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Enhanced Reactivity of a B–Z Junction for Cleavage by the Restriction Enzyme *Mbo*I†

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ABSTRACT: We have been investigating the structure, dynamics, and ligand-binding properties of the interface that exists between a right-handed conformation and a left-handed conformation (i.e., a B–Z junction) in synthetic DNA oligomers. Since exo- and endonuclease activity is known to be sensitive to the conformation of the template DNA, we have designed and synthesized a DNA oligonucleotide of 20 base pairs (designated as BZ-III) with an *Mbo*I recognition site (GATC) at the location of a potential B–Z junction. The activity of the *Mbo*I enzyme toward this molecule and DNA oligomers that contain multiple *Mbo*I sites located at B–Z junctions was monitored in the absence and presence of the Z-conformation-inducing reagent cobalt hexaammine. In all cases, the activity of the enzyme was *enhanced* in the presence of cobalt hexaammine. The activity of *Mbo*I toward BZ-III, in the presence and absence of cobalt hexaammine, was also examined when the DNA oligomer is also in the presence of the DNA binding drugs actinomycin D, ametantrone, or ethidium bromide. In all cases, the activity of the enzyme was inhibited in the presence of drug. The results suggest that B–Z junctions are structurally unique and that this uniqueness may alter nuclease activity at sites in or near the junction.

Our laboratories have been investigating the structure, dynamics, and ligand-binding properties of B–Z conformational junctions in short (i.e., 16–20 base pairs) synthetic DNA oligomers. Recent ^{1}H NMR (Sheardy & Winkle, 1989), Raman (Dai et al., 1989), gel mobility (Winkle & Sheardy, 1990) and ligand-binding studies (Guo et al., 1991; Suh et al., 1991) have suggested that such a conformational junction is structurally different from either flanking B or Z segments. Furthermore, the segment of the DNA oligomer that ultimately forms the junction may be structurally anomalous even when the oligomer is under B-like conditions (i.e., low salt). It has been suggested that this structural peculiarity predisposes this segment to junction formation when the DNA oligomer is subjected to Z-forming conditions such as high salt (Sheardy & Winkle, 1989; Winkle & Sheardy, 1990).

Various endo- and exonucleases have been shown to be sensitive to the conformation of template DNA. For example, exonuclease III has previously been employed to map drug-binding sites and DNA structural anomalies (Royer-Pokora et al., 1981). Furthermore, the activity of Bal31 nuclease is inhibited by the presence of Z-DNA (i.e., enzymatic cleavage ended where Z structure began) (Kilpatrick et al., 1983). Type II restriction enzymes are also sensitive to the local environments of their cleavage sites. It has been reported that cleavage at the recognition sites of the restriction enzymes *Bsp*MI, *Eco*II, *Hpa*II, *Nae*I, *Nar*I, and *Sac*II is inhibited even when the respective site contains the "correct" sequence (Gingeras & Brooks, 1983; Hattman et al., 1979; Kruger et al., 1988; Pein et al., 1989; Oller et al., 1991). The sequences flanking these sites have been implicated as playing roles in the inhibition observed.

Changing the conformation of the cleavage site also alters its cleavability by endonucleases. In particular, the restriction enzyme *Hha*I is inhibited when its recognition site, CGCG, is in a Z conformation (Vardimon & Rich, 1984). Thus, it is likely that the anomalous structures present in B–Z junction molecules would provide substrates for DNA cleavage enzymes which are distinctive structurally from the common cleavage site for these enzymes.

Small molecules have also been used to probe DNA

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structures for localized anomalies. Recently, chemical probes such as (methidiumpropyl-EDTA)-iron(II) [MPE-Fe(II)], phenanthroline-copper, and porphyrin complexes were used to map branched junctions in oligonucleotide structures created to model recombination architecture (Lu et al., 1990; Guo et al., 1989, 1990). The results reported suggest that certain small molecules may show a preference for binding to the structural anomalies present in these branched junctions. Other investigators (Walker et al., 1985a; Shafer et al., 1984) have demonstrated that the binding of a small molecule such as ethidium bromide can influence the B–Z transition in poly(dG–dC). Furthermore, Krugh and co-workers (Walker et al., 1985b), presented evidence suggesting that actinomycin or ethidium may bind initially to small regions of β-like DNA embedded in the predominantly Z-like conformation of poly(dG-5medC). Thus, ethidium and actinomycin may bind preferentially at or near B–Z junctions.

The cleavage specificity of MPE-Fe(II) toward BZ-II, a sequence analogue of BZ-I also capable of B–Z junction formation at high salt, has been thoroughly investigated by Kallenbach et al. (1991). The results reported suggest that the middle of the BZ-II sequence (i.e., that which ultimately forms the B–Z junction at high salt) is a preferential binding site for the above probe under both B-like and Z-like conditions. Chaires et al. (1991) have examined the reversible binding of ethidium to BZ-I under both B-like and Z-like conditions. This study indicates that ethidium displays unusual binding to the oligomer under all conditions but displays *enhanced* binding to the oligomer under high-salt conditions. Together, these two studies indicate that the junction-forming oligomers present preferential binding sites to certain chemical ligands under both low- and high-salt conditions. It is thus likely that B–Z conformational junctions or DNA segments capable of forming such conformational junctions have unusual structure.

Since junction molecules apparently present preferential binding sites to small ligands at the potential junction site, we wanted to investigate the interactions of large ligands with this general class of DNA molecules. To this end, we have synthesized an oligonucleotide, BZ-III (Figure 1A), which possesses an *Mbo*I cleavage site (GATC) at the location of the potential B–Z junction. It should be noted that the apparent *Hha*I sites (CGCG) in the Z-forming segment of BZ-III should display inhibited cleavage by this enzyme due to the methylation of the cytidine residues—a modification which facilitates Z formation. Thus, *Hha*I cleavage of BZ-III was not examined. The cleavage of BZ-III and polyjunction molecules created by ligation of the concatemers formed in an equimolar mixture of the upper strand of BZ-III and the upper strand of BZ-I (Winkle & Sheardy, 1990) was examined under both B-like and Z-like conditions [i.e., in the absence or presence, respectively, of 50 μM $Co(NH_3)_6^{3+}$]. As shown below, the cleavage of these DNA molecules by *Mbo*I is enhanced in the presence of Z-like-producing conditions.

To further probe the structural anomalies of B–Z junction molecules, cleavage of BZ-III and polyjunction molecules by *Mbo*I was also examined in the presence of actinomycin D, ametantrone, and ethidium (Figure 1B). Actinomycin has been previously shown to have a preference for binding to the sequence GC (Krugh et al., 1980) and displays a diminished affinity for Z-DNA (Walker et al., 1985b). Ametantrone shows a slight preference for GC and CG sequences (Lown et al., 1985) while ethidium has no great sequence preference but displays diminished binding to Z-DNA (Walker et al., 1985a; Shafer et al., 1984). These molecules (which have

A. DNA Sequences

C*-G--C*-G--C*-G--C*-G--A-C-T-G-A-C-T-G
G--C*-G--C*-G--C*-G--C*-T-G-A-C-T-G-A-C

BZ-I

A-T-C*-G--C*-G--C*-G--C*-G--A-T-C-A-G-T-C-A-G-T
T-A-G--C*-G--C*-G--C*-G--C*-T-A-G-T-C-A-G-T-C-A

BZ-III

B. Drugs

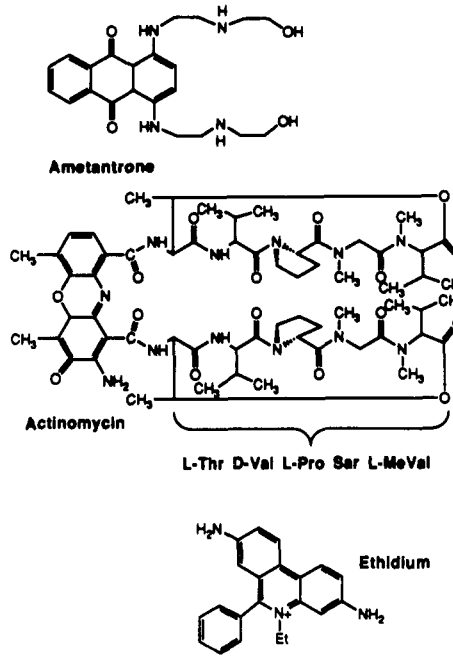

FIGURE 1: (A) Sequences of the DNA oligomers BZ-I and BZ-III. C* is 5-methylcytidine. (B) Structures of the DNA-binding molecules used in this study.

different binding characteristics) differ in their effects on *Mbo*I cleavage of the junction molecules under all conditions (e.g., actinomycin gives a significantly higher degree of inhibition of *Mbo*I activity). The results of these enzyme studies presented below suggest that the structural anomalies of B–Z junction molecules, as suggested in previous studies, may be recognized by enzymes that use DNA as a substrate.

Experimental Procedures

Materials. The oligomers BZ-IA, BZ-IB, BZ-IIIA, and BZ-IIIB were synthesized in our laboratory on an Applied Biosystems 380B DNA synthesizer using phosphoramidite chemistry (Carathurs, 1982) and were purified via reverse-phase HPLC with a C-18 column as previously described (Sheardy, 1988). Poly (BZ-[IA+IIIA]) was produced by ligation of BZ-IA and BZ-IIIA using T4 ligase (IBI) as previously described (Winkle & Sheardy, 1990). Individual ligation products were isolated by separation of the ligation mixture on a 12% polyacrylamide gel followed by staining with ethidium bromide and visualization of the bands using a UV transilluminator. The bands containing individual ligation products were cut out and the DNA was liberated from the gel slices by soaking in 0.5 M ammonium acetate. The DNA fragments were isolated by precipitation with two volumes of 95% ethanol followed by three washes with 70% ethanol. DNAs were ^{32}P-end-labeled with [γ-^{32}P]ATP (New England

Nuclear) and T4 kinase (IBI). For DNA size markers, ϕX174 RF DNA (BRL) or SV40 DNA (IBI) was cleaved with *Hin*fI (IBI) and the fragments were end-labeled with [γ-^{32}P]ATP and T4 kinase or with [α-^{32}P]dATP and Klenow fragment (IBI). The restriction enzyme *Mbo*I was obtained from IBI. Actinomycin D and ethidium bromide was obtained from Sigma. Purities were checked using UV spectroscopy and TLC. The ametantrone was synthesized in our laboratory from 1,4,9,10-tetrahydroxyanthracene (Aldrich) and 2-[*N*-(2′-hydroxyethyl)amino]ethylamine (Aldrich) following the method of Murdoch et al. (1979). The purity and authenticity of the ametantrone was verified by TLC, UV spectroscopy, and ^{1}H NMR. Concentrations of all drugs and the DNAs were determined using UV spectroscopy: actinomycin D, ϵ_{440} = 24 400 M^{-1} cm^{-1}; ethidium bromide, ϵ_{480} = 5280 M^{-1} cm^{-1}; ametantrone, ϵ_{625} = 9700 M^{-1} cm^{-1}; all DNAs, ϵ_{260} = 6500 M^{-1} cm^{-1} in phosphate.

Buffer Conditions. To examine cleavage under B-like conditions, the enzyme reactions were conducted in the buffer provided with the *Mbo*I enzyme (10 mM Tris and 50 mM NaCl, pH 7.6). To examine cleavage under Z-like conditions, enzyme reactions were conducted in the same reaction buffer with sufficient $Co(NH_3)_6Cl_3$ added to give a final cobalt hexaammine concentration of 50 μM.

Preparation of Z-DNA. DNAs were converted to the hybrid form (both B and Z conformations present) by heating in the presence of 50 μM $Co(NH_3)_6^{3+}$ for 5 min at 80 °C, followed by slow cooling to room temperature. Conversion of the oligomers to the hybrid forms by these conditions was monitored using ^{31}P NMR on a GE QE300 NMR spectrometer. Broad-band ^{1}H–^{31}P-decoupled ^{31}P NMR spectra were obtained at 121 MHz at various temperatures (16K data points; sweep width 10 000 Hz; 60 000–100 000 transients).

Restriction Enzyme Digestions. Restriction enzyme digestions were carried out using the conditions specified above and in the figure legends. The enzyme *Mbo*I was used at a ratio of 1 unit of enzyme/μg of DNA. Enzymatic cleavage was halted by the addition of SDS to a final concentration of 0.25%, followed by the addition of tracking dye and quick chilling to −40 °C. As a control for the activity of *Mbo*I, digestions of SV40 DNA samples in the absence or presence of $Co(NH_3)_6^{3+}$ and/or drugs were carried out as specified in the figure legends.

Drug Binding. Reactions with the restriction enzyme *Mbo*I in the presence of actinomycin D, ametantrone, or ethidium bromide were carried out at the drug concentrations specified in the figure legends. Prior to reaction with an enzyme, the drug/DNA mixtures were equilibrated for at least 15 min at the enzyme reaction temperature as specified in the figure legends.

Polyacrylamide Gel Electrophoresis. The results of the enzymatic studies were assayed via PAGE on 12% polyacrylamide (29:1 acrylamide:bisacrylamide). For visual observation of the results, autoradiography was used. For quantitation, either bands were excised from the gel and the ^{32}P band was determined using a Beckman LS scintillation counter or the gel was scanned with an Ambis β scanner. To determine the percent digestion, $cpm_{cleavage\ product}/cpm_{total}$ (for each sample) was used. For the controls with SV40 DNA, bands were visualized by ethidium bromide staining and the cleavage was quantitated using a densitometer.

Results

Previously reported gel electrophoresis results demonstrated that ligation of BZ-IA and BZ-IIIA produces a set of polyjunction molecules (Winkle & Sheardy, 1990). Cobalt hexaammine has been shown to convert poly(dG-dC) from a B conformation to a Z conformation at micromolar concentrations and this transition is facilitated when the cytidine moieties are methylated (Behe & Felsenfeld, 1981; Butzow et al., 1984). Circular dichroism studies have indicated that poly(BZ-[IA+IIIA]) contains a mixture of both B and Z forms under Z-like conditions (i.e., 4.5 M NaCl), as expected from the sequence (Winkle & Sheardy, 1990). The ^{31}P NMR spectrum of BZ-I in 50 μM $Co(NH_3)_6^{3+}$ is characterized by the presence of resonances characteristic of both B- and Z-DNA phosphates. Exonuclease III and Bal31 nuclease digestion studies with BZ-I and BZ-III in our laboratory also indicate that 50 μM $Co(NH_3)_6^{3+}$ induces the B to Z transition of the (5medC-dG)$_4$ segment (Winkle et al., 1991). Thus, $Co(NH_3)_6^{3+}$ was chosen as the Z-inducing agent since it induces the transition at a low concentration that does not affect the activity of the enzyme being assayed (as shown below).

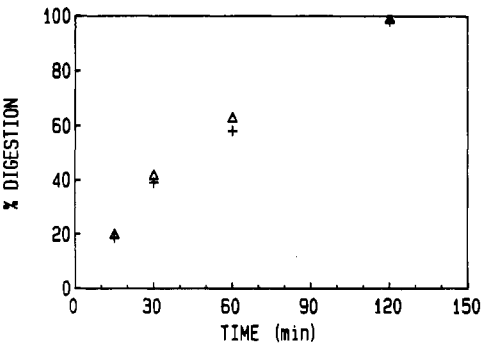

FIGURE 2: Plot of percent digestion vs time for the cleavage of SV40 DNA with *Mbo*I. Digestions were carried out at 37 °C in the absence of $Co(NH_3)_6^{3+}$ (+) or in the presence of 50 μM $Co(NH_3)_6^{3+}$ (△). Digestions were monitored by separation of the cleavage products on a 6% polyacrylamide gel followed by ethidium staining.

To determine whether the presence of $Co(NH_3)_6^{3+}$ affects the cleavage of DNA by *Mbo*I, we examined the digestion of SV40 by *Mbo*I in the absence and presence of this complex. The results of the digestion of SV40 as a function of time, as shown in Figure 2, indicate that the addition of $Co(NH_3)_6^{3+}$ at a concentration of 50 μM results in no apparent alterations in the effectiveness of *Mbo*I cleavage. When cleavage at individual sites of SV40 is examined (there are eight *Mbo*I sites), the cleavage observed at each site is independent of the presence of $Co(NH_3)_6^{3+}$. None of these sites have flanking $(CG)_n$ segments, i.e., those segments capable of forming Z conformations. These results suggest that $Co(NH_3)_6^{3+}$ at a concentration of 50 μM does not affect the activity of the *Mbo*I enzyme. Further, this reagent does not, in general, alter the DNA structure at or near the *Mbo*I cleavage site in a manner discernible to the enzyme.

The results for the *Mbo*I cleavage of BZ-III in the absence and presence of $Co(NH_3)_6^{3+}$ are shown in Figure 3. BZ-III possesses a (5medC-dG)$_4$ segment with the most interior G of this segment as the G of the 5′-GATC-3′ recognition site of *Mbo*I. Thus, the *Mbo*I cleavage site is located at the B-Z junction when BZ-III is in a Z-like environment [such as 50 μM $Co(NH_3)_6^{3+}$]. The data presented in Figure 3 indicate that the cleavability at the *Mbo*I site of BZ-III in the absence of $Co(NH_3)_6^{3+}$, when BZ-III is entirely in a B conformation, is similar to that found for the *Mbo*I sites on SV40. However, in the presence of 50 μM $Co(NH_3)_6^{3+}$, at which the (5medC-dG)$_4$ segment assumes a Z conformation, the cleavage of BZ-III at the *Mbo*I site by the enzyme is *enhanced.*

A similar enhancement of cleavage is observed with the ligated multimers of poly(BZ-[IA+IIIA]). For example, as shown in Figure 4, the data for the *Mbo*I cleavage of the

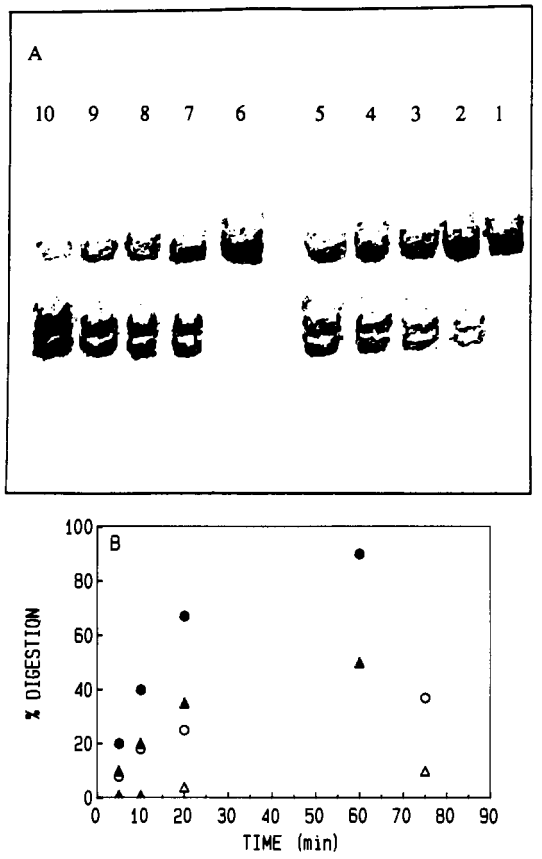

FIGURE 3: (A) Typical Ambis β scan of ^{32}P from 12% polyacrylamide electrophoretic gel for the reaction of BZ-III with *Mbo*I at 37 °C at the specified times. Lanes 1–5, no $Co(NH_3)_6^{3+}$: (1) 0 min; (2) 5 min; (3) 10 min; (4) 20 min; (5) 60 min. Lanes 6–10, 50 μM $Co(NH_3)_6^{3+}$: (6) 0 min; (7) 5 min; (8) 10 min; (9) 20 min; (10) 60 min. (B) Plot of percent digestion vs time for the cleavage of BZ-III with *Mbo*I. Digestion conditions were 37 °C, no $Co(NH_3)_6^{3+}$ (▲); 37 °C, 50 μM $Co(NH_3)_6^{3+}$ (●); 4 °C, no $Co(NH_3)_6^{3+}$ (△); and 4 °C, 50 μM $Co(NH_3)_6^{3+}$ (○).

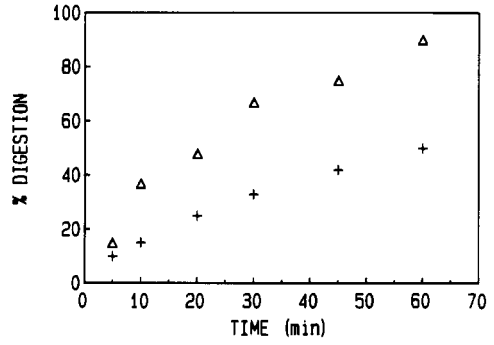

FIGURE 4: Plot of percent digestion vs time for cleavage of the ligated multimer (BZ-[IA+IIIA]$_4$) with *Mbo*I at 37 °C in the absence (+) or presence (△) of 50 μM $Co(NH_3)_6^{3+}$. Digestions were monitored by ^{32}P counting of the bands from electrophoresis of reaction mixtures on 12% polyacrylamide gels.

multimer (BZ-[IA+IIIA]$_4$) (where 4 is the number of repeating IA + IIIA units) are displayed. Similar results with other multimers of BZ-[IA+IIIA] were obtained.

Figure 5A shows the results of the cleavage of BZ-III by *Mbo*I in the presence of either actinomycin D, ametantrone, or ethidium bromide in the absence of $Co(NH_3)_6^{3+}$. The results indicate that all three drugs inhibit the cleavage of BZ-III by *Mbo*I. Figure 5B shows the results of the cleavage of BZ-III by *Mbo*I in the presence of either actinomycin D, ametantrone, or ethidium bromide *and* in the presence of 50 μM $Co(NH_3)_6^{3+}$. Again, all three drugs inhibit the cleavage.

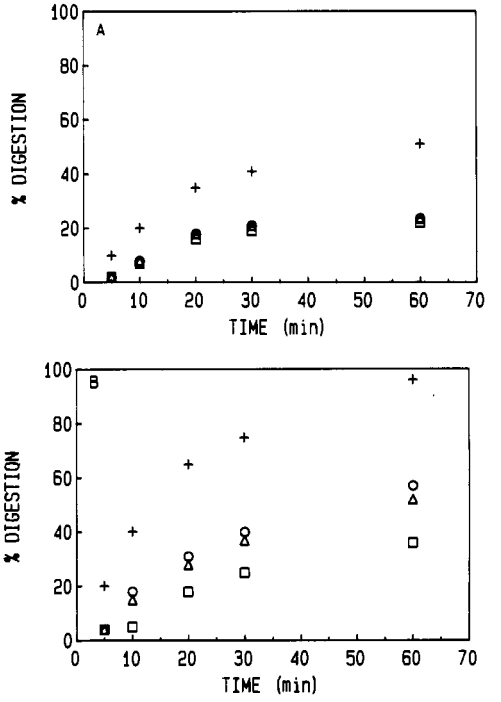

FIGURE 5: (A) Plots of percent digestion vs time for the cleavage of BZ-III with *Mbo*I at 37 °C in the presence of the specified drug and in the *absence* of $Co(NH_3)_6^{3+}$. All [drug] = 6.0 μM and [DNA] = 6.3 μM in base pairs. Samples were preequilibrated with the drugs for 15 min prior to enzyme addition. Digestion conditions: no drug (+); actinomycin D (□); ametantrone (△); and ethidium (○). (B) Plots of percent digestion vs time for the cleavage of BZ-III with *Mbo*I at 37 °C in the presence of the specified drugs and at 50 μM $Co(NH_3)_6^{3+}$ at digestion conditions specified above.

Table I: Rate Constants[a] for *Mbo*I Digestions

substrate	k (s^{-1}), no $Co(NH_3)_6^{3+}$	k (s^{-1}), 50 μM $Co(NH_3)_6^{3+}$
SV40	0.015	0.016
(BZ-[IA + IIIA]$_4$)	0.013	0.037
BZ-III	0.022	0.049
BZ-III/Act	0.0080	0.0086
BZ-III/Amet	0.010	0.016
BZ-III/Eth	0.010	0.020

[a] Pseudo-first-order rate constants (±0.002) determined from plots of ln [DNA]/[DNA]$_{initial}$ vs time. [DNA] and [DNA]$_{initial}$ were determined from ^{32}P cpm of bands on electrophoresis gels of the enzyme reaction mixtures. The [drug] = 6 μM for all three drugs.

Pseudo-first-order rate constants for the *Mbo*I cleavages were determined from plots of ln [DNA]/[DNA]$_{initial}$ vs time. The ratios of [DNA]/[DNA]$_{initial}$ were determined from the percent digestions used in Figures 2–5. These rate constants are presented in Table I and are used as another means of qualitatively assessing the cleavage of the DNAs by *Mbo*I under the various conditions specified. In general, the rate constants determined support the conclusion that the presence of a Z-like sequence flanking the *Mbo*I cleavage site enhances the cleavage of that site by the enzyme.

Discussion

The results presented in Figures 2–5 and in Table I indicate that the presence of a Z-like structure adjacent to the *Mbo*I cleavage site enhances the reactivity of that site to *Mbo*I. Previously, Sheardy and Winkle (1989) reported ^1H NMR results suggesting that the B–Z conformational junction present in BZ-I comprises the base pairs at positions 7, 8, and 9 (the -5meC-G-A- sequence for the upper strand of BZ-I) and might possess flexibility properties different from either

B or Z segments. The results of Peticolas et al. (1989) with a similar molecule indicated that the B–Z junction in their sequence also encompasses only three base pairs. Manning has predicted that conformational junctions may behave differently from flanking sequences (1988). The distinctive temperature dependence of the NMR imino resonance of the G-8 residue of BZ-I (Sheardy & Winkle, 1989) and the unusual *enhanced* gel mobilities displayed by poly(BZ-[IA+-IIIA]) lend further support to the possibility of peculiar flexibility properties for junctions (Winkle & Sheardy, 1990). Finally, studies on the interactions of chemical probes with BZ-I and BZ-II have also indicated that these junction-forming molecules present preferential binding sites to the ligands under both B-like and Z-like conditions (Guo et al., 1991; Suh et al., 1991).

The G10 and A11 bases of BZ-III, which are sequence equivalent to the G8 and A9 bases in the junction of BZ-I, are also the G and A base in the recognition site of *Mbo*I (i.e., 5′-GATC-3′). Perhaps the different flexibility properties suggested above for B–Z junctions enhance the reactivity of this site to *Mbo*I cleavage relative to *Mbo*I sites not adjacent to Z-like segments. Several NMR studies have suggested that the structures of sequences recognized by restriction enzymes are distinguishably different from the structures of flanking sequences (Hosur et al., 1985a,b; Ravikumar et al., 1985; Chary et al., 1987, 1988). Thus, a site which is more accessible than other sequence-equivalent sites for a particular restriction enzyme might show enhanced reactivity to that enzyme. Examination of the computer-generated structures of B–Z junctions by Lavery (1988) suggest that these junctions may be more sterically accessible than other structures. The enhancement shown by the *Mbo*I site at the junction could indicate that this site is more accessible and perhaps more "open".

It should be noted that previous studies on the effects of flanking sequences on restriction enzyme activity have all reported inhibitory effects (Hattman et al., 1979; Kruger et al., 1988; Pein et al., 1989; Gingeras & Brooks, 1983; Oller et al., 1991). The effect of superimposing a junction between conformationally distinct DNA segments on a restriction site can cause enhancement of cleavage, at least for the case under study. B–Z junctions would appear, therefore, to be notable in their effect on DNA structure and function.

A specifically germane case of restriction enzyme inhibition is that of *Hha*I cleavage of Z-DNA (Vardimon & Rich, 1984). When the cleavage site of *Hha*I (CGCG) is located in a Z conformation, the enzyme cannot cleave it. Thus, the reactivities of restriction enzymes for Z-DNA and junction DNA are markedly different.

The inhibition of *Mbo*I activity on B-form BZ-III by actinomycin, ametantrone, and ethidium is expected and is similar to that observed previously with various restriction enzymes and DNA-binding molecules (Hardwick et al., 1984; Ushay et al., 1981; Barton & Paranawithana, 1986; Mallamaci et al., 1991). Inhibition by these agents of *Mbo*I cleavage of BZ-III containing both B and Z conformations is also observed. On the basis of the rate constants given in Table I, the amount of inhibition found with ametantrone and ethidium appears to be slightly greater with the junction-containing molecule than with all B molecules. For ametantrone, the ratio k_{drug}/k is 0.33 for the junction molecule and 0.45 for the B molecule. For ethidium, the ratios are 0.41 and 0.45, respectively. With actinomycin D, the change is more dramatic: k_{drug}/k is 0.18 for junction molecule and 0.36 for the B molecule. Since equilibrium binding data for all three of these drugs to DNA oligomers is not yet available, caution should be taken in comparing the inhibition produced by their binding to BZ-III.

It is apparent that all three drugs bind to BZ-III under both B-like and Z-like conditions. Recently, Chaires and co-workers have reported the binding isotherms of ethidium to BZ-I (Suh et al., 1991). The results presented indicate that ethidium shows *enhanced* binding to the oligomer under conditions of high salt (i.e., 4.5 M NaCl). It is likely that, at high salt, ethidium and ametantrone bind to the B-domain of the junction molecule. Kallenbach has also recently reported that methidium (an analogue of ethidium) may bind to the Z-domain of a similar junction molecule under conditions of high salt. Furthermore, the junction itself may be a binding site.

Perusal of the sequence of BZ-III reveals three preferential actinomycin-binding sites (i.e., CG), all of which are located in the potential Z-forming segment. Furthermore, it has been reported by Krugh and co-workers (Walker et al., 1985b) that actinomycin does not bind to Z-DNA. These authors have also suggested that, with poly(dG-5medC) under Z-like conditions, actinomycin may bind to small B-like regions embedded in the predominantly Z like structure. Our results indicate that actinomycin binds to BZ-III under Z-like conditions even though its preferred binding sites are in the Z conformation. Recent exonuclease III digestion studies and optical titration studies on BZ-I plus actinomycin D suggest that, under conditions favoring the existence of the junction, BZ-I has an increased number of binding sites (two in the fully B like form and three in the hybrid form) (S. A. Winkle, unpublished results). These data, to be presented elsewhere, suggest that actinomycin can bind to sites other than GC.

In summary, our previous studies indicated that B–Z junctions may possess structural properties different from those of either B or Z conformations. In this paper, we present a specific example of this uniqueness. Previous work on the effects of DNA structure and sequence on restriction enzyme activity have all noted inhibitory effects. In contrast, cleavage by the restriction enzyme *Mbo*I at the junction between B and Z conformations is enhanced, providing further evidence for the distinctiveness of junction DNA. The inhibition of *Mbo*I cleavage at the B–Z junction by actinomycin (even though the preferred actinomycin binding sites are occluded) suggests that the presence of junctions can change drug-binding specificities.

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Articles

Thermodynamic and Structural Properties of Pentamer DNA·DNA, RNA·RNA, and DNA·RNA Duplexes of Identical Sequence†

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ABSTRACT: Four pentamers with the general sequence $^{5'}$CU(T)GU(T)G/$^{5'}$CACAG have been prepared by chemical synthesis in order to generate duplex structures with common sequences. The four duplexes studied include the DNA·DNA duplex ($^{5'}$dCACAG/$^{5'}$dCTGTG) and the RNA·RNA duplex ($^{5'}$rCUGUG/$^{5'}$rCACAG) as well as the two corresponding DNA·RNA heteroduplexes ($^{5'}$rCUGUG/$^{5'}$dCACAG and $^{5'}$CACAG/$^{5'}$dCTGTG). The measured entropy, enthalpy, and free energy changes upon melting are reported for each pentamer and compared to the predicted values where possible. Results show that the two DNA·RNA heteroduplexes are destabilized ($\Delta G°_{25} = -4.2 \pm 0.4$ kcal/mol) relative to either the DNA·DNA duplex ($\Delta G°_{25} = -4.8 \pm 0.5$ kcal/mol) or the RNA·RNA duplex ($\Delta G°_{25} = -5.8 \pm 0.6$ kcal/mol). Circular dichroism spectra indicate that the RNA and the two heteroduplexes adopt an A-form conformation, while the DNA conformation is B-form. Imino proton NMR spectra also show that the heteroduplex structures resemble the RNA·RNA duplex.

Double-stranded RNA sequences adopt an A-form conformation, while double-stranded DNA is usually B-form. DNA can exhibit B to A transitions either through sequence effects (Leslie et al., 1980) or as a result of the solvent conditions (Drew et al., 1980), while the corresponding A to B transition for RNA has not been observed. Significant changes in the salt conditions for solutions of either DNA or RNA can additionally effect a B(A) to Z transition (Pohl & Jovin, 1972;

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