DNA Distortion in *bis*-Intercalated Complexes†,‡

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ABSTRACT: The *bis*-intercalators Flexi-Di and ditercalinium are synthetic dimers that *bis*-intercalate into DNA and cause cell death in prokaryotes from futile and abortive repair of DNA. Each is composed of two 7*H*-pyridocarbazole units and a linker. Flexi-Di has a flexible spermine-like linker while ditercalinium has a rigid *bis*(ethylpiperidinium) linker. This report, describing the 2.5-Å X-ray structure of Flexi-Di complexed with $[d(^{Br}CGCG)]_2$, appears to be the first report of a three-dimensional structure of a DNA complex with a *bis*-intercalator with a flexible linker. DNA complex formation with a ditercalinium analog having a flexible linker was not anticipated to yield unstacked and bent DNA as was observed in the previously reported ditercalinium·$[d(CGCG)]_2$ complex. Surprisingly, the DNA in the Flexi-Di complex is bent to a degree exceeding that of the ditercalinium complex. A comparison of the DNA complexes of Flexi-Di and ditercalinium has allowed us to propose a mechanism by which these *bis*-intercalators distort DNA. We propose that this class of *bis*-intercalators *pulls* the internal base pairs into the major groove and *pushes* the external base pairs into the minor groove. The result is a bend toward the minor groove. It appears that hydrogen bonds between the linker and the internal guanines effectively pull the central base pairs of the complex out into the major groove. At the external regions of the complex, stacking interactions between the chromophores and terminal base pairs effectively push the terminal base pairs into the minor groove. The result of this push/pull combination is to bend the DNA.

Many chemotherapeutics bind to DNA. Small molecules can interact with DNA via the minor groove or by intercalation. Some widely used drugs such as daunomycin and adriamycin do both. Intercalation into DNA necessarily results in radical changes in DNA conformation, separating base pairs along the helical axis. Intercalation can inhibit DNA replication, transcription, and/or topoisomerase activities. Relationships between structures of intercalators and conformations of intercalated DNA complexes are subtle and unresolved.

The *bis*-intercalators Flexi-Di (Figure 1A) and ditercalinium (Figure 1B) are synthetic dimers that were designed in pursuit of novel antitumor agents (Pelaprat et al., 1980a, 1980b). Each compound is composed of two 7*H*-pyridocarbazole units and a linker. Flexi-Di and ditercalinium differ only in the compositions of their linkers. Flexi-Di has a flexible spermine-like linker while ditercalinium has a rigid *bis*(ethylpiperidinium) linker. Structure–activity studies of the ditercalinium family suggest correlations between linker rigidity, among other features, and activity (Garbay-Jaureguiberry et al., 1992; Leon et al., 1987, 1988; Pelaprat et al., 1980a, 1991). Ditercalinium is active in both prokaryotes and eukaryotes while Flexi-Di is active only in prokaryotes (Lambert et al., 1988a,b, 1990; Pothier et al., 1991).

In prokaryotes, both Flexi-Di and ditercalinium cause cell death by an unprecedented mechanism (Lambert et al., 1988b). Flexi-Di and ditercalinium form reversible, *noncovalent* complexes with DNA that are recognized as *covalent* lesions by prokaryotic DNA repair systems. These excision-repair systems are unable to excise DNA lesions that form reversibly (Lambert et al., 1988a, 1989, 1990). Cell death results from the futile and abortive repair of DNA. We are interested in Flexi-Di and ditercalinium in part because three-dimensional structures of their DNA complexes can provide detailed views of substrates of DNA repair systems. Structures of these complexes should help us understand how an enzymatic

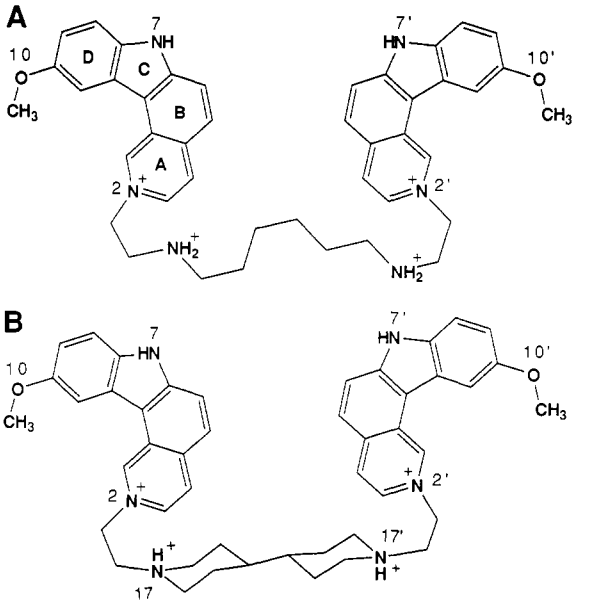

FIGURE 1: DNA *bis*-intercalators (A) Flexi-Di with rings of one chromophore labeled A–D and (B) ditercalinium.

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system can recognize a diverse array of covalent DNA damage. We want to understand what structural features distinguish damaged from undamaged DNA.

The crystal structure of a ditercalinium·$[d(CGCG)]_2$ complex was previously solved by X-ray crystallography (Gao et al., 1991; Williams & Gao, 1992). We described structural features distinguishing the ditercalinium complex from conventional DNA complexes (Williams & Gao, 1992). These features, possible elements in the unusual activity of the ditercalinium family, are (a) poor base stacking, (b) accessibility of the minor groove, (c) mimicry of the minor groove of unperturbed DNA, (d) helical bending, and (e) placement and interactions of the linker. The features that merit serious consideration as possible repair system recognition elements are the complete lack of stacking between each chromophore of ditercalinium and some of the adjacent bases and the lack of interaction with the minor groove of DNA (Williams & Gao, 1992). When viewed from the minor groove, the ditercalinium complex appears to mimic normal DNA. The location of the linker in the major rather than the minor groove was initially observed by NMR in the solution structure of the ditercalinium·$[d(CGCG)]_2$ complex (Delbarre et al., 1987a,b).

The DNA in the ditercalinium complex is bent. Initially we reasoned that much of the helical distortion results from the rigidity of the *bis*(ethylpiperidinium) linker of ditercalinium. By this reasoning, one might expect significant structural differences in complexes of Flexi-Di and ditercalinium with DNA to arise from the dissimilar chemical compositions of their linkers. We anticipated that DNA complex formation with a ditercalinium analog having a flexible linker such as Flexi-Di would not yield unstacked and bent DNA.

This report describes the three-dimensional X-ray crystal structure (2.5-Å resolution) of a Flexi-Di·$[d(^{Br}CGCG)]_2$ complex. Surprisingly, the DNA in this complex is bent to a degree exceeding that of the Ditercalinium·$[d(CGCG)]_2$ complex. This result indicates that rigidity of the linker does not cause DNA bending in these complexes. Here, we propose a novel structural basis for DNA bending by small molecules: it appears that DNA bending in both complexes is caused by contributions from specific hydrogen bonding and base pair–chromophore stacking. The results of these complementary forces are best described as a push/pull effect (Figure 2).

MATERIALS AND METHODS

Crystal Growth. Flexi-Di was synthesized as previously described (Garbay-Jaureguiberry et al., 1992; Pothier et al., 1991). The ammonium salt of reverse-phase HPLC-purified $d(^{Br}CGCG)$ was obtained from the Midland Certified Reagent Co. (Midland, TX). Single crystals of Flexi-Di plus $[d(^{Br}CGCG)]_2$ were grown at room temperature in sitting drops by the vapor diffusion method. The mother liquor consisted of 18 mM sodium cacodylate buffer, pH 7.0, 0.7 mM DNA (single-stranded concentration), 0.2 mM Flexi-Di, 3.9 mM $MgCl_2$, and 3.1% 2-methyl-2,4-pentanediol (MPD) equilibrated against a reservoir solution of 10% MPD. Orange, rectangular crystals and long, thin plates were observed within 3 weeks. The crystal used for data collection was ~0.25 mm × 0.32 mm × 0.20 mm. The crystal was mounted in a glass capillary with a droplet of mother liquor and sealed with epoxy.

Data Collection. X-rays were generated with a Rigaku RU-200 rotating anode generator (Cu Kα, λ = 1.54 Å), and intensity data were collected with a San Diego Multiwire Systems (SDMS) area detector (Xuong et al., 1985). Attempts to collect data at low temperature were unsuccessful because upon shock cooling the gross crystal morphology changed and the peaks broadened and in some cases split. Thus data were collected at room temperature (20 °C).

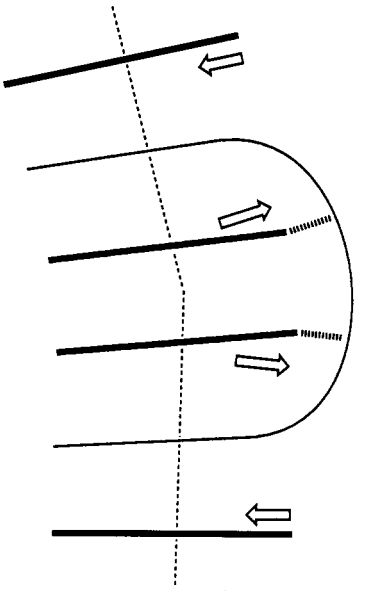

FIGURE 2: Schematic diagram of a Flexi-Di·DNA complex illustrating the push/pull effect and the bend in the DNA helical axis. In the ditercalinium·DNA complex hydrogen bonds are observed at only one end of the complex.

The crystal system is tetragonal with unit cell dimensions a = 27.09 Å and c = 81.89 Å and space group $P4_12_12$. The unit cell and space group suggested that the crystal is isomorphous with the ditercalinium·$[d(CGCG)]_2$ crystal (a = 26.88 Å and c = 82.60 Å) (Gao et al., 1991; Williams, & Gao, 1992).

Refinement. A total of 2139 reflections, reduced to 1183 unique reflections, at 2.5-Å resolution and $F_o > 2\sigma(F_o)$ were used for the refinement. The data are 98% complete at 2.5-Å resolution. The R-merge, a measure of the intensity discrepancy between equivalent reflections ($\Sigma|I_{av} - I_{obsd}|/\Sigma I_{av}$), is 6.63%. The initial model consisted of the duplex tetramer of DNA and two chromophore moieties of the ditercalinium·$[d(CGCG)]_2$ complex. The model was constructed, manipulated, and visualized using the program CHAIN (Sack, 1990) on a 4D70GT Silicon Graphics workstation. Refinement of this model, using the program of Konnert–Hendrickson (Hendrickson & Konnert, 1981) as modified for nucleic acids, resulted in an R-factor of 39.1%. Fourier sum ($2F_o - F_c$) (for example, Figure 3A,B) and difference ($F_o - F_c$) (Figure 3C) maps were generated using X-PLOR (Brunger, 1988). The difference maps showed intense peaks near the C5 positions of the terminal cytosines. Addition of bromine atoms to the model at the positions of these peaks, followed by refinement, resulted in a 7% drop of the R-factor. At this point an elongated string of density spanning the major groove and connecting the two chromophores indicated the position of the linker. The linker was constructed from coordinates of a spermine molecule and added to the model. After further refinement the R-factor dropped to 27.7%. The final refined model contains 27 water molecules and no ions. The RMS deviation of bond lengths from ideality is 0.021 Å, and the RMS deviation of planes from ideality is 0.012 Å. The final R-factor is 23.9%.

RESULTS

The DNA in the Flexi-Di·$[d(^{Br}CGCG)]_2$ complex is bent. This bend is somewhat surprising because Flexi-Di has a

flexible spermine-like linker. DNA bending in the ditercalinium·[d(CGCG)]$_2$ complex was originally attributed to the rigidity and conformational restraints of the *bis*(ethylpiperidinium) linker. With the solution of the structure described here, this proposal became untenable. The distinctions between the Flexi-Di·[d(^{Br}CGCG)]$_2$ and the ditercalinium·[d(CGCG)]$_2$ complexes are complicated and subtle. A detailed comparison of the two complexes has suggested an unexpected mechanism of DNA bending.

Both Flexi-Di and ditercalinium *bis*-intercalate into DNA at successive CpG steps. In the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex, this is a ^{Br}CpG step (Figure 3A,B). Unlike most intercalators and groove binders, both Flexi-Di and ditercalinium bind to DNA with their linkers in the major groove. The minor groove is completely unobstructed in both complexes. The hydrogen bond donor pattern as viewed from the minor groove in both intercalated DNA complexes is similar to that of unperturbed B-DNA. The N7 and N7′ nitrogen atoms of these *bis*-intercalators substitute for the two amino nitrogen atoms of guanine (Figure 5). The potential to form 2-fold symmetric complexes exists in both complexes, but both are asymmetric. In both complexes, the linker spans the major groove diagonally (Figure 3C). The phosphate backbone in both complexes is extended to accommodate the chromophores.

The DNA in both complexes forms a right-handed but underwound double helix. Values for helical parameters, as defined at the 1988 EMBO meeting on DNA curvature (Dickerson et al., 1989), were obtained using the program CURVES (Lavery & Sklenar, 1989). In the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex, the DNA has a helical twist of 20° at the first step, 28° at the second step, and 23° at the third step. Given that the average helical twist of the uncomplexed B-DNA dodecamer [d(CGCGAATTCGCG)]$_2$ is 36°, Flexi-Di unwinds this DNA fragment by a total of 37°. Ditercalinium unwinds [d(CGCG)]$_2$ by a total of 36° (Gao et al., 1991; Williams & Gao, 1992).

The most striking feature of the Flexi-Di[d(^{Br}CGCG)]$_2$ complex is the bend in the DNA helical axis. This axial bend exceeds that of the ditercalinium·[d(CGCG)]$_2$ complex. A comparison of the extent of DNA bending in the Flexi-Di·[d(^{Br}CGCG)]$_2$ and ditercalinium·[d(CGCG)]$_2$ complexes is presented in Figure 4. The direction of the axial bend is toward the minor groove in both complexes.

The specific positions of all nucleotides in a DNA fragment must be considered to describe irregular helical parameters (Lavery & Sklenar, 1988). Although accurate determination of a nonlinear helical axis is problematic with relatively short fragments of DNA, differences in the extent of DNA bending can easily be estimated from a side-by-side comparison of two bent DNA fragments (Figure 4). An estimate of the axial bend was obtained by determining the deviation of the helical axis from linearity. By this method the axial bend of the Flexi-Di·[d(^{Br}(CGCG)]$_2$ complex is approximately 15° while that of the ditercalinium·[d(CGCG)]$_2$ complex is approximately 8°. These values may underestimate the true degree of axial bending because the contribution from the internal base pairs dampens the calculated helical bend. In a previous report (Gao et al., 1991; Williams & Gao, 1992) using only the best planes of the external base pairs, we estimated the axial bend to be 15°. By this method the axial bend in Flexi-Di·[d(^{Br}CGCG)]$_2$ is about 21°.

The hydrogen bonds to the linkers of Flexi-Di and ditercalinium appear to be significant factors contributing to DNA bending. Differences in hydrogen-bonding interactions appear to lead directly to differences in axial bending. In the Flexi-

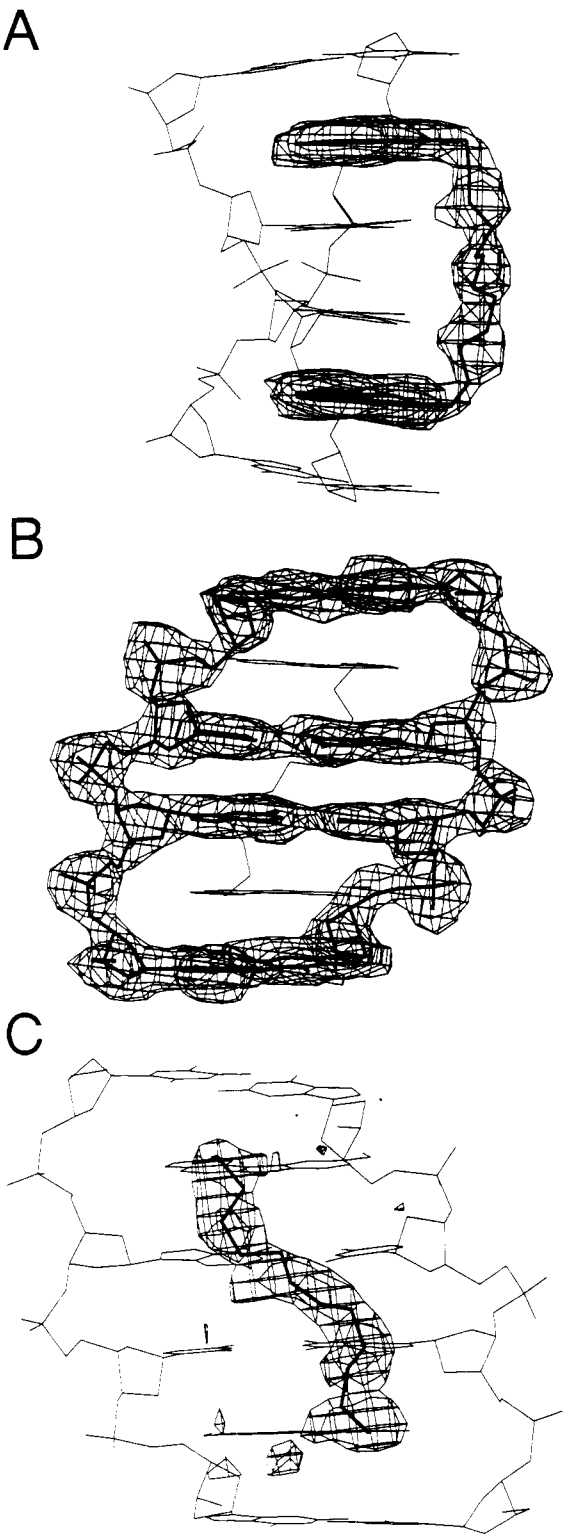

FIGURE 3: Stick drawing of the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex showing (A) Fourier sum ($2F_o - F_c$) density surrounding Flexi-Di and (B) sum density surrounding the DNA. (C) A refined omit map ($F_o - F_c$) of the linker with a stick drawing of the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex. The linker is drawn in bold lines.

Di·[d(^{Br}CGCG)]$_2$ complex, both linker nitrogens form hydrogen bonds to the floor of the major groove. One linker nitrogen forms a hydrogen bond (2.98 Å) to the N7 position of residue G(2). The other linker nitrogen forms a bifurcated hydrogen bond to the N7 (2.94 Å) and O6 (2.84 Å) positions of G(6). It appears that the hydrogen bonds between the internal guanines and the linker nitrogens effectively *pull* the central region of the DNA out into the major groove. These

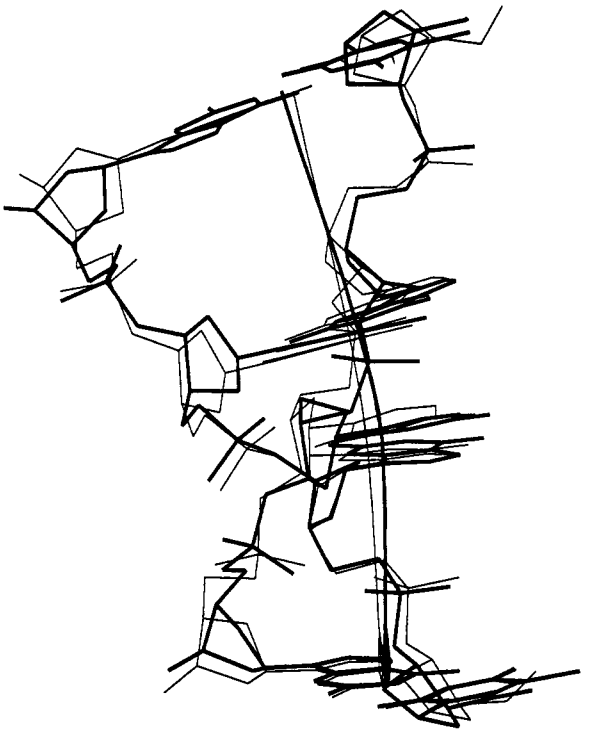

FIGURE 4: Stick drawing of the DNA from the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex drawn in bold lines and from the ditercalinium·[d(CGCG)]$_2$ complex drawn in thin lines. The helical axis of Flexi-Di·[d(^{Br}CGCG)]$_2$ is drawn with a bold arrow, and the helical axis of ditercalinium·[d(CGCG)]$_2$ is drawn with a thin arrow. Helical axes were generated using the program CURVES (Lavery & Sklenar, 1989).

hydrogen bonds, in part, cause the large, ~1.0-Å shifts of the internal base pairs into the major groove, relative to the terminal base pairs. In contrast, in the ditercalinium·[d(CGCG)]$_2$ complex, only one end of the linker forms hydrogen bonds to the floor of the major groove. Only the end of the ditercalinium complex that forms hydrogen bonds between the linker and the floor of the major groove displays a significant shift of that internal base pair into the major groove. Thus, in comparison to the ditercalinium complex, the greater axial bending of the Flexi-Di complex appears to result partially from hydrogen bonding between the DNA and both ends of the linker.

Stacking contacts between the planar moieties of the DNA and the *bis*-intercalators appear to be a second significant factor contributing to DNA bending. Stacking can be qualitatively visualized by the extent of planar overlap when viewing along the normals of the planes. Axial views of base pair–chromophore and base pair–base pair steps in both complexes are shown in Figure 5. The terminal cytosines in both complexes overlap the A-ring (Figure 5A,E,F,J). The terminal guanines partially overlap the C- and D-rings (Figure 5A,E,F,J). The pattern of overlap is essentially reversed at the interior steps (Figure 5B,D,G,I) where the guanines overlap the A- and B-rings of the chromophores. The cytosines are poorly stacked and only partially overlap the D-rings (Figure 5B,D,G,I). At the central step in both complexes (Figure 5C,H), the base pairs display extensive overlap, as seen in uncomplexed B-DNA.

At both ends of the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex, the terminal base pairs are shifted into the minor groove relative to the internal base pairs. This shift, which averages 1.06 Å [from CURVES (Lavery & Sklenar, 1989)] in the Flexi-Di complex, is observed at only one end of the ditercalinium complex. This shift is induced by the *bis*-intercalator and is absent at either set of analogous steps of the dodecamer [d(CGCGAATTCGCG)]$_2$ (Drew et al., 1981). Although DNA bending is also observed in the structure of the dodecamer [d(CGCGAATTCGCG)]$_2$, the helical axis at the [d(CGCG)]$_2$ regions of the dodecamer is not bent in the same manner as in the complex described here. Using the position of the chromophore as a reference, the terminal base pairs of Flexi-Di are shifted further toward the minor groove than are the terminal base pairs of the ditercalinium complex (Figure 5A,E,F,J). A clear result of this shift is to move the bromine atoms of the terminal cytosines out of a solvent-accessible region of the major groove and into a position overlapping the termini of the linker of Flexi-Di. This apparent bromine-induced shift of the terminal base pairs underscores the importance of stacking on the conformation of these complexes.

In both DNA complexes, van der Waals interactions are disrupted in part by planar group displacement along the helical axis. Therefore, axial views must be accompanied by an analysis of interplanar spacings and specific van der Waals contacts. The interplanar spacing component of stacking is partially quantitated by the helical rise. Relative to the ditercalinium complex, the DNA of the Flexi-Di complex is compacted in the center and extended at the termini. The helical rise of the internal step is 3.20 Å while those of the termini are 7.03 and 6.92 Å. In contrast, the helical rise of the internal step of the ditercalinium complex is 3.55 Å while those of the termini are 6.92 and 6.75 Å. Dividing the terminal helical rise values in half gives an estimate of the base pair–chromophore step sizes. These values are greater than the average but are within the range of those observed in B-DNA (Saenger, 1984).

The maximum number of van der Waals contacts between atoms of any base and of Flexi-Di is 18, occurring at the I2-G(6) step (Figure 5E). In contrast, 26 contacts are observed at the I1-G(2) step of the ditercalinium·[d(CGCG)]$_2$ complex (Gao et al., 1991; Williams & Gao, 1992). However, only 10 contacts are made between the terminal cytosines and the adjacent chromophores in the Flexi-Di·[d(^{Br}CGCG)]$_2$ structure, and none are formed at these positions in the ditercalinium·[d(CGCG)]$_2$ structure.

In both the Flexi-Di and ditercalinium complexes infinite helices are formed by the arrangement of the complexes in the crystal lattice (Figure 6). The complexes are aligned with the 5′-ends of a given strand stacked over the 3′-end of the adjacent strand. With this stacking arrangement, the pyrimidine–purine repeat is maintained throughout the lattice, and intercalation sites occur after every two base pair steps within an infinite helix. Axial contacts between complexes are similar to those within DNA. The stacking distance between terminal residues of adjacent complexes is similar to that at the G(2)-C(7) to C(3)-G(6) step. No direct lateral interactions are formed between infinite helices within the crystal.

DISCUSSION

We initially reasoned that much of the helical distortion in the ditercalinium complex resulted from the rigid nature of the *bis*(ethylpiperidinium) linker. Flexi-Di, with a flexible spermine-like linker, was expected to form a linear, more relaxed, complex with DNA. However, as described here, Flexi-Di bends DNA to a degree exceeding that of ditercalinium.

A comparison of the structures of Flexi-Di and ditercalinium bound to DNA has allowed us to propose a mechanism by

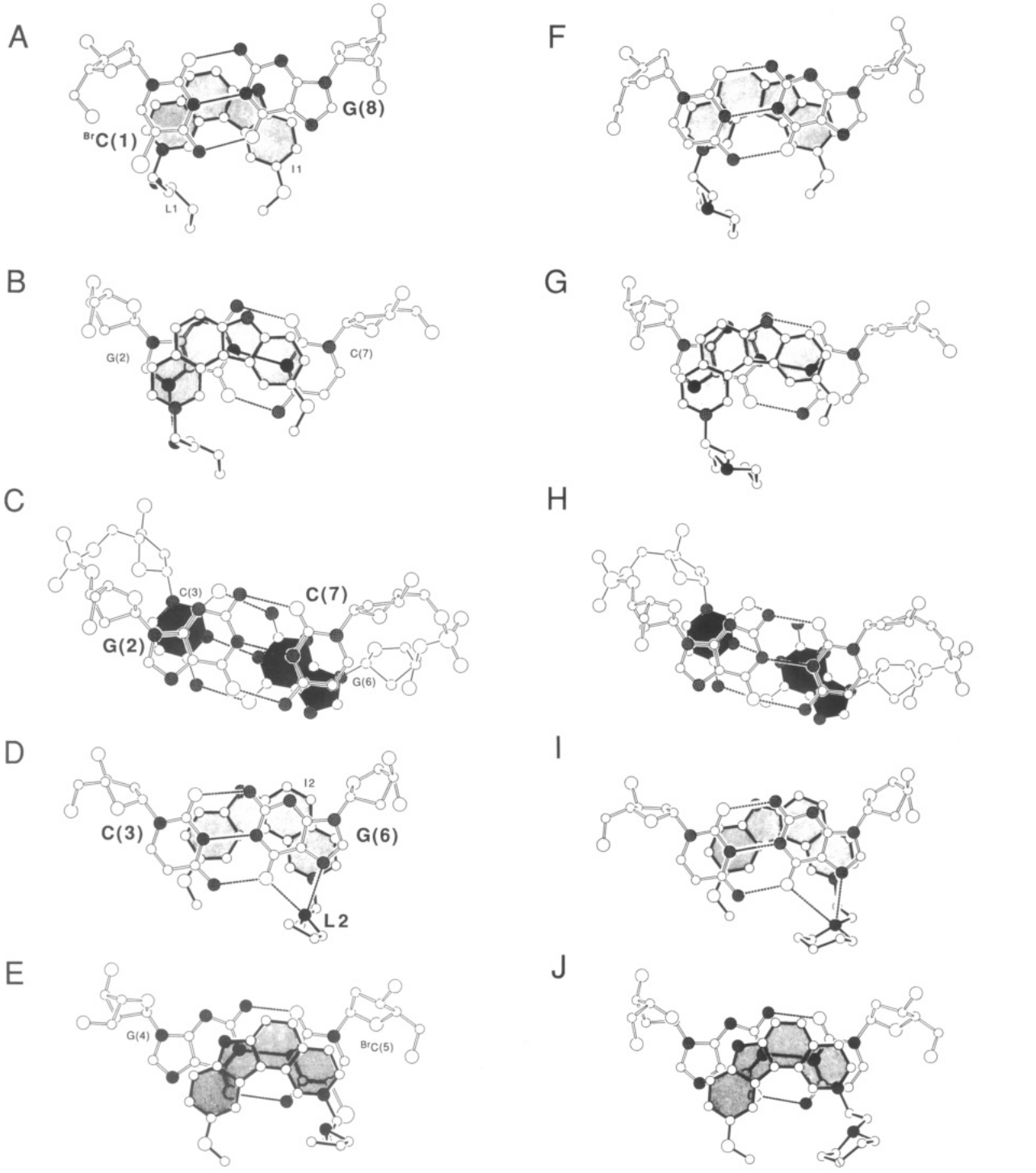

FIGURE 5: ORTEP figures of base pair–chromophore and base pair–base pair steps in the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex (A–E) and in the ditercalinium·[d(CGCG)]$_2$ complex (F–J). Each figure is viewed along the normal to the best plane of the uppermost base pair or chromophore. Residues in the Flexi-Di·[d(^{Br}(CGCG)]$_2$ complex are labeled by base, chromophore (I for intercalator), and linker (L); analogous steps in the ditercalinium·[d(CGCG)]$_2$ complex are shown in the same order. The chromophores of both Flexi-Di and ditercalinium are drawn in thick, solid bonds and are shaded. The DNA is drawn with hollow bonds except the innermost steps (C and H) where the lower base pair is drawn with thin solid bonds and is shaded. Atoms are sized by type with Br > P > O > N > C. Linker nitrogens are shaded in black and all other nitrogens are stippled. Hydrogen bonds are indicated by dashed lines. The labels of the residues that are nearer to the viewer are larger than those further from the viewer.

which these *bis*-intercalators bend DNA. In this model DNA bending does not arise as a consequence of linker rigidity. We propose that this class of *bis*-intercalators *pulls* the internal base pairs into the major groove and *pushes* the external base pairs into the minor groove. The result is a bend toward the minor groove in the DNA helical axis. It appears that hydrogen bonds between the linker and the internal guanines effectively pull the central base pairs of the complex out into the major groove. The position of each internal base pair relative to its adjacent chromophore is determined by base pair–linker hydrogen bonds. At the external regions of the complex, stacking interactions between the chromophores and terminal base pairs effectively push the terminal base pairs into the minor groove. The combination of pulling the internal base pairs into the major groove plus pushing the external base pairs into the minor groove, shown in Figure 2 and 4,

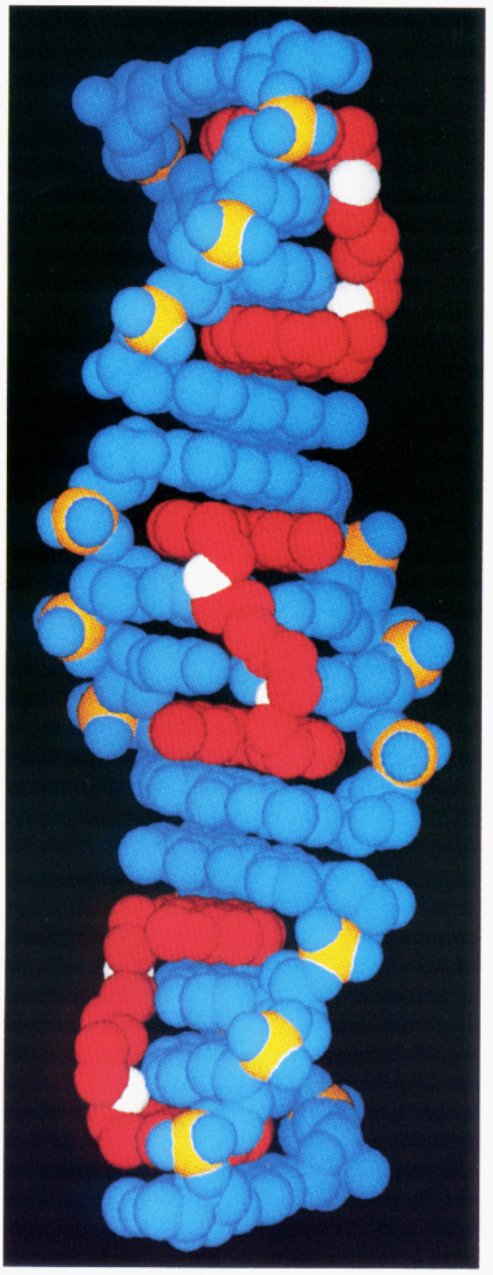

FIGURE 6: Space-filling model of a continuous helix in the Flexi-Di·[d(^{Br}CGCG]$_2$ complex. Terminal complexes were generated by the following symmetry operators: (i) $y - {}^1/_2, {}^1/_2 - x, z - {}^1/_4$ and (ii) ${}^1/_2 - y, x + {}^1/_2, z + {}^1/_4$. Flexi-Di atoms are colored red except linker nitrogens which are white. The DNA is blue with gold phosphorus atoms to highlight the helical backbone. The three representations of the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex were generated using the program INSIGHT II (1991).

results in a bend in the DNA helical axis toward the minor groove.

Support for this mechanism of DNA bending is provided by the greater extent of bending observed in the Flexi-Di complex compared to the ditercalinium complex. The push/pull combination is observed at both ends of the Flexi-Di complex, but only at one end of the ditercalinium complex. One of the linker nitrogens of ditercalinium forms hydrogen bonds to the DNA while the other does not. Ditercalinium cannot form hydrogen bonds simultaneously with both linker nitrogens. The molecular basis of this asymmetry is in the stereochemical restraints of the *bis*(ethylpiperidinium) ring system. When one piperidinium nitrogen forms a hydrogen bond to the floor of the major groove, preferences for chair conformation and staggered exocyclic rotamers direct the proton of the second nitrogen away from the floor of the major groove. Thus the second nitrogen cannot achieve proper geometry for hydrogen bond formation. (The relative positions of the two protons are shown schematically in Figure 1B.) The proscription against simultaneous hydrogen bonds at both ends of the linker of ditercalinium decreases the bend in that complex compared to the Flexi-Di complex. The shift of the terminal base pair of the Flexi-Di·[d(^{Br}CGCG)]$_2$ complex may be somewhat exaggerated by the presence of the bromines at the 5-position of the terminal cytosines. In the Flexi-Di·[d^{Br}CGCG)]$_2$ complex, the bromines stack upon the intercalator. This stacking is facilitated by the shift of the terminal base pairs into the minor groove. Halogenation of nucleosides has been shown to alter DNA conformation. Base stacking patterns with bromo derivatives show the bromines overlapping adjacent bases (Bugg & Thewalt, 1969; Bugg et al., 1971).

The interactions of both Flexi-Di and ditercalinium appear to be specific for 5′ Py-Pu-Py-Pu 3′. In general, intercalated complexes are more stable at 5′ Py-Pu 3′ steps than at other sites in DNA and nearly universally most stable at 5′ C-G 3′ over 5′ T-A 3′ steps (Kastrup et al., 1978; Krugh & Reinhardt, 1975; Krugh et al., 1975; Muller et al., 1975; Muller & Crothers, 1975). These stacking specificities of the Flexi-Di and ditercalinium complexes are in concert with the hydrogen-bonding specificities. The linkers, which donate hydrogen bonds to the floor of the major groove, span the groove diagonally. Thus complex formation requires internal purines to lie diagonally across the groove (i.e., a 5′ Pu – Py 3′ step) for correct placement of hydrogen bond acceptors.

Protein Recognition. There are several unusual structural features of DNA complexes of Flexi-Di and ditercalinium. Base unstacking and minor groove mimicry of unperturbed DNA are two features of these complexes that possibly contribute to the unique activity of these intercalators. Although Flexi-Di and ditercalinium form stable complexes with DNA, poor stacking within the complexes might represent regions of local instability. This feature is unusual for intercalated-DNA complexes and may serve to mimic covalent DNA damage. In addition, the availability of the minor groove of Flexi-Di· and ditercalinium·DNA complexes may be a critical feature for protein recognition. Both Flexi-Di and ditercalinium intercalate into DNA via the major groove, leaving the minor groove clear for binding by other agents. Furthermore, a distinct B-DNA-like pattern of hydrogen bond donors is present in both intercalated-DNA complexes. The presence of a bulky linker in the major groove is an unlikely feature for protein recognition since DNA complexes with nogalamycin, a monointercalator with its bulky sugar in the major groove, are not recognized by prokaryotic DNA repair enzymes [discussed in Williams and Gao (1992)].

A significant distinction between ditercalinium and Flexi-Di is that ditercalinium is likely to form a dynamic complex, with its linker flipping between two isoenergetic states, while Flexi-Di is likely to form a static complex with a single stable ground state. It is reasonable to imagine that in solution the ditercalinium complex rapidly interconverts between two conformers in which first one linker nitrogen, then the other, forms hydrogen bonds to the DNA. Thus this complex would be in dynamic equilibrium. A dynamic ditercalinium·DNA complex and a static Flexi-Di·DNA complex are consistent with the longer lifetimes determined from NMR data for Flexi-Di as compared to ditercalinium in DNA complexes (Pothier et al., 1991).

Influence of Crystal Packing. In DNA crystallography, the influence of crystal packing on conformation must always

be considered. Lattice forces are known to induce bending of B-DNA (DiGabriele et al., 1989) and alterations of backbone torsion angles and other helical parameters in A-DNA (Ramakrishnan & Sundaralingam, 1993). In contrast, the effects of lattice on conformation and hydration of DNA·anthracycline complexes (Williams et al., 1990) and Z-DNA (Egli et al., 1991) have been shown to be less significant. It may be that DNA conformation of drug complexes is less malleable than DNA alone due to the tendency of drugs to stabilize and stiffen DNA. However, it is clear that a complete understanding of the class of *bis*-intercalators described here requires crystallization in additional crystal forms and with longer fragments of DNA.

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