Nonintercalative Binding of Proflavin to Z-DNA: Structure of a Complex between d(5BrC-G-5BrC-G) and Proflavin†

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ABSTRACT: The crystal structure of a disordered 1:1 complex between the tetradeoxyoligomer d(5BrC-G-5BrC-G) and proflavin has been determined and refined to an R factor of 26.9% for 474 reflections initially in space group $P6_5$ and to an R factor of 22.2% for 475 reflections in space group $P2_1$, both at 2-Å resolution with $F_{obsd} \geq 4.0$. The unit cell constants are $a = b = 17.9$ Å, $c = 44.5$ Å, and $\gamma = 120°$. The final models are essentially the same in the two space groups with greater disorder in space group $P6_5$. In space group $P2_1$, the asymmetric unit is a tetranucleotide duplex, two sandwiched proflavin molecules, and four "outside-bound" proflavins. The tetranucleotide duplex is in the Z conformation and is located at the origin of the unit cell with a pair of proflavins sandwiched between the tetranucleotides. Thus, the tetranucleotides and proflavin dimers stack alternatively forming a quasi-continuous helix with the helix axis coincident with the c axis. The structure analysis revealed the presence of outside-bound proflavins as well. It is interesting that one type of outside-bound proflavins occupies a similar environment as the cobalt hexaammines in their complex with the decadeoxyoligomer d(CGTACGTACG) [Brennan, R. G., Westhof, E., & Sundaralingam, M. (1986) *J. Biomol. Struct. Dyn. 3*, 649]. Crystals of the latter are isomorphous to the present complex. The outside-bound proflavins penetrate the deep minor groove, thereby closing it off, and provide a visualization of a quasi-internal mode of binding of proflavin to a nucleic acid.

Even before the discovery of left-handed Z-DNA by X-ray crystallographic work (Wang et al., 1979, 1981; Crawford et al., 1980; Drew et al., 1980), solutions of poly(dG-dC) and ethidium bromide or proflavin were studied by absorption and circular dichroism (Pohl & Jovin 1972; Pohl et al., 1972). This work indicated that ethidium bromide converts with high cooperativity the high-salt form (later recognized as the left-handed form) to the low-salt form (or classical right-handed form). Subsequently, it was concluded that Z-DNA does not bind ethidium bromide (Van de Sande & Jovin, 1982; Patel et al., 1982). It has been shown also that intercalating drugs inhibit the salt-induced B to Z transition (Mirau & Kearns, 1983) or convert the left-handed form to the right-handed one (Rio & Leng, 1983). Van de Sande and Jovin (1982), however, described a different left-handed Z form which has the property to bind intercalating drugs like ethidium bromide. A more recent report (Shafer et al., 1984) concluded from absorption, fluorescence, and circular dichroism studies that the complex between ethidium bromide and Z-DNA closely resembles that between intercalating drugs and B-DNA. From this, as well as from another study (Genest & Malfoy, 1986), it was concluded that ethidium bromide binds to Z-DNA by intercalation. A modeling study has also implicated an intercalative mode of binding to left-handed DNA (Gupta et al., 1983). For a review on the B–Z transition, see Jovin et al. (1987).

In this work, we report the crystal structure of a complex between the intercalating drug proflavin and the tetranucleotide d(5BrC-G-5BrC-G) in the Z conformation. The proflavin drugs are found not to intercalate the Z-DNA but rather are sandwiched between adjacent stacked tetranucleotide duplexes. Besides being sandwiched, the proflavins are also found to bind on the outside in the deep (minor) groove of the Z-DNA. In space group $P6_5$ the structure is heavily disordered while in space group $P2_1$ it is not except possibly for the proflavin molecules. The low resolution and limited quantity of the data permitted the determination of only the main geometric and interaction parameters. The general difficulty in obtaining good crystals of oligonucleotides, especially of complexes, makes the structure analysis of such crystals challenging. The development of powerful constrained refinement programs able to deal with low-resolution data and disordered models has enabled the analysis of such structures.

Experimental Procedures

Single crystals of the tetranucleotide d(5BrC-G-5BrC-G) (colorless) and its complex with proflavin (yellowish in color) were grown by the liquid diffusion of acetone into equimolar solutions of the tetranucleotide and proflavin hemisulfate (Sigma Chemical Co.). Crystals of both the free tetranucleotide and the complex grew at pH 7.0 in 25 mM cacodylate buffer as hexagonal rods in 2–3 weeks. Crystals of the complex are yellow when seen sideways and deep red when seen down their hexagonal cross section, confirming the presence of proflavin molecules. Surprisingly, unit cell dimensions of both crystals are identical and equal to $a = b = 17.9$ Å, $c = 44.5$ Å, and $\gamma = 120°$.

We chose to pursue further investigations on only the crystals of the drug–nucleotide complex since they diffracted better. A hemisphere of diffraction data to 2-Å resolution was

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collected, from a crystal of size 0.03 × 0.03 × 0.40 mm, on the CAD-4 diffractometer in the $\omega/2\theta$ scan mode with a constant ω scan of 20/54 deg/min. Friedel mates of all the strong reflections were measured on the -2θ side at the same scan speed. Lorentz, polarization, decay, and absorption corrections were applied during data reduction by the empirical φ curve method. For the observed monoclinic Laue symmetry of $2/m$ with c as the unique axis, R_{sym} was 5.5%. The systematic absences were in the $00l$ reflections, $l = 2n + 1$. Of the 1308 unique monoclonic reflections, 852 were observed at the 3σ level on F and 689 at the 4σ level. At the 4σ level, there are 490 reflections with $F_{obsd} \geq 1.0$. However, the reflection 0 0 12 is by far the strongest of all the reflections. For reflections with $F > 4\sigma(F)$, the data exhibited pseudo-Laue symmetry of $6/m$ with most of the $00l$ reflections wth $l \neq 6n$ absent (R_{sym} = 11.0%). Thus, space group $P6_5$ (see below) was considered in the initial structure determination. The hexagonal lattice has been observed several times (Drew et al., 1978; Crawford et al., 1980; Brennan & Sundaralingam, 1985; Fujii et al., 1985) and is always associated with crystallographic disorder of the oligomers which are stacked end to end. Interestingly, the hexagonal lattice is obtained by ethanol diffusion (Drew et al., 1978; Brennan & Sundaralingam, 1985) as well as by vapor diffusion in the presence of high concentrations of organic solvents (Fujii et al., 1985) or of high salts (Crawford et al., 1980; Fujii et al., 1985).

Structure Determination. It was recognized that the cell dimensions of this crystalline complex are identical with those of the complex between the decadeoxyoligonucleotide d-(CGTACGTACG) and cobalt hexaammine (Brennan & Sundaralingam, 1985; Brennan et al., 1986) which belongs to space group $P6_5$. The proflavin–tetranucleotide complex was also isomorphous to the cobalt hexaammine–decanucleotide complex that occurred in the Z-DNA conformation. Therefore, the initial structure determination was attempted in space group $P6_5$. As in the decamer crystals, the very intense meridional 0 0 12 reflection of the present complex suggests that there are 12 molecular planes per cell, separated by about 3.7 Å and perpendicular to the c axis. This is evidence for the presence of a Z-DNA-like structure. In the decamer crystals, the 12-nucleotide duplex is constituted by borrowing either 2, 4, 6, 8, or 10 nucleotides from an adjacent decamer so that it may complete its pseudo-12-nucleotide length (Brennan & Sundaralingam, 1985). The sequence repeats after five continuous unit cells, and the borrowing results in the disordering of the asymmetric unit in such a way that each pyrimidine–purine base pair is occupied $^3/_5$ of the time by a C-G pair and $^2/_5$ of the time by a T-A pair. In the decamer crystals, the unit cell is thus constituted of a pseudododecamer in the left-handed Z-DNA conformation with the asymmetric unit being a disordered dinucleotide fragment YpR in which each duplex fragment pyrimidine–purine base-pair is occupied 60% of the time by a C-G pair and 40% of the time by a T-A pair.

In the tetramer–proflavin complex, the 12 planes can be visualized as being comprised of 8 base pairs from 2 tetranucleotide duplexes in the Z conformation and 4 sandwiched proflavin molecules, leading to a 1:1 tetranucleotide–drug complex. The tetranucleotide and the two sandwiched proflavin molecules stack alternatively along the c axis:

$$[(CGCG)\cdot Pf\cdot Pf]_2$$

In five unit cells, all the combinations will be exhausted. In this case, the crystallographic asymmetric unit would consist of the dinucleotide d(5BrC-G) duplex $^2/_3$ of the time and of two stacked proflavin molecules $^1/_3$ of the time. However, these proportions will not give a balance of charges. This aspect will be discussed below after the description of the refinement which also revealed the presence of outside-bound proflavin molecules that satisfied the charge balance in the structure.

Restrained Refinement in $P6_5$. On the basis of the similarity in cell dimensions and disorder between the decamer–cobalt hexaammine complex and the present structure, the refinement was started with coordinates for the d(5BrC-G) part taken from the decamer structure. The coordinates for the proflavins were obtained by fitting each proflavin in the position occupied by a C-G pair in the decamer with the program FRODO (Jones, 1982). The refinement was carried out with the restrained least-squares program of Hendrickson and Konnert (1979) developed for nucleic acids, NUCLSQ (Westhof et al., 1985). Covalent bond lengths, covalent bond angles, planar groups, chirality of sugar atoms, and nonbonded contacts were restrained. Temperature factors were not restrained. The occupancies of the disordered groups were either varied with restraints so that the whole group would vary in a chemically meaningful way or kept fixed at an average value. Because of the disorder and the lack of data, the refinement was not straightforward. In the program NUCLSQ, variable but fixed occupancies can be easily handled as well as the geometry, stereochemistry, and nonbonded contacts between the various components with variable occupancies.

At the beginning, the refinement converged to an R factor 35% for 500 reflections at the 4σ level. In difference maps, strong peaks were observed above or below the 3′-phosphate of the cytosine bases. At first, it was thought that these peaks were hydrated magnesium ions. The inclusion of magnesium ions to represent those peaks dropped the R factor. But, in the $2F_o - F_c$ maps, the peaks appeared much too flat and elongated for being magnesium ions. Interestingly, these peaks were close to the positions occupied by the cobalt hexaammine in the decamer crystals previously reported (Brennan et al., 1986). It was decided to represent these peaks by proflavin molecules. The peaks on each strand were not exactly similar, and consequently, at first only one additional proflavin molecule was added. Upon refinement, the R factor dropped to values slightly below 30%, and the electron density appeared meaningful in $2F_o - F_c$ maps. The other set of peaks was assigned to a second proflavin, and on refinement, the R factor dropped to a final R factor of 26.9% for 474 reflections between 4.5- and 2.0-Å resolution at the 4σ level. It is noteworthy that, at the end of the refinement, the removal of the outside-bound proflavins raises the R factor to 37%, a value considerably higher than the final value. This further provides credence for the outside-bound proflavins. Because of the disorder, the $2F_o - F_c$ maps showed occasional discontinuities in electron density.

Occupancies and Balance of Charges in $P6_5$. The occupancy of all atoms was also varied at various stages of the refinement. During this process, the temperature factors were kept constant. The best results were obtained with an average occupancy for the dinucleotide fragment d(5BrC-G) of 60% and for the proflavin dimer of 40%. These values depart from the values of 66.7% and 33.3%, respectively, used at the beginning of the refinement. They imply that the tetranucleotide:drug ratio is less than 1. In other words, over the extended lattice consisting of five unit cells along in c axis (see above), instead of 20 CpG dinucleotides and 10 proflavin dimers in the starting model, the refined model consists of 18 CpG dimers and 12 proflavin dimers or 24 proflavins. For the outside-bound proflavin molecules, the total occupancy added to 1, with one

Table I: Summary of Refinement Parameters

	in $P6_5$	in $P2_1$[a]
mean F_{obsd}	7.9	8.6, 8.8, 8.4
mean ($F_{obsd} - F_{calcd}$)	2.2	1.9, 1.8, 2.1
mean positional shift (Å)	0.01	0.02
mean thermal shift (Å^2)	0.20	0.60
R factor[b]	26.90	22.2, 20.7, 23.8
correlation coeff between F_{obsd} and F_{calcd}[b]	95.06	95.8, 96.6, 94.8
$K = \sum F_o / \sum F_c$	1.076	1.006
resolution range (Å)	4.0–2.0	4.0–2.0
no. of reflections	474	475, 447, 490
no. of parameters	745	1033
no. of atoms	186	258

[a] In this space group, the first number refers to observed data larger than 4.0, the second number to data larger than 5.0, and the third number to data larger than 1.0. [b] The R factor is defined as $\sum|F_{obsd} - F_{calcd}|/\sum|F_{obsd}|$. The correlation coefficient is given by $\sum[(F_{obsd} - \bar{F}_{obsd})(F_{calcd} - \bar{F}_{calcd})]/([\sum(F_{obsd} - \bar{F}_{obsd})^2][\sum(F_{calcd} - \bar{F}_{calcd})^2])^{1/2}$.

proflavin having an occupancy of 75% and the other 25%. The presence of the outside-bound proflavins gives the balance of charges which could not be obtained in the starting model, thereby reinforcing the correctness of the refined model. Indeed, the nucleic acid dimer of the asymmetric unit accounts for 1.8 minus charges, $(-2 \times 0.6) + (-2 \times 0.3)$ (the latter represents the intermediate phosphate between CG dimers which is only present half the time), and the proflavin dimer accounts for $+2 \times 0.4$ or 0.8 plus charge. This leaves one minus charge, which is exactly compensated by the two outside-bound proflavin molecules whose occupancies add to 1. The reason for the different occupancies for the outside-bound proflavins, of 75 and 25%, respectively, may stem from the intermolecular contacts which the two proflavins make with neighboring molecules, which are less favorable in the lower occupied case.

Since the level of bromination was not guaranteed to be 100% (D. Patel, private communication), the occupancies of the two bromine atoms were also varied. The refinement led to 60% bromination for cytosine C1 and 75% bromination for cytosine C3, confirming that the tetrameters were not fully brominated.

Restrained Refinement in $P2_1$. The restrained refinement was next carried out in space group $P2_1$, where the asymmetric unit is a full tetranucleotide duplex with two sandwiched proflavin molecules. The outside-bound proflavin molecules appeared early in the refinement. Four outside-bound sites for the proflavins were found in the electron-density maps. The two proflavin molecules bound at the terminal base pairs of the tetranucleotide have a similar orientation and similar interactions with the nucleic acid but different from those adopted by the outside-bound proflavin molecules (see below).

With bromination of the cytosines around 70%, the R factor decreased to 20.7% for 447 reflections between 4 and 2 Å ($F_{min} = 5.0$), to 22.2% for 475 reflections ($F_{min} = 4.0$), and to 23.8% for 490 reflections ($F_{min} = 1.0$) at 4σ on F_{obsd}. In this model, the six positively charged proflavin molecules (two sandwiched and four outside) exactly balance the six negative charges of the tetranucleotide molecule. The summary of the refinement parameters is given in Table I. Because of the low ratio of observations to adjustable parameters, the deviations of geometrical parameters from standard values are given in Table II. The fractional coordinates of the asymmetric unit will be deposited in the Brookhaven Protein Data Bank.

The presence of the outside-bound proflavin molecules might be sufficient to explain the strong color of the crystals without the need for the proflavin molecules being sandwiched between tetramer molecules. Therefore, one could also imagine a model in which the 12 planes are occupied by three tetramer molecules each binding four outside proflavin molecules. Such a model can be excluded on the basis of the balance of charges, since in such a case the six negative charges of each tetramer would not be compensated by the four positive charges of the proflavin molecules.

Table II: Agreement Statistics for Geometrical Parameters with the σ's Applied after the $P2_1$ Refinement

geometrical parameter	RMS deviation from ideality	σ applied in refinement
bond distances[a]	0.009	0.025
bond angles	0.028	0.050
hydrogen bonds	0.031	0.050
base planarity	0.012	0.030
chiral volumes[b]	0.068	0.100
single-torsion contacts	0.182	0.063
multiple-torsion contacts	0.181	0.063
isotropic thermal factors[c]	3.065/3.588/3.339/4.090	

[a] A total of 15 of the 968 distances and 10 of the 800 nonbonded contacts deviate by more than 2σ. If not specified, values are in angstroms. [b] Values are in cubic angstroms. [c] First number is for atoms connected by a bond length, the second number for atoms connected by a bond angle, the third number for P–O bonds, and the fourth number for atoms involved in hydrogen bonding or in phosphate angles. No restraints were applied on the B factors. Values are in squared angstroms.

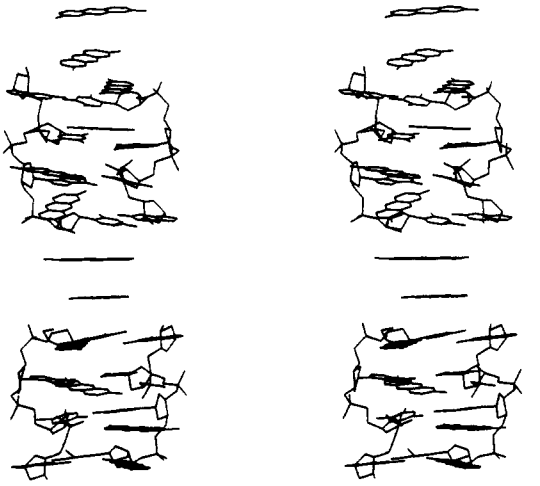

FIGURE 1: Stereo view of two stacked complexes of the tetradeoxyoligomer with proflavin molecules, with 12 planes spanning the length of the c axis.

Results and Discussion

Figure 1 is a stereo view of the structure showing the c axial repeat (12 planes) of the alternatively stacked tetranucleotide, the two sandwiched proflavins, and the four groove-bound proflavins. The tetranucleotide belongs to the ZI-DNA family as can be seen from Table III. The base pairs are skewed with a noticeable propeller twist. Similar distortions, although not always so large, were noted previously (Crawford et al., 1980; Brennan et al., 1986). An overlay of the tetranucleotide and the two sandwiched proflavins on the hexanucleotide portion of the previously determined pseudododecamer structure (Brennan et al., 1986) is illustrated in Figure 2. The deviation between the corresponding atoms after least-squares fitting is 0.53 Å. Views of the sandwiched (left) and groove-bound (right) proflavins are shown in Figure 3. Figure 4 shows a view of the outside-bound proflavins looking down the c axis.

Tetramer and Sandwiched Proflavins. A view of the stacking pattern of the sandwiched proflavin dimer is shown in Figure 5. Such stacking geometries have not been found

Table III: Conformational Torsion Angles (deg) in the Asymmetric Unit of the Tetramer in Space Group $P6_5$[a]

residue	σ	φ	ψ	Ψ	φ'	σ'	χ	τ	P
C1		-27.7	90.4	142.3	-74.3	104.1	25.3	31.7	162.5
G2	162.9	121.7	123.7	84.6	-108.2	-23.8	125.1	32.0	62.5
C3	177.6	170.1	88.1	132.2	-87.0	139.2	23.9	31.2	160.9
G4	-27.6	-95.4	-169.2	92.9	-15.1	-39.3	134.0	30.7	62.1
C5	-93.4	-11.2	110.9	137.4	-113.1	111.9	-0.2	30.5	165.6
G6	50.7	-137.4	-171.3	77.9	-117.4	-23.4	120.3	38.3	60.9
C7	-140.4	-172.6	49.5	130.6	-91.1	105.3	5.7	30.9	156.7
G8	-12.5	-124.7	-126.8	92.4	0.0	0.0	134.9	27.2	53.3

[a] The nomenclature is that from Sundaralingam (1969), and the pseudorotation angles (Altona & Sundaralingam, 1972) were calculated with the algorithm of Rao et al. (1981).

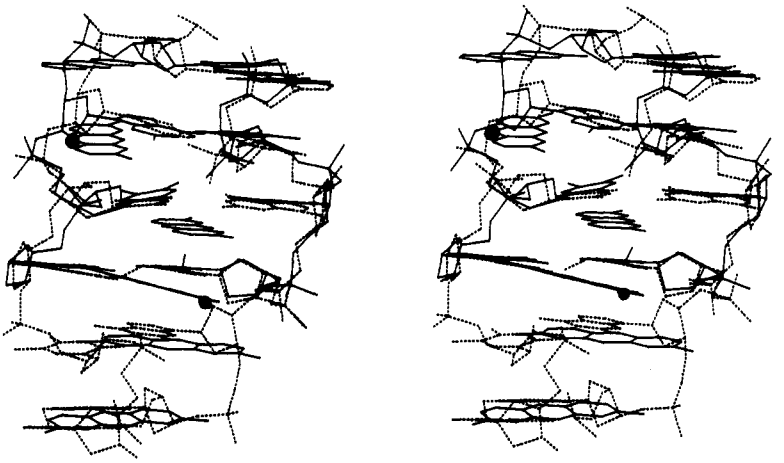

FIGURE 2: Superposition of the present tetramer duplex structure with two sandwiched proflavins (solid) on a hexanucleotide stretch of the pseudododecamer structure (dashed). The solid circles represent the positions of cobalts in the cobalt hexaammine bound to the dodecamer. The rms deviation between the backbone atoms of the two structures, after a least-squares fitting, is 0.53 Å.

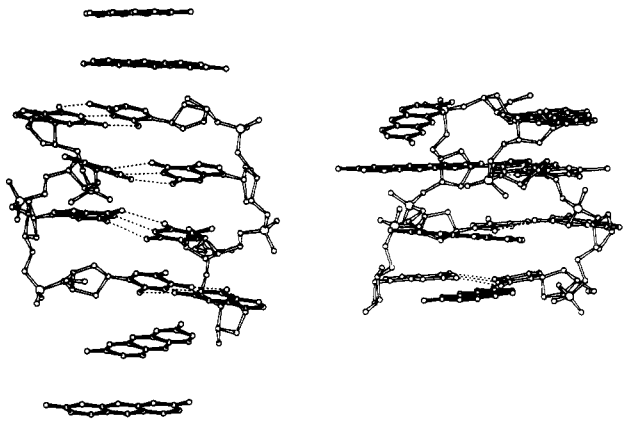

FIGURE 3: View of the sandwiched proflavins (left) and outside proflavins (right) bound to the tetramer duplex.

in previous structures of complexes between nucleic acid fragments and proflavin [for a review, see Neidle and Berman (1983)]. In the structure of the 1:2 complex between ApA and proflavin (Shieh et al., 1982), the two proflavin cations are stacked almost directly on top of one another without the pronounced sliding seen in Figure 5 (top). The structure of proflavin dichloride (Obendorf et al., 1974) presents a somewhat related stacking geometry between two proflavin molecules with the two proflavin molecules parallel and with a rotation of 180° between each proflavin. The sandwiched proflavin molecules have a more pronounced stacking with the cytosine nucleotides of the first and last base pairs of the symmetrically related tetranucleotide molecules between which they are located (Figure 4). The presently observed stacking pattern is mainly dictated by the fact that the sandwiched proflavin molecules have to mimick the C-G base pairs of a

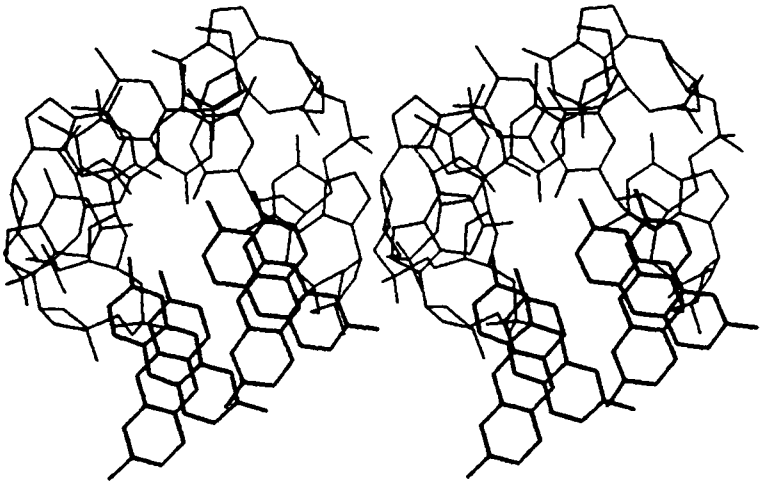

FIGURE 4: Stereo view (down the c axis) of the tetradeoxynucleotide with the outside-bound proflavins.

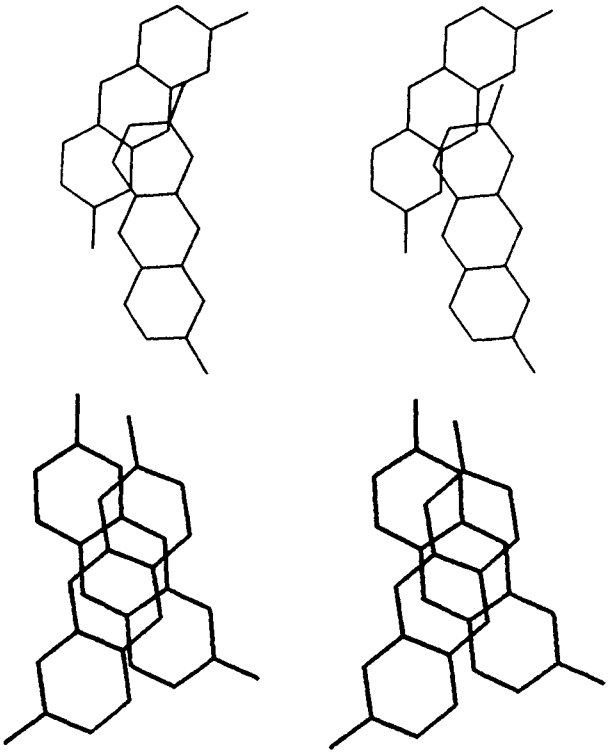

FIGURE 5: Stereo view of the stacking pattern of the proflavin molecules: (Top) sandwiched molecules; (bottom) two outside-bound molecules.

Z-DNA dimer. It is known that, in Z-DNA, there is a slide between two consecutive C-G base pairs so that the two cytosine bases stack on top of each other with the guanine bases stacking on the sugars (Wang et al., 1979).

Outside Binding of Proflavin on Z-DNA. The four proflavin molecules bound on the minor groove side can be divided into two groups (Figure 4). Figure 5 (bottom) shows a view of the stacking pattern between two outside-bound proflavin molecules in one of these groups. The stacking patterns observed between the proflavin molecules are similar to those seen in the structure of proflavin hemisulfate (Jones & Neidle, 1975) and in the complex between ApA and proflavin (Shieh et al., 1982). The contacts made by the outside-bound proflavin molecule are given in Table IV. The proflavins appear to interact mainly electrostatically, and most of the contacts involve the phosphate groups and the rings of the proflavin. There are two instances where a proflavin amino group interacts with the exocyclic O2 atom of a cytosine base. Some of the close contacts between proflavin amino groups and guanine amino groups are at van der Waals separation. The proflavin rings are located above the anionic phosphate oxygens, and in one case, there is a hydrogen bond between one exocyclic amino group and a phosphate oxygen. There are several C–H···O intermolecular contacts between C–H groups on the proflavin and anionic oxygen atoms of guanine bases. The intramolecular or intermolecular contacts made by the outside-bound proflavins, although not exactly the same, are very similar. In a crystallographic study of the binding of proflavin to yeast Phe-tRNA, it was found that proflavins bind in a nonintercalative mode through electrostatic interactions to contiguous helical phosphate groups (Sundaralingam, 1980; Liebman and Sundaralingam, unpublished results). Classically (Lerman, 1961; Peacocke, 1973), outside binding involves strong electrostatic interactions between stacked assemblies of drugs and the sugar–phosphate backbone of double-helical nucleic acids.

Table IV: Environment of the Outside-Bound Proflavins

atom 1	atom 2	residue	code[a]	distance (Å)
PfO1–C7	O2P	G6	0 0 0	2.9
PfO1–C8	O2P	G6	0 0 0	2.6
PfO1–N15	O5′	C5	−1 0 0	2.7
PfO1–N18	O2	C5	0 0 0	2.4
PfO2–C1	O1P	G8	0 0 0	2.8
PfO2–C12	O1P	G8	0 0 0	3.0
PfO2–N15	N2	G6	0 0 0	2.7
PfO3–C1	O2P	G8	0 0 0	3.0
PfO3–N10	O1P	G4	0 0 0	3.0
PfO3–C13	O1P	G4	0 0 0	3.0
PfO3–C14	O1P	G4	0 0 0	2.8
PfO3–N15	N2	G6	0 0 0	2.8
PfO3–N15	O2	C7	0 0 0	2.5
PfO3–N18	C2′	G6	−1 −1 0	2.5
PfO3–N18	O3′	G6	−1 −1 0	2.9

[a] The symmetry operation to be applied on the second atom. The three numbers refer to the translation along x, y, z.

CONCLUSIONS

There are two aspects to this work. First, it shows that it is possible to extract important structural information from low-resolution data with refinement methods which have been developed for nucleic acids (Westhof, et al., 1985; Hendrickson-Konnert, 1979). The use of energy calculations simultaneously or in parallel with the restrained refinement appears to be the next step for properly characterizing the geometry and the interactions present in such disordered structures. Second, this work shows that proflavin, which binds to B-DNA by intercalation and outside binding (Lerman, 1961; Peacocke, 1973), binds to Z-DNA neither by intercalation nor by the classical outside-binding mode. The binding is mainly electrostatic to the sugar–phosphate backbone, and the bound proflavin blocks the entrance to the minor groove of Z-DNA. The sites bound to the internal base pairs are close to those of the cobalt hexaammines in their complex with the decamer d(CGTACGTACG) (Brennan et al., 1986) or to sites B of the ruthenium hexaammine complex with d(CGCGCG) (Ho et al., 1987). Thus, it would appear that the cationic drug proflavin is able to stabilize the Z-DNA conformation of alternating 5BrC-G sequences in a fashion similar to cobalt and ruthenium hexaammines.

If the outside binding of proflavin to Z-DNA observed in the present crystal structure is a possible model for ethidium bromide, it could help in resolving discrepancies present in the literature. Hydrodynamic properties indicate a large increase in the chain stiffness of Z-DNA compared to B-DNA (Thomas & Bloomfield, 1983). On the other hand, fluorescence anisotropy measurements of ethidium bound to DNA indicate that B-DNA is 6 times more rigid than Z-DNA (Ashikawa et al., 1984); i.e., the dye is more mobile when bound to Z-DNA than when bound to B-DNA. If ethidium is involved in outside binding as described here for proflavin, the ethidium molecule would be less firmly bound to Z-DNA, although at the same time it is similarly protected from the solvent in both forms (Ashikawa et al., 1984; Genest & Malfoy, 1986), thereby explaining the fluorescence data. The latter study concluded that the first ethidium molecules intercalate between Z-DNA base pairs rather than between residual B-DNA base pairs (Genest & Malfoy, 1986). However, another study concluded that the cooperative binding of ethidium under Z-form conditions is associated with a sequential conversion of three to four base pairs of left-handed form to right-handed form (Walker et al., 1985). The very first step in the binding of intercalating drugs to Z-DNA, before its induced conversion to B-DNA, is probably portrayed

in the present crystal structure.

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SUPPLEMENTARY MATERIAL AVAILABLE

A table containing the atomic coordinates of the atoms in the structure (5 pages). Ordering information is given on any current masthead page.

Registry No. Proflavin, 92-62-6.

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