Articles

Drug Binding to a DNA BZ Molecule: Analysis by Chemical Footprinting†

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ABSTRACT: The polymorphism in a DNA 16-mer (designated BZ-II) has been investigated by means of circular dichroism (CD) spectroscopy and chemical footprinting. CD spectra indicate that, in low salt, the oligomer is fully right-handed whereas, in high salt, it possesses a B–Z conformational junction: half of the duplex is right-handed while the other half is left-handed. Treatment of BZ-II with diethyl pyrocarbonate (DEPC) confirms the existence of a left-handed segment of the duplex in high salt: enhanced DEPC scission occurs at the G residues in the alternating CG sequence. The scission patterns of the upper and lower strands in BZ-II by the reactive chemical probe MPE·Fe(II), and the antitumor antibiotics dynemicin and Fe(II)·bleomycin, are different under low salt conditions. The 3′-terminal region of both upper and lower strands and the middle region of the upper strand of BZ-II are preferential cleavage sites in low salt. This result suggests that the methylated cytosines or the alternating CG domain in the molecule perturbs the DNA structure. Under high salt conditions, the reactivity of the Z-DNA segment of BZ-II for MPE·Fe(II) and Fe(II)·bleomycin is dramatically enhanced, while it is reduced in the case of dynemicin. Excess propidium (PI) eliminates preferential cleavage by each of these chemical probes in high salt conditions. This is due in part to conversion of the BZ-DNA molecule into B-DNA, as is seen by a DEPC modification experiment. At low PI concentrations, the DEPC experiment suggests that the Z conformation remains intact; thus, at sufficiently low drug concentrations, we believe the cleavage data reveal a preferential binding mode of these drugs to the Z sequence itself that does not require concomitant Z → B isomerization.

Variant states and structures of DNA have biological and physical properties that differ from those of bulk DNA. Examples include sites of base mismatches (Hunter et al., 1986), bulges (Williams & Goldberg, 1988a,b), sequences that induce bending of the helix axis (Hagerman, 1986; Koo et al., 1986), cruciforms (Mizuuchi et al., 1982), H-DNA (Htun & Dahlberg, 1988) and other branched species (Seeman, 1982), left-handed Z-DNA [for review, see Rich et al. (1984)], and synthetic B–Z DNA junctions (Sheardy, 1988; Sheardy & Winkle; 1989; Dai et al., 1989; Doktycz et al., 1990). Some of these states have been implicated in biological functions including mutagenesis, control of transcription, or genetic recombination. At present, however, the biological role of Z-DNA remains unclear. Whether or not left-handed Z-DNA is a functional conformational element in vivo (Jaworski et al., 1987), the mechanism of the structural conversion of right-handed B-DNA to left-handed Z-DNA is of physicochemical interest (Singleton et al., 1983; Jovin & Soumpasis, 1987; Manzini et al., 1987; Chen et al., 1987; Zacharias et al., 1988; Luthman & Behe, 1988; Soumpasis, 1988; Sheardy, 1988).

Short synthetic DNA oligomers that exhibit salt-dependent polymorphism have made it possible to determine a number of features of the interface between B and Z sequences (Sheardy, 1988; Sheardy & Winkle, 1989; Dai et al., 1989; Poktycz et al., 1990). These oligomers contain a run of alternating CG or similar Z forming sequences abutting a region that lacks this feature (see Scheme I). ^{1}H NMR spectroscopy of an oligomer of this type, referred to as BZ-I, indicates that the interface, or B–Z junction, spans three base pairs, one of which appears to be highly distorted (Sheardy & Winkle, 1989). CD characterization of the high salt form of this molecule is consistent with the idea that the molecule contains both left-handed and right-handed sequences. Raman spectroscopy on another short oligomer capable of forming a B–Z junction confirms that the junction consists of approximately three base pairs (Dai et al., 1989). Thermodynamic analysis of the salt-induced B–Z transition shows that the free energy of forming a single B–Z junction is about 4.7 kcal/mol per junction (Doktycz et al., 1990). All these results suggest that short oligomers of appropriate sequence can accommodate a B–Z junction under high salt conditions.

Scheme I: Upper and Lower Strands of the Duplex BZ-II, with C* Denoting 5-Methyldeoxycytidine

5′-C*-G--C*-G--C*-G--C*-G--A--T--C--G--A--C--T--G-3′ (upper)

3′-G--C*-G--C*-G--C*-G--C*-T--A--G--C--T--G--A--C-5′ (lower)

An important issue with respect to characterizing B–Z DNA molecules concerns how they respond to different ligands capable of interacting with double-stranded DNA. The salt-

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induced B to Z transition can be influenced by drugs, including intercalators (Pohl et al., 1972; Mirau & Kearns, 1983), carcinogens (Nordheim et al., 1983; Rio & Leng, 1983), and minor-groove binding ligands (Zimmer et al., 1983). We have been particularly interested in the ligand binding properties of the interface or junction between B and Z sequences in synthetic DNA hybrid oligomers. This is a region with unique structural properties (Sheardy & Winkle, 1989) that in principle has the potential to interact selectively with drugs (Walker et al., 1985; Suh et al., 1991). Certain drugs have in fact been found to bind cooperatively to Z-DNA, inducing formation of right-handed DNA (Walker et al., 1985a,b; Chaires, 1985). Walker et al. (1985a,b) investigated the effect of ethidium and actinomycin D on the B–Z transition in poly(dG-dC)·poly(dG-dC) and concluded that cooperative binding of these drugs to left-handed Z-DNA reverses the conformation of the helix into right-handed B-DNA. Subsequently, Chaires and his co-workers have investigated the interaction between ethidium bromide (EB) and BZ-I using fluorescence spectroscopy (Suh et al., 1991). They report that ethidium binding is highly cooperative in high salt but surprisingly does not seem to involve drug–drug interactions. The binding constant is tighter in the presence of high salt rather than the opposite. However, the specific sites involved could not be identified in these experiments. In order to do so, and in particular to define the drug binding properties of the B–Z junction region itself, we have performed footprinting experiments, using a mixed-sequence molecule designated BZ-II that has a sequence at the junction different from that of BZ-I (see Scheme I).

A variety of cleavage reagents are used as probes in DNA footprinting experiments, including the enzyme DNase I (Galas & Schmitz, 1973), UV irradiation (Becker & Wang, 1984), and a number of chemicals that modify DNA (Ogata & Gilbert, 1977; Siebenlist et al., 1980) or induce radicals in the presence of light or redox agents (Dervan, 1986; Ward et al., 1986; Sigman, 1986; Tullius, 1987). In general the "footprint" signal obtained with a particular reactive probe depends on both the nature of the active species and the mode and strength of interaction between the probe and DNA. In the case of a polymorphic DNA substrate such as BZ-II, we are interested in the differential properties of regions of the sequence under low and high salt conditions. The probe MPE·Fe(II) consists of an intercalating ring system covalently tethered to an EDTA moiety that binds iron and can generate OH radicals in the presence of H_2O and peroxide or oxygen (Hertzbert & Dervan, 1982, 1984). MPE·Fe(II) cleaves double-stranded B-DNA with little sequence specificity (Hertzberg & Dervan, 1982; van Dyke & Dervan, 1983) but reacts preferentially at the junction region in branched DNA oligomers (Guo et al., 1989, 1990) as well as at sites of base bulging in duplex DNA (Williams & Goldberg, 1988a,b). Dynemicin is a potent anthracycline antitumor antibiotic that induces single-strand breaks in DNA by formation of phenyl diradicals from its enediyne core in the presence of a thiol compound (Sugiura et al., 1990). Dynemicin cleaves normal duplex DNA with relatively low sequence specificity (Sugiura et al., 1990) and has also been shown to interact preferentially at the branch point in synthetic DNA junctions (Lu et al., 1991). A second antitumor antibiotic, Fe(II)·bleomycin, also cleaves B-DNA with moderate sequence and/or structural selectivity (Kuo & Haidle, 1974; Giloni et al., 1981; Burger et al., 1982; Wu et al., 1983). These reagents are used here in conjunction with diethyl pyrocarbonate, which has been shown to discriminate B from Z sequences (Johnston & Rich, 1985), to probe the structural state and reactivity with drugs of BZ-II.

Experimental Procedures

Materials. Bleomycin was provided by Dr. Richard Burger of the Publich Health Research Institute, New York. Dynemicin was kindly supplied by Dr. Konishi of the Bristol-Myers Squibb Research Institute, Tokyo, Japan. Propidium diiodide and diethyl pyrocarbonate were purchased from Sigma. All other chemicals used were of commercial reagent grade.

Synthesis and Purification of Oligonucleotides. Oligonucleotides used in this study were synthesized on an Applied Biosystems 380 B DNA synthesizer by use of "trityl-on" phosphoramidite chemistry (Caruthers, 1982) and purified by reverse-phase HPLC. Product purity was verified by analytical HPLC and polyacrylamide gel electrophoresis. Oligonucleotides were labeled at their 5′ terminus with use of T4 polynucleotide kinase (Boehringer), and the labeled strands were isolated by polyacrylamide gel electrophoresis.

Annealing Reactions. The duplex of BZ-II was formed by mixing equimolar quantities of the individual strands at 15 μM concentration in a buffer containing 50 mM Tris-HCl, pH 7.5, and 4.5 M NaCl in a total volume of 15 μL. An Eppendorf tube containing the solution was immersed in boiling water for 3 min and allowed to cool slowly. The B-form duplex was formed similarly, in 50 mM Tris-HCl, pH 7.5, and 100 mM NaCl.

CD Spectroscopy. Circular dichroism spectra were recorded with an AVIV Model 60DS CD spectropolarimeter at 25 °C. DNA samples ([DNA] = 10^{-4} M in base pairs) were prepared in 0.01 M phosphate buffer (1 mM EDTA, pH 7.0) in either low salt ([NaCl] = 0.10 M] or high salt ([NaCl] = 5.0 M). For salt titrations, aliquots of the high salt solution were added to the low salt solution to obtain DNA samples of varying NaCl concentration.

DNA Cleavage Reactions. DEPC: DNAs were modified by diethyl pyrocarbonate following the procedure of Herr (1985). The 15 μL of DNAs (15 μM) with or without 45 μM propidium was suspended in a buffer of 50 mM Tris-HCl (pH 7.5), together either with 4.5 M NaCl or with 100 mM NaCl. Two microliters of DEPC was added to each sample. The samples were incubated for 60 min at 25 °C. Because DEPC is relatively insoluble in water, the samples were thoroughly mixed at the beginning as well as halfway through the 60-min incubation. Following DEPC reactions, the DNA was incubated with 100 μL of 1 M piperidine at 90 °C for 30 min to cleave the sites of DEPC modification, followed by extensive lyophilization. *MPE·Fe(II)*: Our procedure followed that of van Dyke and Dervan (1983) and Guo et al. (1989). For cutting both BZ- and B-DNA molecules, 15 μL of DNA (15 μM) was exposed to 5 μM Fe(II) and 5 μM MPE in a buffer containing 10 mM Tris-HCl, pH 7.5, and 50 mM NaCl with or without 45 μM propidium for 15 min at 25 °C, followed by addition of 4 mM DTT for 30 min. The reaction was stopped by extraction with 1-butanol and precipitation with ethanol. *Dynemicin*: Dynemicin was dissolved in 100% methanol. The reaction samples contained 15 μM DNAs, 50 mM Tris-HCl (pH 7.5), together with 4.5 M NaCl or with 100 mM NaCl in a 20-μL final volume. The cleavage reaction was initiated by addition of 0.1 mM dithiothreitol, and the samples were incubated with 0.1 μg of dynemicin at 25 °C for 45 h with or without 45 μM propidium. Reactions were terminated by 1-butanol extraction and two sequential rapid ethanol precipitations. *Fe(II)·Bleomycin*: Bleomycin sulfate (Bleoxane) contained approximately 60% bleomycin A_2, 30%

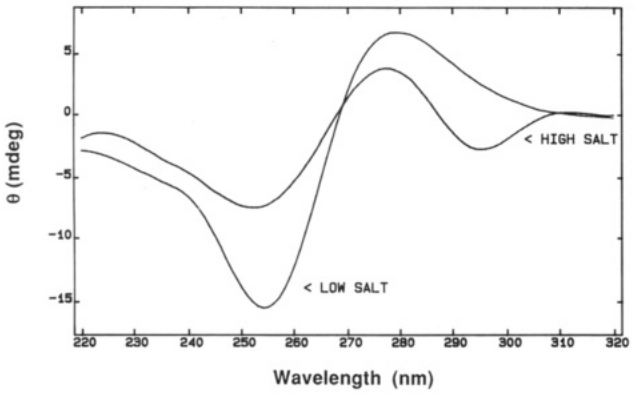

FIGURE 1: CD spectra of BZ-II in phosphate buffer at low salt (100 mM NaCl) and high salt (5.00 M NaCl) at 25 °C. The low salt spectrum is characterized by a trough at 254 nm and a peak at 281 nm, while the high salt spectrum is characterized by a trough at 254 nm, a peak at 279 nm, and a shallow trough at 295 nm. These spectra are nearly identical with those of BZ-I, which has been shown to possess a B–Z conformational junction at high salt (Sheardy, 1988; Sheardy & Winkle, 1989).

bleomycin B_2, and 10% various other bleomycins. Reaction mixtures contained 15 μM DNAs, 5 μM bleomycin, 5 μM Fe(II), and 50 mM Tris·HCl (pH 7.5) together either with 4.5 M NaCl or with 100 mM NaCl. The cleavage reaction was initiated by addition of 1 mM dithiothreitol; the samples were incubated at 25 °C for 30 min with or without 45 μM propidium. Reactions were stopped by 1-butanol extraction and ethanol precipitation. *Sequencing Reaction*: Purine (A+G) sequencing ladders were generated from each 5′-^{32}P-labeled oligonucleotide with use of the piperidine formate reaction (Maxam & Gilbert, 1980).

Gel Electrophoresis. Samples of products of cleavage reactions were taken up in formamide loading buffer, heated briefly to 90 °C, cooled, then run on a 20% denaturing polyacrylamide gel for 3 h at 2000 V (ca. 50 V/cm) and 45 °C. No dyes were added in these runs. The gel was dried immediately on a vacuum drying apparatus (Hoefer) and exposed at room temperature to X-ray film (Kodak X Omat) without an intensifier screen. Autoradiograms were scanned on a Hoefer GS300 densitometer, without base-line corrections.

Results

BZ-II Has a Hybrid Structure in High Salt. The CD spectra of the BZ-II duplex under low and high salt conditions are shown in Figure 1. The conservative low salt spectrum indicates that BZ-II is fully right-handed under low salt conditions. The high salt spectrum is consistent with the presence of a B–Z junction (Sheardy, 1988; Sheardy & Winkle, 1989). Since the mixed-form CD spectrum does not reveal whether half of the population of molecules are B and the remainder are Z or whether the whole population is 50% B, 50% Z, the duplex was exposed to diethyl pyrocarbonate, a reagent that has been found to react preferentially with purines in the Z conformation (Johnston & Rich, 1985). The results in Figure 2 show that only the G bases residing in the $(C^*G)_4$ sequence of BZ-II display enhanced reactivity under high salt conditions. This is consistent with the repeated (meCG) sequence of the duplex taking on a Z conformation, and not the whole sequence. This differential reactivity does not extend into the B segment, implying that the three base pairs abutting the Z segment are not "open" (Herr, 1985). The midpoint for the conformational transition obtained by NaCl titration of BZ-II occurs at 2.9 M NaCl (data not shown), different from that reported for BZ-I. Thus, the free energy

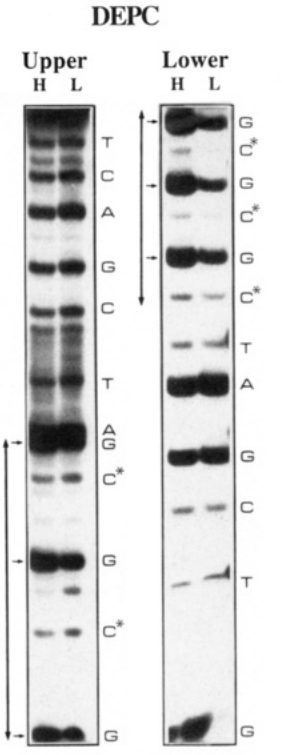

FIGURE 2: Diethyl pyrocarbonate modification reactions of the two strands of BZ-II at low salt (L) and high salt conditions (H). This experiment was performed as described under Materials and Methods. The autoradiograph of the gel is shown of the upper strand (left) and the lower strand (right) of BZ-II. The small arrows indicates sites of differential enhanced reactivity of the Z-DNA region in the high salt form relative to the low salt form.

of a B–Z junction depends on the sequence (R. D. Sheardy, unpublished results).

Cleavage of BZ-II by MPE·Fe(II) and the Effect of Propidium. In this experiment the probability of chain scission at different positions in BZ-II is monitored by use of the reactive probe MPE-Fe(II) (van Dyke & Dervan, 1983; Hertzberg & Dervan, 1984) and 5′-^{32}P-labeled strands under low and high salt conditions. The extent of cleavage at a given site is normally taken to reflect the relative strength of binding the methidium moiety (van Dyke & Dervan, 1984; Guo et al., 1989, 1990). In low salt, we recall that the duplex appears to be fully right-handed B-DNA according to its CD spectrum, while in high salt the C*G-containing segment switches to a Z conformation. Comparison of the strands of BZ-II exposed to MPE·Fe(II) should reveal sites of potential difference in drug binding between the B-DNA and Z-DNA domains and define a postulated interaction at the junction itself (Walker et al., 1985a,b; Suh et al., 1991). Two kinds of comparison are described: first, reaction of MPE·Fe(II) with B-DNA vs BZ molecule, looking for differential effects in the latter that are absent in B-form duplex; and second, reaction of B-DNA + PI vs BZ molecule + PI, monitoring the effect of propidium on transition of BZ molecule into B-DNA [see Suh et al. (1991)].

Figure 3 shows the results of the cleavage of BZ-II by MPE·Fe(II), in which the experiments were carried out at a molar ratio of 1 MPE·Fe(II) per 3 duplexes (5 μM:15 μM). At this concentration, the intercalation of methidium is not accompanied by a highly cooperative left- to right-handed conformational switch of the polynucleotide as detected by CD spectroscopy. The autoradiograph of the cleavage products and the corresponding densitometric scans shown in Figure 3A reveal striking differences between the cleavage reactions

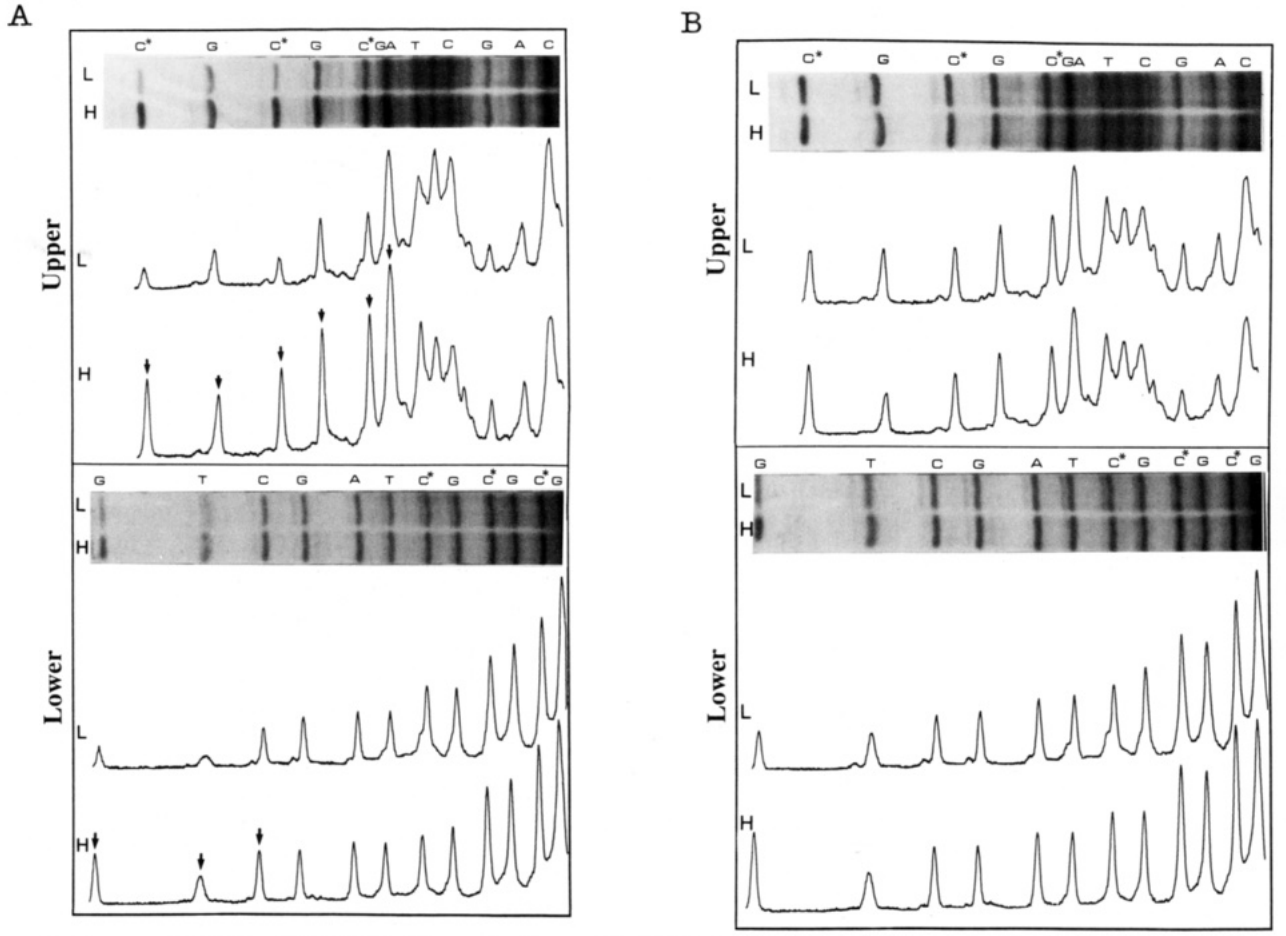

FIGURE 3: Cleavage of BZ-II by MPE·Fe(II) with or without excess PI. (A) Autoradiograph and the corresponding densitometric scans showing cleavage of the upper strand and lower strand of BZ-II at low salt (L) and high salt (H) conditions. The band that appears between the T and C residues in the upper strand is apparently an artifact of the cleavage reaction. The arrows indicate sites of enhanced cleavage in the high salt form relative to the low salt form. (B) Autoradiograph and the corresponding densitometric scans showing cleavage of the upper strand and lower strand of BZ-II at low salt and high salt conditions in the presence of PI. The 3-fold excess PI ([DNA]/[PI] = 1:3) was incubated with the DNA samples for 1 h at room temperature before the cleavage reaction was initiated.

under low and high salt conditions. The smearing in the gel that is seen in the upper strand in both lanes is a consequence of the presence of methylated C, since C does not show this. The gels were repeated at higher T to try to eliminate the smearing, without success. The proximity of the G and A bands varies slightly and allows us to distinguish the two (in the sequence gel in Figure 2 for example). Under low salt conditions, residues at positions 8–11 and 14 in the upper strand, and 11–14 in the lower strand, are preferentially cleaved (Figure 3A). Under high salt conditions the pattern is different: residues at positions 3–9 of the upper strand display enhanced reactivity, while residues at positions 3–5 of the lower strand show minor enhancement relative to low salt form (Figure 3A). This suggests that the methidium moiety binds preferentially to the Z-DNA region and possibly to the B–Z junction region also, providing the concentration of MPE is not high enough to drive the BZ hybrid into fully B-form DNA.

The fact that ethidium and other interacalators can interact specifically with Z-DNA, converting Z-DNA into B-DNA (Walker et al., 1985a,b; Chaires, 1985), raises the possibility that MPE·Fe(II) is doing this in BZ-II. To test this, we added excess propidium to the BZ-II solution. If the excess propidium results in apparent conversion of the B–Z hybrid structure into B-form DNA, differential cleavage of BZ-II by MPE·Fe(II) at high salt conditions should be eliminated. In order to test this prediction, we added a 9-fold excess of propidium diiodide over MPE·Fe(II) to the reaction mix, equilibrated the mix for 1 h, and repeated the MPE·Fe(II) cleavage experiment. The resulting densitometric traces are shown in Figure 3B. Comparison of the cleavage profiles under low salt conditions in the presence and absence of PI shows an interesting feature: the cutting profiles of the upper strands under high salt conditions actually lack some of the distinctive features of the profiles shown in Figure 3A. In the presence of excess PI, competition for the DNA binding sites tends to make all sites equivalent to the methidium; thus all sites on both strands are cleaved nearly equally as observed. The results obtained under high salt conditions might either be due to conversion of BZ-II to a fully right-handed conformation by the excess propidium or represent a "footprint" of propidium (van Dyke & Dervan, 1983a,b).

Monitoring the Conversion of B–Z Conformation DNA into B-Form DNA in the Presence of Excess PI by DEPC Modification. DEPC reacts more strongly with purines within the Z-DNA region of BZ-II (Figure 2). It reacts with G to cause opening of the imidazole ring and carbethoxylation at N7 (Vincze et al., 1973). Since this lies in the major groove, the modification pattern of BZ-II in the presence of excess propidium should probe features of the major groove and potentially yield a footprint of bound PI. The results of DEPC modification experiments with or without excess PI are shown in Figure 4. In the absence of propidium, the characteristic enhancement of cleavage in the G's of the Z region of BZ-II is seen in high salt (Figure 4A); in the presence of excess propidium (DNA:propidium = 1:3), this enhancement dis-

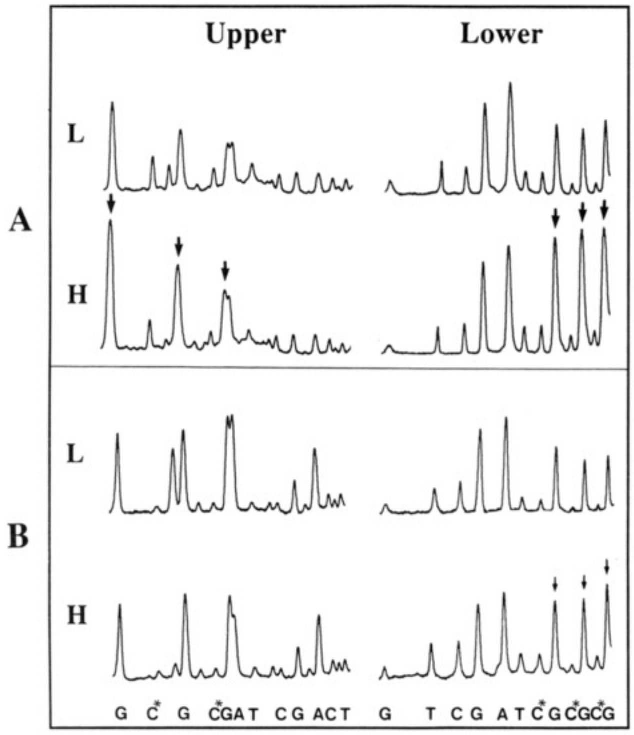

FIGURE 4: Verification of the conversion of B–Z conformational DNA into B-form DNA in the presence of excess PI by DEPC modification reaction. Densitometer scans are shown of the DEPC modification experiment of BZ-II in the absence (A) and presence (B) of PI. The 3-fold excess PI ([DNA]/[PI] = 1:3) was incubated with the DNA samples for 1 h at room temperature before the modification reaction was initiated. The same conventions apply as in Figure 2.

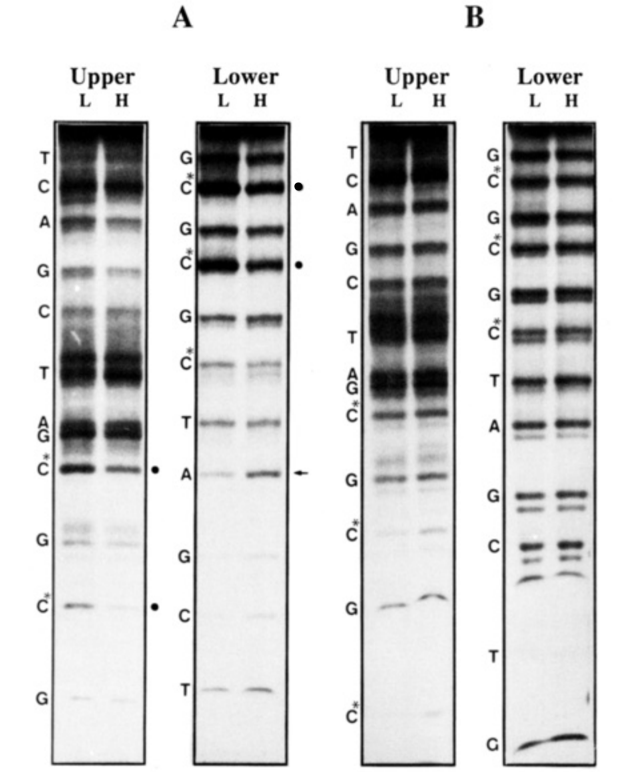

FIGURE 5: Cleavage of BZ-II by dynemicin with or without excess PI. The autoradiograph of the gel is shown of the cleavage products of BZ-II in the absence (A) and presence of PI (B). The same conventions apply as in Figure 3. The sites of differential protection of the cleavage at high salt form relative to low salt form are indicated by solid circles.

appears completely for the upper strand but not the lower strand (Figure 5B). This result suggests that excess propidium only partially switches the BZ-II hybrid conformation to right-handed DNA and that the two strands in the complex remain inequivalent. A possible "footprint" of PI in the upper strand can be seen at high salt, where the A flanking the Z segment is protected from DEPC modification.

What is the effect of a lower concentration of PI on BZ-II? We repeated the experiment shown in Figure 4 using a ratio of 2:3 for PI:BZ-II to answer this question. The result indicates that the enhanced scission by DEPC still occurs on both strands at this lower ratio (data not shown). Thus, we infer that there is some threshold concentration required for the conformational switching of Z $\rightarrow$ B in this molecule. Since BZ-II is an oligonucleotide, this is probably a range of values rather than a single critical concentration. Below this range, preferentially binding to the CG domain can occur in high salt, while the structure can remain in the Z state. Above this range, conformational switching does occur, although not uniformly over the sequence of the molecule. The lower right-hand panel in Figure 4B shows partial conversion of the lower strand sequence relative to the upper.

Cleavage of BZ-II Propidium by Dynemicin and the Effect of PI. Dynemicin is a combination of an anthraquinone with an enediyne unit; it cleaves normal duplex DNA with relatively low sequence specificity (Sugiura et al., 1990). This drug preferentially interacts with the branch of synthetic DNA junctions (Lu et al., 1991). It is of interest to know how dynemicin reacts with the two different conformations of BZ-II. Autoradiographs of dynemicin cleavage experiments in the presence and absence of excess propidium are shown in Figure 5. Under low salt conditions when BZ-II displays normal B-DNA conformation, dynemicin preferentially cleaves residues near the 3′ termini of both strands as well as residues in the B–Z junction region of the upper strand: at positions 7–10 and 13–15 of the upper strand and at positions 11–14 of the lower strand (Figure 5A). These results, together with those of MPE·Fe(II) cleavage (Figure 3), suggest that *under B-form conditions* the junction presents a conformational anomaly that is detected by the intercalative drugs. Previous studies have pointed out that the junction region of BZ-I may have unusual structural and dynamical properties under such conditions (Sheardy, 1988; Winkle & Sheardy, 1989); the cleavage patterns observed for BZ-II show that there is a structural perturbation in this region. Under high salt conditions, the Z region of both strands of BZ-II displays reduced reactivity to the dynemicin reagent.

Figure 5B shows the cleavage pattern of BZ-II generated by dynemicin after pretreatment with excess propidium (DNA:PI = 1:3). The cleavage patterns of BZ-II under low salt conditions with excess propidium are altered: residues at position 7 of the upper strand and at position 11 of the lower strand are clearly protected (Figure 5B). Elimination of the protection in the Z region of BZ-II under high salt conditions in the presence of propidium is consistent with the interpretation that the excess propidium converts BZ-II to a fully right-handed conformation, resulting in nearly equivalent cleavage of the two strands.

Cleavage of BZ-II in the Presence and Absence of Propidium by Fe(II)·Bleomycin. Bleomycin cleaves double-stranded DNA with the sequence specificity for 5′–3′ GC and CT in the presence of Fe^{2+} and molecular oxygen (D'Andrea & Haseltine, 1978; Kross et al., 1982; Wu et al., 1983, 1985). Since bleomycin is not an intercalator, its action on BZ-II at both low and high salt conditions is of interest. Fe(II)·bleomycin is used here to footprint both the normal B form and B–Z forms of BZ-II. At low salt conditions, Fe(II)·bleomycin

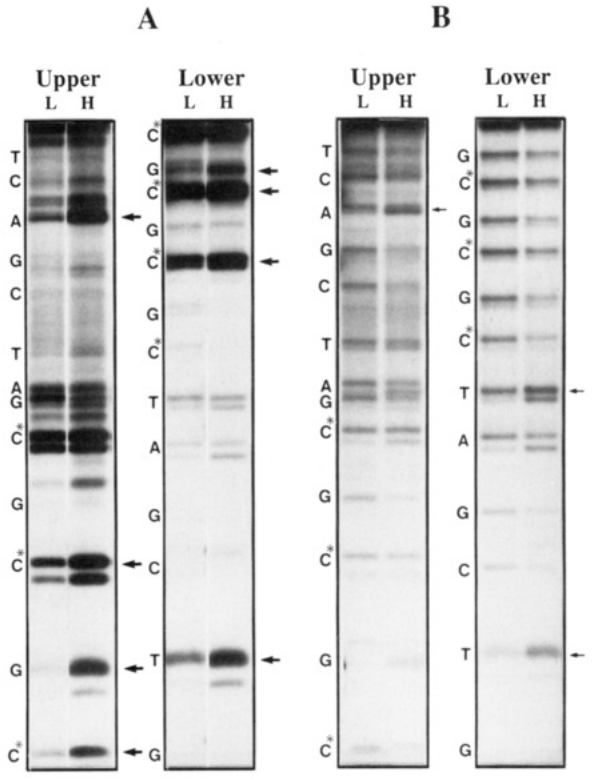

FIGURE 6: Cleavage of BZ-II by Fe(II)·bleomycin without or with excess PI. The autoradiograph of the gel is shown of the cleavage products of BZ-II by Fe(II)·bleomycin in the absence (A) and presence of PI (B). The same conventions apply as in Figure 3.

shows preferential cutting at sites 5, 7–9, and 13 of the upper strand, and 4, 11, and 13–15 of the lower strand (Figure 6A). However, at high salt conditions, drastically enhanced cleavage is observed at both strands: 3–5 and 13 in the upper strand, and 4, 11, 13, and 14 in the lower strand (Figure 6B). Most of these hyperreactive sites are located in the Z-DNA segment, suggesting that Fe(II)·bleomycin binds preferentially to the Z-DNA even in the high salt conditions.

It has been reported that intercalators such as ethidium bromide can alter the sequence-specific cleavage of DNA by bleomycin (Sugiura & Suzuki, 1982). If bleomycin is sensitive to the presence of intercalators, can bleomycin detect the difference in PI binding to BZ-II between low and high salt conditions? We therefore repeated the cleavage reaction after equilibrating the DNA samples with excess PI for 1 h. As shown in Figure 6B, the cleavage pattern in the presence of excess PI is dramatically altered (Figure 6, part A vs part B). Interestingly, the hyperreactive sites in the high salt form at the Z-DNA segment of both strands disappear, while the enhanced cutting in the presumed B-form region (13 of the upper strand and 4 of the lower strand) remains.

Discussion

Some aspects of the interaction between ethidium bromide and a BZ-DNA oligomer have been discussed recently by Suh et al. (1991). The interaction is a complex one, and evidence for the presence of multiple conformational states in the reaction has been presented. The possibility that the junction itself represents a site of exceptional binding properties has been raised, with no unequivocal supporting evidence as yet (Walker et al., 1985a,b; Chaires, 1985; Suh et al., 1991). For these reasons we have used chemical footprinting to characterize this interaction with higher structural resolution than was available in that study.

Dynemicin (Figure 5) and Fe(II)·bleomycin (Figure 6) both interact with BZ-II under low salt conditions in a manner that distinguishes the upper from the lower strand, resulting in preferential scission of the junction region in the upper strand only and the 3′-terminal region in both upper and lower strands. This unexpected behavior implies that the BZ molecule already possess a structural anomaly in low salt, presumably in its fully B form. The difference in the pattern of cleavage of the upper and lower strands shows that the two strands are nonequivalent, presumably because of some intrinsic structural difference, possibly involving axial bending or solenoidal deformation of the duplex (Hagerman, 1990). But they both detect hyperreactivity of the junction itself, suggesting that this region can interact selectively with drugs. The fact that the same sequence GAT at the junction of the upper strand occurs also in the lower strand but does not show the enhancement indicates this is a structural effect and not a sequence selectivity. The effect is not so clearly demonstrated in the MPE profiles (Figure 3), but we believe it exists also in this case. Under high salt conditions, MPE·Fe(II) and Fe(II)·bleomycin show enhanced reactivity in the Z-DNA segment of BZ-II, while dynemicin has the opposite effect. Suh et al. (1991) noted that ethidium interacts more strongly with BZ-I in high salt, consistent with the observation we make here. In the presence of excess propidium, the differential cleavage between the low and high salt due to these probes is eliminated, consistent with the idea that PI at the high concentration flips Z-DNA to B-DNA, or more properly a B-like state (Suh et al., 1991). This is confirmed by a diethyl pyrocarbonate modification experiment. This agent sensitizes purines to cleavage, and those in Z-DNA react more rapidly than in B-DNA (Johnston & Rich, 1985). Contrary to our initial hypothesis, it turns out that the two strands in BZ-II are nonequivalent in low salt as well as in high salt. This might be an intrinsic consequence of the methylation of the C residues or some structural property of the junction complex.

In summary, we find that the structure of the BZ-II duplex is unusual in that the two strands are not equivalent in both low salt and high salt conditions. The interface between the two segments is a distinct structure in both conditions and is shown to have discernible selective drug interactions both at low and high salt concentrations. This means that the properties of this region are not determined by the B–Z interface, as has been postulated. This suggests that a detailed structure determination of the properties of BZ-II at low salt would be of interest. The B–Z junction thus does not behave like a bulge, for example, or a similar induced structure in this respect (Williams & Goldberg, 1988a,b). On the other hand, the Z domain in high salt itself appears to be responsible for the preferential binding of methidium and bleomycin detected by ourselves and by others (Walker et al., 1985a,b; Chaires, 1985; Suh et al., 1991). Detailed isotherms measured by Suh et al. (1991) show clearly that the high salt form binds ethidium more tightly than the low salt form. This argues that the differential effects seen in Figures 3 and 6 reflect enhanced scission in the Z domain and the junction rather than some form of inhibition of scission of the B domain.

Registry No. MPE·Fe(II), 83789-87-1; BZ-II, 135972-24-6; dynemicin, 124759-75-7.

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