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Molecular structure of the octamer d(G-G-C-C-G-G-C-C): Modified A-DNA

(sugar pucker/x-ray diffraction/sequence-dependent DNA conformation/base stacking/benzo[*a*]pyrene)

ANDREW H.-J. WANG†, SATOSHI FUJII†, JACQUES H. VAN BOOM‡, AND ALEXANDER RICH†

†Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; and ‡Gorlaeus Laboratory, Leiden University, Leiden, The Netherlands

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ABSTRACT **The deoxynucleotide fragment d(GpGpCpCpGpGpCpC) was synthesized and crystallized, and its three-dimensional structure was determined by x-ray diffraction techniques to a resolution of 2.25 Å. The molecule forms a right-handed double helix in which the two base pairs at either end of the molecule are in the conventional A-DNA conformation, while the central four base pairs have a modified form in which alternate residues have sugar conformations that are closer to those in B-DNA than in A-DNA. The molecules have an intermolecular contact in which the planar terminal guanine·cytosine base pair lies on the flat minor groove surface of the A-DNA helix.**

In 1953 Franklin and Gosling reported that two different diffraction patterns could be produced from DNA fibers (1). One of these patterns, called A, was found in fibers exposed to air with 75% relative humidity. The second pattern, found in more hydrated fibers, was termed B-DNA. These two conformations differ, among other things, by the tilt of the base pairs, the pucker of the deoxyribose rings, and the position of the base pairs relative to the helical axis. These two forms readily interconvert, showing that DNA is clearly polymorphic. It is usually assumed that most double-helical DNA exists in the B form in biological systems, but the evidence for this is not strong.

Recently, study of crystalline oligonucleotides has revealed a variety of DNA conformations, including some oligomers that were found to have more than one conformation in their polynucleotide backbone. In some structures there are variations in the sugar ring pucker in alternating nucleotides (2, 3). There are two families of ring-pucker conformation: the C2′ *endo* conformation found in B-DNA has phosphate groups almost 1 Å further apart than is seen with the C3′ *endo* conformation found in A-DNA (and most RNAs) (4). A single crystal of a DNA dodecamer (5) was found to have adopted a B conformation. More recently, a tetramer (6) and an octamer (7) have been found to adopt the A-DNA conformation. In the present communication, we describe the structure of two closely related crystal forms of a DNA octamer, d(GpGpCpCpGpGpCpC), that adopts a modified A-DNA conformation. The central four nucleotides of the octamer are found to have a conformation in which alternate residues adopt a ring pucker closer to B-DNA than A-DNA. This alternation in ring conformation is found even though there is no alternating purine-pyrimidine sequence. In addition, the molecules have an interesting packing mode in which the terminal base pair of one molecule stacks on a flat portion of the shallow minor groove A-DNA surface of an adjoining molecule. The area occupied by the purine·pyrimidine base pair is virtually the same as the area of the benzo[*a*]pyrene molecule, suggesting a model for the binding of this carcinogen to DNA.

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Experimental

Two octanucleoside septaphosphates, d(GpGpCpCpGpGpCpC) and d(CpCpCpCpGpGpGpG), were synthesized by the modified triester method (8). After final purification, the oligomers were converted into ammonium salts and lyophilized. A solution was made containing 25 mM Na cacodylate (pH 7), 0.6 mM spermine, 3 mM $MgCl_2$, and the octamers at 1.2 mM. Crystals were grown by equilibrating the solution to 30% 2-methyl-2,4-pentanediol. Truncated tetragonal bipyramidal crystals formed and were initially examined by precession photography with CuK_α x-rays. The space group and unit cell dimensions are given in Table 1. The two octamers crystallize in the same space group with lattice dimensions that are virtually identical to each other. In the present investigation, attention was focused on d(G-G-C-C-G-G-C-C). Three-dimensional x-ray diffraction data were collected to a resolution of 2.25 Å at −8°C with a Nicolet x-ray diffractometer. Later experimentation with data collection at lower temperatures revealed that a transition took place in which there were significant changes in all three cell dimensions. This transformation was a slow process, and it took over 3 days at the lower temperature to stabilize the second lattice. A second data set was then collected at −18°C. The structure was solved by using the rotation–translation search method. Because of the symmetry constraints of the lattice, the molecules were judged to form a double helix with a two-fold axis that coincided with the crystallographic two-fold axis. Thus, only two variables had to be examined—the position of the molecule along the two-fold axis and the orientation of the helix on the axis. In the search procedures, both A-DNA and B-DNA models (9) were examined initially by using data in the 6.5- to 15-Å resolution range (59 reflections). The global minimum *R* factors were 46% and 56%, respectively. Consequently, the coordinates derived from the A-DNA model were used in refinement. After 20 cycles of refinement, the *R* factor dropped to 31%. The Hendrickson and Konnert restrained refinement (10) was then continued to higher resolution. Several cycles of sum-function Fourier ($2F_o - F_c$) and difference Fourier ($F_o - F_c$) calculations revealed a number of water molecules, and they were included in the refinement. At present the molecule has been refined to 2.25-Å resolution, and the current *R* values are 0.175 and 0.154 at −8°C and −18°C, respectively. In the course of this refinement, 75 water molecules appeared in the −8°C structure and 84 in the −18°C structure. At this resolution we were not able to make definitive assignments for ionic positions in the lattice.

Table 1. Characteristics of A- and B-DNA crystals

Compound	Space group	Cell dimensions, Å			Resolution, Å	Reflections,* no.	Current *R* value†
		a	b	c			
d(G-G-C-C-G-G-C-C) (−8°C)	$P4_32_12$	42.06	42.06	25.17	2.25	1,224 (920)	0.174
d(G-G-C-C-G-G-C-C) (−18°C)	$P4_32_12$	40.51	40.51	24.67	2.25	1,221 (874)	0.158
d(C-C-C-C-G-G-G-G)	$P4_32_12$	42.1	42.1	25.1			
d(G-G-T-A-T-A-C-C) (7)‡	$P6_1$	44.97 45.08	44.97 45.08	41.76 41.72	2.25	2,320	0.18
d(IC-C-G-G) (6)‡	$P4_32_12$	41.1	41.1	26.7	2.1	1,486	0.205
d(C-G-C-G-A-A-T-T-C-G-C-G) (5)‡	$P2_12_12_1$	24.87	40.39	66.20	1.9	2,818	0.178

* The number of reflections collected is listed. For this work, the number of observed reflections [$I > 3\sigma(I)$] used in the refinement is given in parentheses.

† R factor is defined as $\Sigma||F_o| - |F_c||/\Sigma|F_o|$, where F_o is the observed structure amplitude and F_c is the calculated structure amplitude.

‡ Reference source of data.

Molecular conformation

The external form of the octamer can be seen in a van der Waals side view of the molecule (Fig. 1 *b* and *c Upper*) together with comparison views of A-DNA (Fig. 1*a*) and B-DNA (Fig. 1*d*) (9). In these views the minor groove is at the top of the helical fragment, and the major groove is seen at the bottom. There are several differences between B-DNA and A-DNA. In B-DNA the base pairs occupy the central axis of the molecule and are almost perpendicular to the axis (the base tilt = −7°). In A-DNA the base pairs are removed from the central axis of the molecule and have a considerable tilt (19°). In B-DNA the rise per residue is 3.34 Å, close to the thickness of the unsaturated purine·pyrimidine base pair. Because of the base pair tilt in A-DNA, the rise per residue is 2.56 Å along the axis, and the diameter of the molecule has increased slightly. The minor groove is considerably flatter and wider because the base pairs are found away from the axis. The major groove in A-DNA is deeper and extends through the central axis of the molecule. The tilt in the base pairs brings the phosphate groups closer together on opposite strands across the major groove in A-DNA than they are in B-DNA. This is indicated in the upper part of Fig. 1 by the arrow at the right of the figure, which shows the distance along the helix axis between the phosphate groups at either end

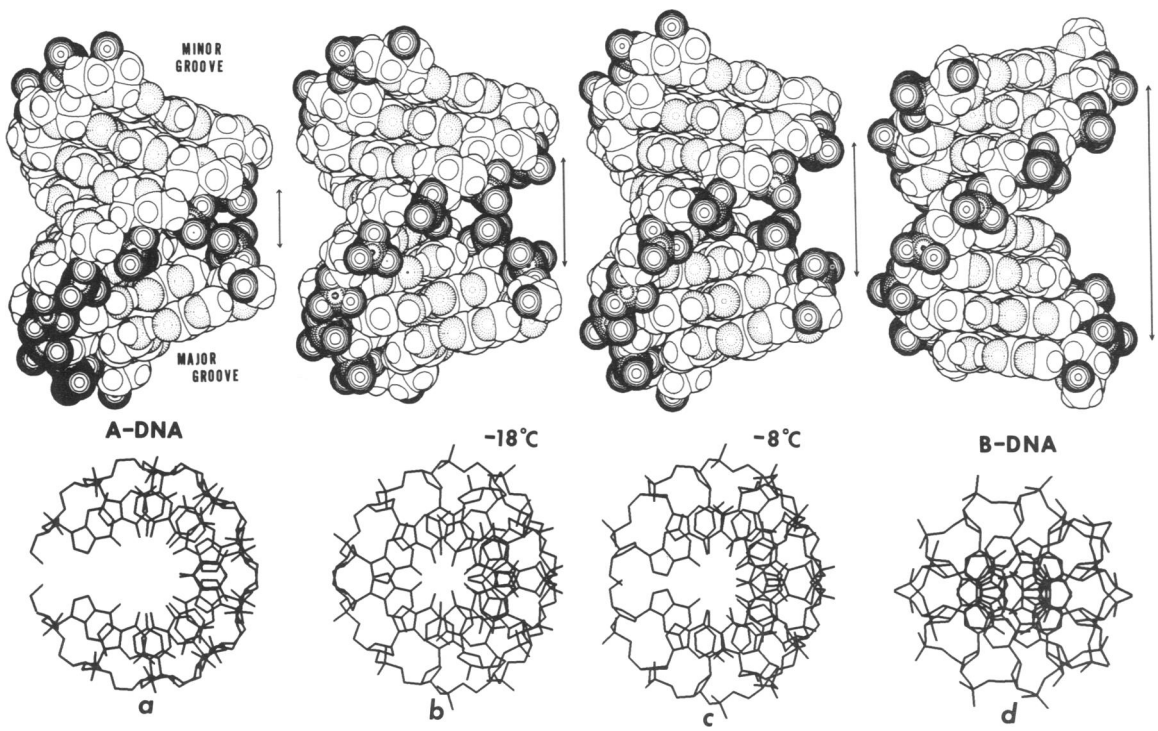

FIG. 1. (*Upper*) Van der Waals diagrams of four different DNA models. The helix axis is vertical, and a horizontal two-fold axis is located in the plane of the paper. All four DNA fragments have the sequence d(G-G-C-C-G-G-C-C). (*a*) A-DNA. The minor groove and major grooves apply to the other three drawings as well. (*b*) Form of the octamer observed experimentally at −18°C. (*c*) Octamer observed at −8°C. (*d*) B-DNA. The arrows at the right of each structure represent the vertical distance from the terminal phosphates of the two chains. This distance is large for B-DNA and small for A-DNA. Intermediate values are found in the two structures reported here. The phosphorus and oxygen atoms have a heavier shading than the other atoms. The tilt of the bases from the horizontal is 19° in A-DNA and −7° in B-DNA, whereas the base pairs in *b* and *c* are tilted approximately 14° and 12°, respectively. (*Lower*) Skeletal views down the helix axis.

of the molecule. In A-DNA (Fig. 1*a*) the vertical distance between the phosphates at the ends of the two chains is much smaller than in B-DNA (Fig. 1*d*). If we look at the two octamer structures shown in Fig. 1 *b* and *c*, we see that the molecule has adopted a conformation that is somewhat intermediate between A-DNA and B-DNA. The molecule looks more like A-DNA, but the tilt of the bases is about 14° in Fig. 1*b* and about 12° in Fig. 1*c*. Likewise the arrow at the right of these figures is longer than that seen in Fig. 1*a* for A-DNA but shorter than that found in Fig. 1*d* for B-DNA. However, the change in base tilt in Fig. 1 *b* and *c* relative to Fig. 1 *a* and *d* is not due to a localized bending of the molecules.

Skeletal model end views of the two octamer crystal structures are shown at the bottom of Fig. 1 together with A-DNA and B-DNA. There is a large hole down the axis of A-DNA; that hole is absent in B-DNA in which the base pairs lie on the axis. The two crystal structures at −8°C and −18°C have a conformation intermediate between A- and B-DNA, although it is closer to A-DNA. The base pairs do not occupy the axis but are closer to it than in A-DNA. The A-DNA end view of 8 base pairs is only part of a circle because there are 11 base pairs per turn. B-DNA has 10 base pairs per turn, and the end view projection of 8 base pairs looks more circular. The two octamer crystal structures show an intermediate state in which the circle of residues no longer has an opening at the left.

Table 2 lists the sugar conformations, torsion angles, and distances between adjacent phosphorous atoms along the polynucleotide chain. Inspection of this table reveals significant differences in the sugar conformation. The normal conformation for A-DNA is a C3′ *endo* conformation; however, residues C3 and G5 adopt a conformation closer to a C2′ *endo* (or C1′ *exo*) than C3′ *endo*. This is shown in the torsion angle δ, which has values closer to that seen for B-DNA than A-DNA. The δ torsion angle is associated with the C3′—C4′ bond of the deoxyribose ring. In the duplex the residues G1 and G2 are paired to C8* and C7* of the opposite strand. These all have conformations close to A-DNA. However, the central segment of four base pairs, C3–G6, consists of sugar residues that have ring puckers closer to B-DNA, followed by A-DNA in an alternating fashion. This difference in ring pucker is also reflected in the distances between adjacent phosphates, where the values for C3 and G5 are closer to that seen for B-DNA than A-DNA. Another significant parameter in describing the conformation of the polynucleotide chain is the manner in which successive base pairs stack. Fig. 2 shows the stacking of successive base pairs along the octamer helix. The stacking diagrams are closer to the stacking found in A-DNA than to that in B-DNA, although the stacking patterns are not identical. Thus, the molecule is seen to have an unusual conformation. Largely A-DNA stacking is retained, while the conformation of the sugar-phosphate backbone is alternating for the central four base pairs.

The effects of the alternating backbone can be seen by inspecting the distances between the C1′ sugar atoms of adjacent nucleotides along the polynucleotide backbone. It can be seen that the projection of the C1′–C1′ distance in the sequence G2pC3 is shorter than the same distance in C3pC4, which is long (Fig. 2). Furthermore, the C1′–C1′ projection distance of C4pG5 is also shorter than the distance in C3pC4. Thus, the backbone is alternately compressed and expanded as one goes down the polynucleotide chain. There is a two-fold axis between base pairs C4·G5* and G5·C4*, so the alternating compression and expansion of the backbone continues through the center of the molecule. This alternating compression and expansion is reflected in differences between twist angles associated with the adjacent base pairs. The twist angles in projection in Fig. 2 for the four base pairs going from top to bottom are 35°, 28°,

Table 2. Conformational parameters of d(G-G-C-C-G-G-C-C)

Residue	Torsion angles, deg* α	β	γ	δ	ε	ζ	χ	Distance P_i–P_{i+1}, Å
G1	—	—	93	124†	202	−74	−142	—
	—	—	62	80	183	−38	−164	—
G2	−77	201	31	120†	198	−99	−145	6.2
	−94	198	54	90	191	−69	−156	6.1
C3	−19	160	30	116	172	−73	−133	6.4
	−57	183	46	125	194	−87	−148	6.4
C4	−131	189	95	80	232	−76	−166	5.7
	−94	187	60	73	191	−41	−152	5.9
G5	−42	171	54	116	175	−101	−141	6.9
	−91	183	66	113	188	−91	−150	6.7
G6	−57	193	40	85	196	−52	−161	5.7
	−77	188	58	83	195	−55	−151	5.8
C7	−92	185	68	70	183	−47	−149	6.1
	−101	178	92	73	191	−63	−163	6.3
C8	−106	195	71	76	—	—	−153	—
	−140	197	118	94	—	—	−157	—
A-DNA	−90	211	47	83	175	−45	−153	5.7
B-DNA	−41	136	38	139	227	−157	−102	6.5

* Torsional angles are defined as

$$03'\text{—}P\text{—}05'\text{—}C5'\text{—}C4'\text{—}C3'\text{—}03'\text{—}P\text{—}05',$$
$$\alpha\quad\beta\quad\gamma\quad\delta\quad\varepsilon\quad\zeta$$

and χ is the glycosyl torsion angle.

† The deoxyribose of these residues was found to have a conformation close to planar, which is an unfavorable one. It is probable that these residues in the crystal adopt a number of sugar puckers but were refined to an average structure because of the disorder in the sugar conformations. Two rows of numbers are given. The upper is for the −8°C structure and the lower, for that at −18°C.

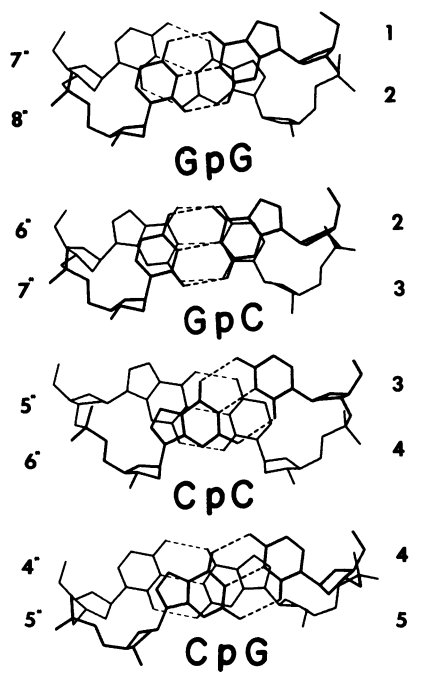

FIG. 2. The stacking of successive base pairs is illustrated for the octamer structure, which has been determined at −8°C. The base pair drawn with the heavier line is closer to the reader than the base pair drawn with thin lines. The sequence refers to the right-hand side of the diagram; the left-hand side has the complementary sequence. In the upper level, the base pair 1·8* is stacked above base pair 2·7*, etc. It should be noted that although the backbone is regular in A-DNA and B-DNA, it is quite irregular in the actual structure of the octamer.

45°, and 16° in the −8°C octamer structure. Similar alternations are found in the −18°C structure. These are to be compared with a 36° twist angle that is found in B-DNA and a 33° twist angle found throughout A-DNA.

Molecular packing

The octamer molecules are organized around a left-handed four-fold screw axis in which the terminal base pair of the octamer is stacked against the minor groove of an adjacent molecule. The end of this adjacent molecule in turn abuts against another molecule related by a 90° turn. The net effect of this stacking is to produce an open lattice with large solvent channels which pass through the crystal parallel to the 4_3 axis (Fig. 3). The aqueous channels between the molecules measure *ca.* 10 by 20 Å. The detailed interaction between two molecules is shown in the stereo diagram of Fig. 4. The lighter line drawing represents one octamer duplex forming a right-handed double-helical fragment. The heavier lines show the position of the terminal base pair of an adjoining octamer where it abuts against the double-helical fragment in the minor groove. The minor groove at the outer part of the octamer forms an almost flat surface upon which the base pair from the adjoining molecule is stacked. This end of the molecule has a conformation close to A-DNA in which the minor groove is shallow; thus, the surface along one side is relatively flat. Measurement of the close contact distances between the terminal base pair and the atoms forming the minor groove shows that there are several atoms in van der Waals contact (<3.2 Å), and there is no hydrogen bonding between them. Probably the closeness of the contacts stabilized largely by van der Waals interactions provides the stability for building this type of lattice.

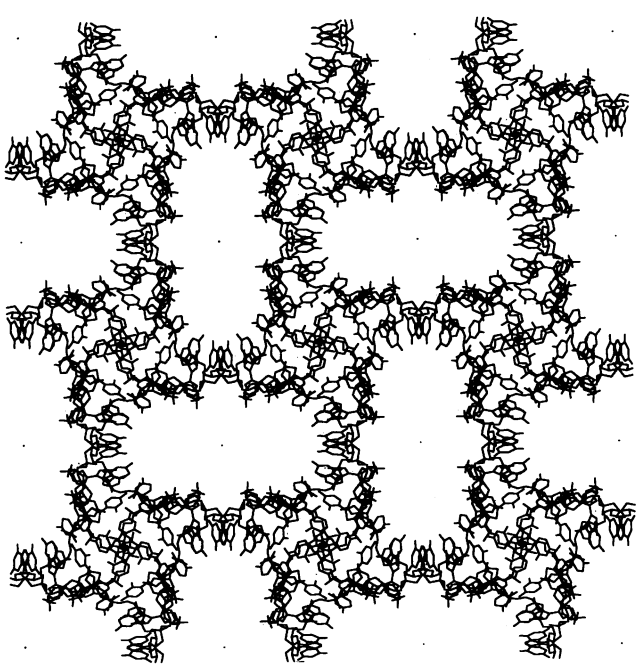

FIG. 3. A lattice-packing diagram illustrating the manner in which the DNA octamers stack together to form large aqueous channels. Four molecules come together in the center of the diagram packing around a four-fold screw axis. Both ends of the molecule are involved in similar interactions at four-fold axes. This produces the oval channels that run through the lattice and measure approximately 10 by 20 Å. These channels are filled with solvent water molecules (not shown).

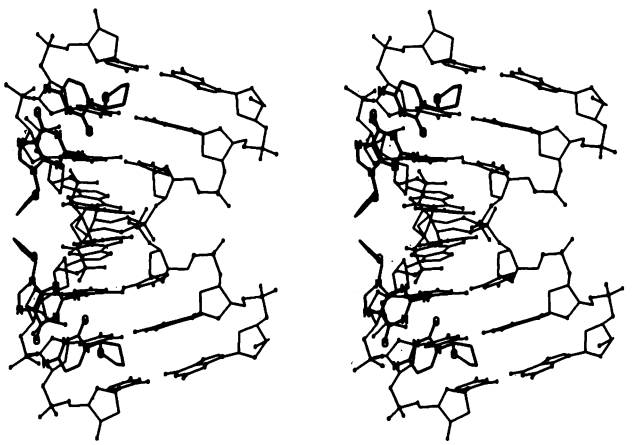

FIG. 4. A stereo diagram of the DNA octamer is shown drawn with thin lines. In addition, two guanine-cytosine base pairs are drawn in heavier lines together with their attached sugar rings. These are the terminal base pairs of adjoining molecules that abut the flat minor groove of the A-DNA segment at the end of the molecule. It can be seen that this base pair is close to the G6 N2 amino group. This diagram shows the −8°C structure, but a similar interaction is found in the −18°C structure.

A-DNA in crystal lattices

Two structures have been determined recently that have an A-DNA conformation. The tetramer reported by Connor *et al.* (6) is deoxy ^{I}C-C-G-G, while Shakked *et al.* (7) have reported the structure of an octamer d(G-G-T-A-T-A-C-C). This octamer shows some conformational features that are similar to those observed in the present structure; it also has an intermediate morphology similar to that which is shown in Fig. 1 *b* and *c*. This octamer also forms a lattice that uses the same abutting interaction in which the terminal base pair binds to the minor groove through a van der Waals interaction. In that case it builds up a 6_1 axis instead of the 4_3 axis seen in the present structure. The tetramer lattice dimensions (table I in ref. 6) are somewhat similar to those seen in the octamer reported here, even though the iodine on the terminal cytosine may modify the lattice interactions somewhat. However, it is probable that that lattice is also built up through a similar abutting mechanism as that found in the present octamer structures.

If one compares in detail the two structures seen at −8°C and −18°C, an interesting displacement occurs. Comparison of the end views shown in Fig. 1 shows that the projected curve of the molecule at −18°C has closed up more than the −8°C structure to make a tighter system with less space at the center of the axis. Analysis of the structure reveals that this is primarily due to movements of residues 1–4, whereas residues 5–8 change their positions very little. The movement of residues 1–4 involves displacements of up to 1.5 Å. The differences in the torsion angles are listed in Table 2, which shows that the largest differences are found in residues 1–4.

The earlier study by Viswamitra *et al.* (3) of the sequence d(pA-T-A-T) revealed a structure in which there was an alternation in sugar pucker involving both C3′ and C2′ *endo* conformations. This led to the proposal of an "alternating B-DNA" conformation for the poly(dA-dT) double helix (11). It is of interest that in d(C-G-C-G-C-G) which forms left-handed Z-DNA (2), alternating conformations are also found involving C2′ and C3′ *endo* residues. Z-DNA also has dramatic differences in the twist angles between adjacent base pairs along the helix axis. It should be noted that the alternating conformation in the present structures is not associated with an alternating purine-pyrimidine sequence.

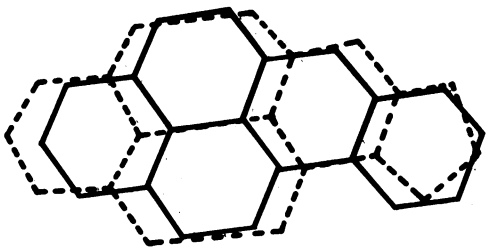

FIG. 5. A comparison of the backbone of benzo[*a*]pyrene drawn in solid lines and the guanine·cytosine base pair drawn in open lines. The areas occupied by these two flat structures are very similar.

The octamer d(C-C-C-C-G-G-G-G) listed in Table 1 crystallizes in virtually the identical lattice as those of the octamers that we have reported here. Further, the virtual identity of the sampled precession diffraction patterns suggest that it is likely to adopt a similar conformation. The octamer d(C-C-C-C-G-G-G-G) has a sequence C-C-G-G in its central four base pairs identical to that found in the structures reported above. This leaves open the possibility that this alternating conformation may be favored by the sequence d(C-C-G-G). It is possible that such a conformation may be recognized by the restriction endonucleases *Hpa* II and *Msp* I, which cleave this sequence. The first five molecules listed in Table 1 adopt the A-DNA conformation [if we assume that d(C-C-C-C-G-G-G-G) has the same structure], whereas the dodecamer at the bottom of Table 1 adopts the B conformation. A common feature seen in the first five molecules is the G-G sequence; this might induce local domains with an A-DNA configuration. This could be an example of a sequence-specific conformation that may occur more generally in DNA.

In the two structures that have been determined, we can see 80 water molecules of an estimated 150. Most of them are found in the large solvent channel area near the major groove of the molecules, as the minor groove is blocked by the abutting base pair (Fig. 4). The water molecules further into the aqueous channels are probably disordered.

We have illustrated the interaction between the abutting base pair and the minor groove of the A-DNA structure (Fig. 4) because it may serve as a model for understanding how planar or near planar molecules can interact with nucleic acid structures. If one looks at B-DNA, there are no planar surfaces; both grooves are deep, and large planar molecules are unlikely to find a flat surface to which they can bind. However, the minor groove of A-DNA forms a flat surface. We mention this point because of the interesting comparison that can be made between the G·C base pair and the benzo[*a*]pyrene molecule, a flat molecule containing five aromatic rings. Fig. 5 shows a superposition of the benzo[*a*]pyrene nucleus and the G·C base pair, and the two have a similar shape. The benzo[*a*]pyrene diol epoxide is known to be a highly active carcinogen that binds covalently to the guanine N2 amino group (12). The G·C base pair abutting the A-DNA molecule is very close to N2 of G6 in the minor groove. This might be a model for the manner in which the benzo[*a*]pyrene diol epoxide is bound to DNA. The carcinogen could bind to segments of DNA that have an A-DNA structure and be positioned so that the epoxy functional group can react with a neighboring guanine N2. This can be studied more thoroughly in structural experiments involving benzo[*a*]pyrene adducts of selected fragments of DNA oligonucleotides.

Single-crystal analyses of DNA oligonucleotides make it possible for us to obtain detailed structural information about the conformation of individual segments of DNA. The general impression obtained from these studies is that the DNA molecule is conformationally active and can adopt a number of different stable conformations. It is likely that these different conformations will be related to the functional biology of DNA.

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