</td>
<td>-30°</td>
<td>-20°</td>
<td>-72°</td>
<td>-34°</td>
</tr>
<tr>
<td>β Sp</td>
<td>-123°</td>
<td>166°</td>
<td>-190°</td>
<td>-185°</td>
<td>-97°</td>
<td>-97°</td>
<td>-97°</td>
<td>-30°</td>
<td>-20°</td>
<td>-72°</td>
<td>-34°</td>
</tr>
</tbody>
</table>

The labeled values have not been used for calculating the average torsional angles due to the following:

- the $Z_{II}$ conformation of P-5;
- the partial $Z_{II}$ conformation of P-9.

Table IV lists a number of helical parameters for both the spermine and the magnesium structure. Tilt defines that angular deviation of the long axis of the base pair from perpendicular to the helical axis, while roll represents the rotation of the base pair about this long axis. As seen previously for Z-DNA and consistent with the more rigid nature of this conformation, there is very little roll or tilt of the base pairs. Also, due to the unusual arrangement of bases relative to the phosphate backbone, there is very little propeller twisting or buckling of the base pairs either. It can be seen that the bases within the base pairs are largely coplanar with only

sides or the $r_{max}$ angle of the cytosine residues (Table IIIb).

The lower pucker amplitude results in a larger $δ$ angle for the same pseudorotation angle ($δ = α + 120°$). The ribose pucker has also been verified by using a different algorithm (i.e. determination of the ring atom with the maximum distance from the mean square plane determined by the remaining four ring atoms).

Table IV lists a number of helical parameters for both the spermine and the magnesium structure. Tilt defines that
Helix Parameters of the magnesium and the spermine (Sp) forms either within 1 base pair or between 2 consecutive base pairs

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Period</th>
<th>CI-2</th>
<th>CI-1</th>
<th>CI-C1</th>
<th>CI-C2</th>
<th>CI-C6</th>
<th>CI-C7</th>
<th>Mean</th>
<th>σ</th>
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</thead>
<tbody>
<tr>
<td>Roll</td>
<td>Mg</td>
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<tr>
<td></td>
<td>Sp</td>
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<td>-3.1</td>
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<td>2.9</td>
<td>2.5</td>
<td>0.2</td>
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<tr>
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<td>-7.6</td>
<td>-7.6</td>
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<tr>
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<td>-8.3</td>
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<td>-6.6</td>
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<tr>
<td>Slip A</td>
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<tr>
<td></td>
<td>Sp</td>
<td>-2.8</td>
<td>-1.9</td>
<td>-2.8</td>
<td>-3.6</td>
<td>-4.2</td>
<td>-3.8</td>
<td>-3.5</td>
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<tr>
<td>Slip B</td>
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<td>-2.8</td>
<td>-4.0</td>
<td>-5.6</td>
<td>-6.5</td>
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<tr>
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<td>Sp</td>
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<td>-1.9</td>
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<td>-4.2</td>
<td>-3.8</td>
<td>-3.5</td>
<td>0.4</td>
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</table>

The helical parameters are calculated by using the crystallographic 2-fold screw axis along c at 0.75 a and 0.5 b as helix axis. The average was calculated by using absolute values to account for the alternating slip of the dinucleotide repeat.

1 The average was calculated by using absolute values to account for the alternating slip of the dinucleotide repeat.
2 Between the last base pair of one DNA molecule and the first one of the next molecule stacked along c.
3 The dihedral angle between base planes can be broken into components of propeller twist and buckle.

a small dihedral angle between them. Furthermore, the distribution of dihedral angles is different from one structure to the other, suggesting that these small differences in base pair orientations are influenced somewhat by the ions that are packing around them.

In Z-DNA, the helical twist angle between successive base pairs varies between small values for CpG pairs (~7 to ~11°) and much larger values for GpC pairs (~49 to ~58°), as can be seen in Table IV. In these structures, there are some slight variations in the individual twist angles which may reflect small changes in the lattice packing associated with different ions. However, it should be noted that the average twist angles are ~8.6° for CpG and ~51.4° for GpC with a sum of ~60° for the dinucleotide repeating unit. This is exactly what one would expect for a helix with six dinucleotide steps/helical turn.

Radial Projections: The Helical Groove—A double helix may be represented by a radial projection of the molecule in order to better recognize its repeating helical features. This projection, as seen in Fig. 6A, can be understood by imagining a cylinder wrapped around the molecule with every atom being projected onto this cylinder by a line extending perpendicularly from the helix axis. In this diagram the groove extends from the upper left to the lower right, indicating a left-handed structure. The base pairs are viewed edge-on, and the zigzag sugar phosphate backbone lining the grooves is readily observable. In the upper part of the groove, a series of average phosphate-phosphate distances has been included. For example, the distance 8.7 Å is the span across the groove between the apical phosphates of the complementary strands of one DNA molecule.

A more realistic picture of the groove is shown in the lower part of the diagram where van der Waals radii have now been appended to the atoms along the edge of the groove. This reveals a very short van der Waals separation of 3.3 Å from one phosphate oxygen to the other phosphate oxygen across the groove. The space available for access to the groove may be visualized as a rectangle that is shown in thin lines. The entrance to the groove may be regarded as being made up of a series of such rectangles with approximate dimensions of 6-7 Å by 7-8 Å, superimposed in a staggered array along the groove. The groove is therefore appreciably narrower than either the major or the minor groove of right-handed B-DNA.

In fact, it is so narrow that in both the magnesium and the spermine crystal structures, nothing penetrates the groove other than water molecules, not even hydrated ions. However, this narrow groove is quite deep (about 9 Å) with the bottom of the groove extending almost to the helix axis.

Ions on a Helix—Fig. 6B is a stereo radial projection which shows, in addition to the DNA itself, four different crystallographically independent types of hydrated magnesium ions, labeled A-D. Some symmetry-related complexes are shown as well and labeled with prime (', ') letters. All hydrogen bonds between the ion complexes and the DNA are drawn in as thin lines, illustrating the DNA-ion interactions. Ion complex A has the interesting property that it forms several hydrogen bonds with three successive phosphates along one strand of the DNA. Another ion complex, B, is sitting at the entrance of the groove linking the phosphate oxygens of the two complementary strands by forming hydrogen bonds through its hydration shell. Finally, in ion complex C, where one of the coordination waters is replaced by an N-7 of guanine, one edge of its coordination sphere is shared with the adjoining ion complex D.

A more detailed view of the way in which these ions surround the Z-DNA molecule is given in the stereo skeletal drawing in Fig. 7A. This diagram shows all four different magnesium hexahydrate complexes (A-D) which interact either directly or through at least one bridging water molecule with a Z-DNA helix composed of two hexamers stacked on top of each other as they are in the crystal lattice. Ion complex A is found connecting three successive phosphate groups of one chain (P-8, P-9, P-10) by five hydrogen bonds and also bridging through a water molecule to the N-2 of guanosine G-8. Its symmetry-related complex A' forms hydrogen bonds with both the phosphate group P and phosphates above O-4' of G-6. Another ion complex, B, is situated at the entrance of the groove linking the phosphate oxygens of the two complementary strands by forming hydrogen bonds through its hydration shell. Finally, in ion complex C, where one of the coordination waters is replaced by an N-7 of guanine, one edge of its coordination sphere is shared with the adjoining ion complex D.

Molecular Structure of Z-DNA at High Resolution
Molecular Structure of Z-DNA at High Resolution

A

B

FIG. 6. Radial projection of the magnesium form of d(CGCGCG), displaying (A) the detailed geometry of the deep groove and (B) in stereo the hydrogen-bonded ion complexes around the DNA helix. In panel A some representative distances (±0.5 Å) between phosphorous atoms are indicated. In addition, van der Waals radii are drawn around the atoms along the lower section of the groove such that the distances correspond to the actual size of the groove opening. In B the four crystallographically distinct magnesium ion complexes are labeled A–D, and their symmetry-related positions are indicated by one- and two-prime labels. Hydrogen bonds between the DNA and the ion complexes are shown in thin solid lines while the ion complexes themselves are emphasized by heavy bars between the central ion and its ligands. The rotation angle of 0° corresponds to the same view as in Figs. 7 and 8, i.e., in the direction of the b axis.

the next residue in the 3′ direction (C-1). This residue actually belongs to the next DNA hexamer which is stacked (along the c axis) directly upon the previous one. Thus this particular ion complex is not only involved, as are all the other ion complexes, in stabilizing the interactions between DNA molecules within the crystal lattice in directions perpendicular to the helix axis but also along the helix axis. This end-to-end intermolecular linkage is enhanced by a bridging water between the same cytidine N-4 atom and another water ligand of ion complex C. In addition to this particular interaction still another ligand water forms a hydrogen bond to the second phosphate in the 5′-direction (P-5), locking it in the ZIII conformation. Again a bridging water, which is also linked to the next ion complex B′′, supports this stabilization. Ion complex D, in addition to the ligand it shares with complex C, is involved through two other ligands in stabilizing the interaction with the next DNA molecule. One ligand forms a hydrogen bond to O-6 of the same guanosine to which complex C is coordinating (G-6), while another one is bonding to the O-6 of the first guanosine residue of the next DNA molecule (G-12). The latter interaction is further stabilized by a bridging water molecule to the N-7 of the same guanosine (G-12).

The symmetry-related ion cluster C′′–D′ faces a different surface of the Z-DNA helix and forms an array of hydrogen-bonding interactions. These include hydrogen bonds to 1) O-6 of a guanosine residue (G-10), 2) O-6 and N-7 positions of the same guanosine (G-10) through bridging waters, 3) N-4 of the next cytidine in the 5′-direction (C-9), 4) the
FIG. 7. A, stereo skeletal diagram of the ionic structure around the magnesium form of d(GCGCGG). Two consecutive DNA molecules are stacked upon each other, with the base pair numbering scheme shown at the right. The magnesium hexahydrate complexes are labeled A–D as in Fig. 6B. Their bonds are drawn with heavy lines while bonds between the complexes and the DNA are drawn with thin lines. B. Identical view of the spermine form of d(GCGCGG). The bonds between spermine atoms are drawn with dark lines while those to the DNA are drawn with thinner lines. The individual spermine molecules are labeled at each end, with the larger number corresponding to the proximal end, as described in the text. Symmetry-related molecules are labeled with one- and two-prime letters.

phosphate group of this same residue (P-9), 5) the guanosine O-6 of the next base pair in the opposite 3'-direction, and finally 6) the ion complex B'. An additional view of the same cluster interacting with DNA can be represented by a different symmetry-related ion cluster C''–D'' which is connected to the DNA by only three bridging waters to two successive phosphate groups (P-11 and P-10) and to the ion complex B.

Interaction of Spermine with Z-DNA—The stereo skeletal drawing of Fig. 7B shows in similar detail the interactions of the two crystallographically distinct spermine molecules with the DNA. Three symmetry-related positions of the first spermine molecule (I, I', and I''), which each interact with
FIG. 8. Stereo van der Waals drawing of (A) the magnesium hexahydrate complexes and (B) the organization of spermine molecules around the Z-DNA helix. In both panels the helix is extended to show three consecutive molecules, of which the bottom two correspond to those shown in Fig. 7. The labeling scheme for magnesium complexes (shown in darker shading) is identical to that of Fig. 7A above, and for spermine molecules (also shown in darker shading) identical to that in Fig. 7B.

the DNA in a slightly different fashion, are shown. While spermine 1, located on the convex surface, interacts only through its first two amino groups with the guanosine N-7 and O-6 positions of two adjacent base pairs (G-4 and G-8), spermine 1' and spermine 1" form multiple direct and water-bridged hydrogen bonds to several phosphate groups of the DNA. The proximal three amino groups of spermine 1' span the opening to the deep groove, and through bridging water molecules, form hydrogen bonds to phosphate groups of both strands (P-6, P-10), while the terminal amino group binds to three successive phosphate groups either directly (P-9) or through bridging waters (P-10 and P-8). The spermine 1" is hydrogen-bonded to two adjacent phosphate groups of one strand (P-5 and P-6) by its terminal two amino groups. The last amino group also bridges through a water molecule to the ribose O-4' of residue G-6. In this way it is located just along the edge of the deep groove.

The crystallographically distinct spermine molecule 2 also interacts in three symmetry-related modes with the DNA. Spermine 2 winds up along the convex surface of the Z-DNA helix, forming hydrogen bonds to three consecutive base pairs. The proximal amino group is hydrogen-bonding to the guanosine O-6 of the first base pair (C-1-G-12). The third amino group is interacting with the cytidine N-4 of the next base pair (G-2-C-11) as well as to the guanosine O-6 and N-7 of the third base pair (C-3-G-10). Finally, the terminal amine...
group bonds directly to the N-7 of G-2 and through a bridging water molecule to the N-4 of C-3. The symmetry-related spermine molecule 2' begins close to the end of spermine 2, exhibiting mainly hydrophobic interactions, as is also clearly seen in the van der Waals diagram of Fig. 8B. Its second amino group is hydrogen-bonded to the N-7 of G-2, and through bridging waters also to the O-6 of the same residue, and to the 5'-terminal hydroxyl group of C-1. Spermine 2', which is translated along the a axis, is forming only one hydrogen bond to one phosphate group (P-11).

Comparison of Mg²⁺ and Spermine Sites of Interaction—An overall impression of the arrangement of the Mg²⁺ ions around the helix can be more readily seen in the van der Waals diagram in Fig. 8A. Here all those complexes which do not bind directly to the DNA are omitted. It can be easily seen in this stereo diagram that there are two general binding modes of the ion complexes to the DNA. Five of the magnesium complexes shown (C, D, B', C', D') are found on the convex surface of the Z-DNA helix coordinating preferentially with the guanosine residues and remaining close to the phosphate groups. The other class of ion complexes (A, B, A') is found at the entrance of the deep groove but never penetrating into it. It is likely that these general ionic binding modes are also present in solution.

The van der Waals diagram of Fig. 8B shows the surface interactions of the spermine molecules. The orientation of the DNA molecule is the same as in Fig. 8A, thus emphasizing the similarity of the spermine sites to those found for magnesium ions in that structure. While spermine 1' winds along the groove (without entering it), spermine 2 wraps along the outer surface of the molecule, possibly also exhibiting hydrophobic interactions between its methylene groups and the less polar nucleotide positions, cytosine C-5 and C-6, guanosine C-8, and ribose C-2'. The most obvious hydrophobic interaction is found between the last methylene groups of spermine 2 and the first ones of spermine 2', which are wrapping around each other. Spermine 1', in contrast, seems to move its hydrophobic groups away from the negatively charged phosphate groups. The other spermine molecules are pointing perpendicularly away from the DNA helix, obviously involved in crystal packing rather than in interactions with one specific DNA molecule.

Movement of the Molecule—The movement of the DNA molecule is reflected in the temperature factors of the atomic components. The average isotropic temperature factors for the bases, sugars, and phosphates along the chain are shown in Fig. 9 for the magnesium form of the crystal. This simply describes in numerical fashion the phenomenon which is shown in Fig. 1 for the spermine form. In general, lower temperature factors are associated with bases and sugars, while higher values are found for the phosphate groups. This was also true for the spermine form, where higher values are represented by the larger thermal ellipsoids of anisotropic vibrations. Fig. 9 shows some variations in the temperature factors for individual residues of the isotropically refined magnesium form. The temperature factor of the phosphate group P-5, which is found in the Z₀ conformation and stabilized by the magnesium ion complex C, is somewhat lower than those of its neighbors. It seems that the constraint of hydrogen bonding to the hydrated magnesium ion has decreased the thermal activity of P₅. Similarly, the explanation for the low temperature factor of phosphate P-10 may be found in its immediate environment. This phosphate is surrounded by ion cluster B as well as ion cluster C and is adjoining a guanosine residue to a neighboring molecule, and therefore its movement is also minimized. Phosphate P-4, on the other hand, is in a region that has no close neighboring DNA molecules. It is therefore not surprising to find that phosphate P-4 has a high temperature factor. Thus the low temperature factors of both P-5 and P-10 are probably due to the packing interactions in the crystal. It is not likely that the decreased motion, i.e. low temperature factor, of phosphate 10 would be maintained when the molecule is in solution.

DISCUSSION

One of the major structural differences between A- or B-DNA and Z-DNA is the alternation of syn and anti conformations in Z-DNA. However, the syn conformation of guanosine does not significantly modify the geometry of the base itself. As shown in this study, the bond distances and angles of these bases are similar to those obtained in small molecule crystals. There are, however, some small but significant differences in the base pair geometry as compared to r(GC), the only crystal structure of comparable resolution containing G-C base pairs in the all-anti A-RNA conformation (17). The most obvious discrepancy is found in the angle between the glycosidic bond and the guanine residue, i.e. C-1'—N-9—C-4. While this angle is found to be 125.7° in r(GC), it is widened to 130 and 131° in the magnesium and spermine form of d(CGCGCG), respectively. A similar increase in this bond angle has been observed in other syn purine nucleoside structures (23, 24) and can thus be attributed to the constraints imposed on the conformation by the close proximity of ribose and base. In contrast, the cytidine residues, which assume the anti conformation in both A-RNA and Z-DNA have very similar glycosidic bond angles (C-1'—N-1—C-2) of 117.7° in r(GC) and 116-118° in d(CGCGCG).

A more subtle change is found in the hydrogen bond distances of the base pairs. Compared to the crystal structure of r(GC), the inner (groove) hydrogen bond is enlarged by 0.030 Å, while the middle (imino) and the outer (surface) hydrogen
bonds are compressed by 0.015 and 0.055 Å (on average). This is consistent with a 1° rotation of the two bases within the plane about a point between the imino and the groove hydrogen bond. Such a rotation, together with the wider glycosidic bond angle, would indeed explain the increased C-1'-C-1' distance (+0.125 Å) and the smaller angles between the glycosidic bond and the C-1'-C-1' axis (G, −5.25°; C, −1.2°). This rotation leads to an opening of the minor groove and is probably induced by the repulsive Coulomb forces between the negatively charged phosphate groups of the two strands, which are very close in Z-DNA.

Laser light-scattering experiments of DNA in solution have shown that the persistence length of Z-DNA is about three times larger than either A- or B-DNA (25). It is interesting to speculate about the structural characteristics responsible for the increased rigidity of Z-DNA. One of the outstanding features of the molecule is the sharp contrast between CpG and GpC base steps. The CpG base steps are strongly sheared without much helical twist between the bases, while the GpC steps have a significant amount of helical twist without much shear. The stacking interactions are strongly intrastrand in the GpC interactions. In the CpG sequences, on the other hand, there are a mixture of interstrand interactions as well as stacking interactions between the six-membered ring of guanine and the O-4' of the sugar residue immediately adjacent to it. These different interactions are in contrast to the rather simple stacking interactions that are uniformly found in right-handed B-DNA.

One consequence of the increased flexibility of B-DNA is that the bases can readily separate to allow planar molecules to be intercalated. In Z-DNA, on the other hand, the bases cannot separate to allow such intercalation and consequently, the addition of an intercalating agent converts Z-DNA into intercalated B-DNA (26-29). In addition, the depth of the groove, as illustrated in Fig. 6A, raises an interesting biological question. Can amino acid side chains penetrate into the groove interacting in ways that lead to sequence-specific recognition with the bases which are found at the bottom of the groove? Examination of these structures indicates that this type of interaction is unlikely. The groove may be too narrow to allow even a long side chain of lysine to enter it. This question of accessibility may be approached with footprinting techniques that would reveal, for example, whether guanine N-3 can be methylated when a segment of DNA is held in the Z conformation.

One of the surprising features of this high-resolution analysis of Z-DNA structures is the fact that many of the cations are found interacting with the DNA in clusters. For example, in the magnesium crystal two of the four magnesium ions (C and D) are sharing one edge of their inner coordination spheres and are linked again through their second shell of hydration to a third one (B'). In the structure of d(m5CGTAm5CG), where the cytosines are methylated at the C-5 position, the cations were found to cluster in a group of four making an extended array with both shared edges and faces in their octahedral coordination spheres (5). However, due to the somewhat lower resolution, they could not be assigned unequivocally as either sodium or magnesium ion.

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**Fig. 10.** Stereo van der Waals diagram of those spermine molecules with three or more direct contacts to the Z-DNA molecule. Three hexamers are stacked end-to-end as in Fig. 8 to give the appearance of a continuous helix. The nitrogen atoms are shown as dotted spheres, oxygen as dashed spheres, phosphorous as solid spheres, and the magnesium complexes as hatched spheres. The spermine molecules are enhanced with dotted shading.
complexes. In the current structure the positions of the magnesium hexahydrate complexes, especially A and B, as seen in Figs. 6D and 7A, suggest that they may be preferred binding sites in solution, since they are making a rather detailed fit involving two or more hydrogen bonds, either bridging across the groove or coordinating to three successive phosphates along the chain. Similarly ion complex C might be expected to be quite stable, and thus also existing in solution, since it directly coordinates with a guanine N-7 and is associated with a Z-DNA GpC conformation.

In addition to the studies of ionic interactions with DNA, there has been a great deal of interest in the manner in which spermine molecules stabilize DNA conformations. This is the first study with high enough resolution to define the path of the spermine molecules attached to the left-handed DNA helix. The only other macromolecular structure in which spermine has been firmly identified is in the three-dimensional structure of yeast phenylalanine transfer RNA. In that structure a number of different spermines was identified occupying unique niches in the molecule (30). However, the assignments of their positions are less definite due to the limited resolution (2.8 Å) of that structure. Similarly, in the B-DNA dodecamer, a spermine was tentatively identified bridging across the major groove, but in that case as well the resolution of only 2 Å limited the available information (31).

Recently, a spermine molecule has also been identified in the major groove of an A-DNA octamer, although again the resolution is not yet high enough to describe this interaction in detail (32).

Summarizing the observations we have described above concerning the interactions of spermine molecules with DNA, we find two typical binding modes which are also likely to be found in solution. These are demonstrated in the stereo van der Waals diagram, Fig. 10, where the spermine molecules with three or more contacts to the DNA helix are shown. The first type of interaction involves hydrogen bonding to phosphate oxygens at the edge of the deep groove. Some spermine molecules wind along one side of the groove where they might be expected to effectively neutralize the negative charges of the phosphate groups. These phosphate groups come very close together in Z-DNA, and their repulsive action might otherwise be a major force in destabilizing the Z conformation. In addition, other spermine molecules actually bridge the opening to the groove by hydrogen bonding to phosphate groups on opposite edges. These specific interactions of spermine molecules, which are likely also to be found in solution, might constitute the major reasons for the very effective stabilization of Z-DNA by spermine molecules. The second general binding mode represented by spermine molecules in this structure does not lead to an equally easy interpretation. However, it seems that these molecules span the convex surface of the Z-DNA helix through hydrophobic interactions with the DNA bases as well as with each other. In addition, specific interactions with hydrogen acceptor groups of the bases further stabilize the DNA conformation both by steric and also electrostatic means.

In addition to the spermine molecules, we find in the spermine structure a hydrated magnesium ion complex at the same position as the complex C in the magnesium structure. The ion also coordinates with N-7 of guanosine G-6 and exhibits the same hydrogen bonding to the phosphate P-5, stabilizing it in the Z$_0$ conformation. Hydrated magnesium ions have also been found at the corresponding position in isomorphically crystallized DNA hexamers of different sequences (5, 20).

Thus it is interesting to ask why this ion complex coordinates to this particular guanosine residue and not to any of the five remaining ones. The answer is found in both the limited length of the fragment and the lattice geometry. As described above, this ion complex is among others characterized by a hydrogen bond to the second phosphate group in the 5′-direction. Due to the missing phosphate group on either end of the helix, G-2 and G-8 are not suitable for this particular interaction. The corresponding analogous residue to G-6, namely G-12, is constrained by a close lattice position to the G-10 residue of a symmetry-related helix. In fact, the N-7–N-7 distance between those two guanosines in only 5.22 Å, making it impossible for hydrated magnesium ions to fit between them. Why the only remaining position, G-4, is not utilized for a comparable ionic coordination is less clear; one reason might be that the N-7 position of this residue is fully exposed to the large solvent channel with its disordered and dynamic solvent structure (see right side in Fig. 2). A specific role in crystal packing might be associated with this ion complex, since it is situated between two stacked DNA helices. Together with the associated magnesium ion complex D it bridges the two stacked helices with several hydrogen bonds not only to each other (as shown in Fig. 2) but also to the magnesium hexahydrate.

An interesting observation is made if one compares in detail the interactions of the spermine molecules and magnesium ions of the spermine form with those of the magnesium ions in the magnesium crystal. Almost every ionic interaction in each structure has its counterpart in the other structure, as can be seen by comparing Figs. 7 and 8. It is thus apparent that the coordination of positively charged counterions is determined largely from the DNA structure, and probably its sequence, and less from the nature of the ions. The major difference between the two ionic structures we are describing here is thus reduced to the fact that each spermine molecule bears twice as many charges as the magnesium ions and thus substitutes on average for two magnesium hexahydrate complexes. Since all four charged nitrogen atoms in the spermine molecule are covalently linked, they have little freedom to move independently. The magnesium hexahydrates, in contrast, are totally independent from each other and represent rather unstable complexes, easily exchanging ligands with the surrounding solvent water. Since, with one exception, all magnesium ions interact only through their hydration shell with the DNA, there are two independent bonds, the coordinative bond between the ion and its ligand and the hydrogen bond between the ligand and the DNA, that, when broken, lead to the same result, namely the dissociation of the ion from the DNA. The spermine, in contrast, hydrogen bonds often directly to the DNA, thus forming a more stable interaction.

Finally, we can thus address the question, why spermine molecules as well as stable highly charged ion complexes, i.e., cobalt hexamine, are several orders of magnitude more effective than sodium or magnesium ions in stabilizing the Z conformation, as many solution studies on polymers of poly(dG-dC) have shown (33, 34). Since the actual coordination sites of the magnesium complexes are comparable to those of spermine and also cobalt hexamine (11), the strength of the interaction seems to be the major driving force for the stabilization of Z-DNA. Finally, as mentioned above, spermine is very effective in stabilizing Z-DNA, and it may be regarded as a complex of four cationic charges covalently linked together. From this study it appears that Z-DNA is in fact generally stabilized by clusters of positive charges rather than by individual positive charges scattered about the molecule. It would be of interest to know whether this is a property of Z-DNA alone or whether it is shared by molecules in other DNA conformations as well.
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