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Anomalous Gel Migration of DNA Oligomers Containing Multiple Conformational Junctions†

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ABSTRACT: We have previously shown that a short 16 base pair DNA oligomer can accommodate a B-Z conformational junction [Sheardy, R. D., & Winkle, S. A. (1989) *Biochemistry 28*, 720-725]. Results from ^{1}H NMR studies indicated that only three base pairs were involved in the junction and that one of these base pairs was highly distorted. Being interested in the nature of this distortion, we constructed DNA oligomers which have the potential to contain multiple B-Z junctions for polyacrylamide electrophoretic studies. We report that the mobilities displayed by these molecules through acrylamide gels in the absence and presence of cobalt suggest that these molecules run shorter than they actually are. This anomalous migration may be due to structural/dynamic properties of the DNA helix manifested by the periodic disortions of the potential B-Z junctions.

The interest in the biophysical properties of unusual DNA structures has been steadily increasing over the past few years. Investigations have focused on the relationship between sequence and conformation for specific DNA segments. One particular area of interest is the study of DNA molecules that contain bends which give rise to global curvature of the DNA molecules. It has been shown that DNA which contains tracts of oligo(dA)-oligo(dT) exhibits bending or curvature (Levene & Crothers, 1983; Wu & Crothers, 1984; Hagerman, 1984, 1985, 1986; Levene et al., 1986; Koo et al., 1986; Rice & Crothers, 1989; Cacchione et al., 1989). One experimental

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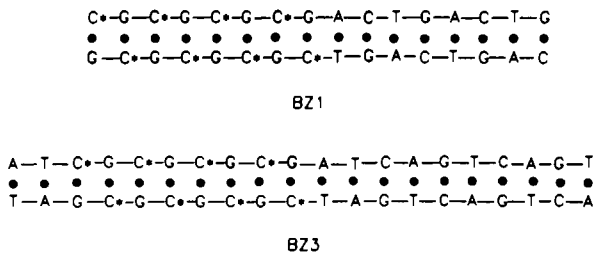

FIGURE 1: Sequences of BZ1 and BZ3. The upper strands of these sequences, referred to as 1A and 3A, run 5′ to 3′. In this report, C* is 5-methyl-2′-deoxycytidine.

result commonly used to invoke DNA bending is the observation of anomalous migration of these molecules through electrophoretic gels (Koo et al., 1986; Hagerman, 1985, 1986; Cacchione et al., 1989). These experiments evidence DNA bending via gel retardation of ligated multimers of sequence-specific oligomers.

We and others have been interested in the dynamic and structural properties of B–Z conformational junctions in short synthetic DNA oligomers (Sheardy, 1986; Sheardy & Winkle, 1989; Doktycz et al., 1990; Dai et al., 1989). We have demonstrated that a short oligomer of appropriate sequence can accommodate a B–Z junction (Sheardy, 1986; Sheardy & Winkle, 1989) under high-salt conditions. In particular, BZ1 (Figure 1) assumes a right-handed double-helical structure at low ionic strength but undergoes a conformational transition as the ionic strength of the media is increased. CD and NMR characterizations of the high-salt form indicate that one end of the molecule is left-handed while the other end of the molecule is right-handed. This molecule, thus, must have a B–Z conformational junction. Furthermore, the junction encompasses only three base pairs as evidenced by NMR studies. BZ3 (Figure 1) also undergoes a salt-induced conformational transition similar to that observed for BZ1 (Sheardy, unpublished results). Peticolas and co-workers recently reported that their Raman results on a short oligomer capable of forming a B–Z junction also suggest that the junction is composed of approximately three base pairs (Dai et al., 1989). Finally, Porschke et al. (1987) suggest that B–Z junctions are neither highly bent nor flexible. Our NMR data, however, indicate that at least one base pair in the junction is highly distorted (Sheardy & Winkle, 1989).

Being interested in the nature of this distortion, we investigated, in a manner analogous to that of Hagerman (1985, 1986) and others, the gel electrophoretic mobilities of DNA oligomers of various lengths that potentially contain multiple B–Z junctions. Due to the observed distortion of the B–Z junction in BZ1, it was hypothesized that the presence of periodically spaced B–Z junctions may produce global distortions in these DNA oligomers that could be detected via anomalous gel migration.

Examination of the sequence of BZ1 reveals that the upper strand (referred to as 1A) can form a semi-self-complementary duplex with a core of 8 G·C* base pairs and dangling ends of eight unpaired bases on the 3′ termini (see Figure 2). This duplex has been detected in solution and characterized by CD and ^{32}P NMR (Sheardy, 1986). The base-paired region of this duplex is fully right-handed at low salt but assumes the left-handed conformation at high salt (>2.0 M NaCl).

The upper strand of BZ3 (3A) also forms a semi-self-complementary duplex with a core of 10 base pairs, $[AT(C^*G)_4AT)]_2$, and dangling ends of 8 unpaired bases on the 3′ termini (see Figure 2). The base-paired region of this oligomer also undergoes the B to Z transition as the ionic

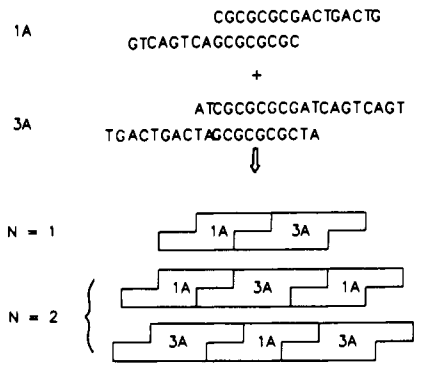

FIGURE 2: Construction of the ligation products. Strands 1A and 3A form the semi-self-complementary sequences shown above. The dangling ends of the 1A duplex are complementary to those of the 3A duplex. Ligation of an equimolar mixture of the two strands gives rise to oligomers, such as 1A + 3A (number of ligations, $N = 1$), of high molecular weight. Note that two ligations give rise to two possible products (see text). The number of ligations observed ranged from 1 to 22.

strength of the medium is increased (Sheardy, unpublished results). However, the NaCl concentration required for the transition is much greater for 3A than 1A. In particular, whereas the midpoint for the transition for 1A is 1.1 M NaCl (Sheardy, 1986; Doktycz et al., 1990), the transitional midpoint for 3A is 3.1 M NaCl (Sheardy, unpublished results). This increase in [NaCl] required for the transition of 3A is probably due to the presence of the two A·T base pairs flanking both ends of the Z-forming region (Chen, 1987).

Examination of the sequences of a particular oligomer reveals that the dangling ends are not complementary to each other. Thus, concatomers would not form from self-association to either 1A or 3A. It should also be noticed, though, that the dangling ends of 1A *are* complementary to those of 3A. Thus, in an equimolar mixture of 1A and 3A, concatomers would form (see Figure 2). Incomplete ligation of these concatomers with DNA ligase would generate a set of oligomers of various lengths that contain blocks of potential Z-forming segments of 8 base pairs separated by blocks of non-Z-forming regions of 10 base pairs. These ligated oligomers could be considered as alternating-block copolymers. Gel electrophoresis of the ligated oligomers would give rise to a "ladder" of discreet DNA bands as observed with similar ligation experiments (Hagerman, 1985, 1986; Wu & Crothers, 1984; Ma et al., 1986; Koo et al., 1986; Cacchione et al., 1989).

It should be pointed out that Rahmouni and Wells (1989) recently detected left-handed conformations for tracts of alternating CG as short as 12 base pairs when inserted into a plasmid. They suggest that tracts of 8–10 base pairs of alternating CG, which exist with high frequency in *Escherichia coli* chromosomes, may be biologically important in terms of gene regulation. Hence, our ligated oligomers provide a starting point into the investigations of the biological role of short alternating CG tracts.

We report initial PAGE and CD results on the ligated oligomers arising from 1A plus 3A. We have examined the relative mobilities of these oligomers through nondenaturing polyacrylamide gels at various acrylamide concentrations and Co^{3+} concentrations. Under all conditions, these oligomers exhibit anomalous migration behavior. However, instead of being retarded in the gel as were previously studied oligomers (Hagerman, 1985, 1986; Wu & Crothers, 1984; Koo et al., 1986; Cacchione et al., 1989), our oligomers displayed *enhanced* mobilities through the gels under all conditions. The CD results indicate that these ligated oligomers undergo a salt-induced conformational transition.

Materials and Methods

Materials

Oligomers 1A and 3A were synthesized and purified as described previously (Sheardy, 1986). [γ-^{32}P]ATP and [α-^{32}P]dATP were obtained from New England Nuclear (specific activity 3000 Ci/mmol). T4 polynucleotide kinase was from Boehringer Mannheim Biochemicals. T4 ligase, Klenow fragment, ϕX174RF DNA, the restriction enzyme *Hin*fI, acrylamide, and BIS were obtained from International Biotechnologies Incorporated. ATP was from Sigma Chemical Co., and cobalt hexamine chloride was from Aldrich Chemical Co.

Methods

Phosphorylation. Solutions containing 15 nmol of one of the oligomers ([oligomer] = 750 nM) in 50 mM Tris-HCl (pH 7.8), 15 mM 2-mercaptoethanol, and 10 mM $MgCl_2$ were combined with ATP to give [ATP] = 300 μM and with [γ-^{32}P]ATP (50 μCi). To each sample was added 50 units of T4 polynucleotide kinase. Reaction mixtures were incubated at 37 °C for 1 h. To each sample were then added additional ATP (bringing the total [ATP] to 600 μM) and an additional 20 units of kinase. At the end of 1 additional h, the reactions were stopped by addition of 2 volumes of cold 95% ethanol. The precipitated oligomers were washed 3 times with cold 95% ethanol.

Ligation. The precipitated oligomers from the 5′ end labeling reactions above were dissolved in ligation buffer provided by International Biotechnologies to give concentrations of ca. 74 μM. Five nanomoles of each oligomer, dissolved in ligation buffer, was combined with sufficient additional ligation buffer to give a total volume of 50 μL. Ten units of T4 ligase was added, and the reaction mixture was incubated at 4 °C for 96 h. The reaction was then quenched by heating at 65 °C for 10 min.

ϕX174RF Marker. Five micrograms of ϕX174RF DNA was digested with 14 units of the restriction enzyme *Hin*fI for 2 h at 37 °C. Fifty microcuries of [α-^{32}P]dATP and 7 units of Klenow fragment were added, and the mixture was incubated at room temperature for 20 min. The reaction was quenched by precipitating the DNA by addition of 2 volumes of cold 95% ethanol. The precipitated DNA was washed 3 times with cold 95% ethanol.

$Co(NH_3)_6$ Addition. Aliquots of the ligation mixture were dissolved in TE buffer [10 mM Tris-HCl (pH 8.0)/1 mM EDTA] combined with the appropriate amount of $Co(NH_3)_6Cl_3$ stock solution (to give [$Co(NH_3)_6$] of 0, 50, 100, or 200 μM). Unligated oligomers 1A and 3A and ϕX174RF marker solutions were similarly made. Solutions were heated for 5 min at 80 °C and then cooled to room temperature.

Gel Electrophoresis. The samples prepared above were electrophoresed on acrylamide gels (acrylamide:BIS ratio = 29:1) using 25-mA current at 22 °C. Sets of samples were run on 6%, 8%, or 12% acrylamide gels containing the same concentration of $Co(NH_3)_6$ as the sample set. $Co(NH_3)_6$ at the appropriate concentration was also in the running buffer [TBE: 0.9 M Tris–borate (pH 8.0), 0.09 M boric acid, and 2 mM EDTA]. Gels were autoradiogrammed and/or were vacuum-dried and scanned using an Ambis β scanner. Samples were also run at the various acrylamide concentrations given above but with the acrylamide:BIS ratio equal to 19:1. Similar results were obtained at both acrylamide:BIS ratios.

CD Studies. Circular dichroism spectra were recorded with an AVIV Model 60DS CD spectropolarimeter at 25 °C. DNA samples (at [DNA] = 10^{-4} M in phosphate) were prepared in phosphate buffer (10 mM phosphate/0.1 mM EDTA, pH 7.0) at either 100 mM NaCl (low salt) or 5.0 M NaCl (high salt). CD spectra were obtained at low and high salt for an equimolar mixture of 1A and 3A (unligated) as well as for the mixture of oligomers obtained from the ligation reactions.

Results and Discussion

Examination of the sequences of 1A and 3A shows that both possible products arising from a single ligation (N = 1: 1A + 3A and 3A + 1A) are identical, having a 28 base pair double-stranded region and an 8-base dangling end at each 3′ terminus. The products of all multiple ligations will also have the dangling ends. In the discussion that follows, the lengths given are those of the double-stranded region. There are two distinct products of two ligations [N = 2: 1A + 3A + 1A (length of 44 base pairs) and 3A + 1A + 3A (length of 48 base pairs)]. Three ligations give a single product of 64 base pairs in length. Four ligations give two products of 80 and 84 base pairs in length. This pattern of an odd number of ligations giving one product and an even number of ligations giving two products continues. Figure 2 shows how the ligated oligomers were constructed.

All products begin and terminate with potential Z-forming blocks and have the general block sequence of Z-(B-Z)$_N$ where N ranges from 1 up to 19. Furthermore, an oligomer created from N ligations will contain N potential B–Z junctions and N potential Z–B junctions. Thus, for example, the oligomer arising from N = 2 has an alternating block sequence of Z-B-Z-B-Z with two B–Z junctions and two Z–B junctions.

As stated above, each potential Z-forming block has eight base pairs, and each intervening B-forming block has 10 base pairs. This phasing gives rise to a potential B–Z junction every 18 base pairs. The phasing chosen was simply due to a matter of convenience in preparing the constituent oligomers. We are currently designing and synthesizing oligomers that will address the question of phasing between conformational junctions.

The lengths of the ligated products were determined by consideration of the number of ligations and the new segment added. At each acrylamide concentration and cobalt concentration, the distances traveled by the ϕX174RF marker fragments were determined from the autoradiograms of the gels and plots made (for each set of conditions) of ln (fragment size) vs distance traveled. Under all experimental conditions, linear plots were obtained for the ϕX174 digest marker bands. The distances traveled by the ligation products were also measured from the autoradiograms. These distances were used with the appropriate ϕX174 marker plot to extrapolate the apparent sizes of the ligation products. For the actual sizes of the ligation products, the numbers of base pairs in the double-stranded regions are used. The assumption is made that the eight-base tails do not affect gel mobility for large fragments. It should be noted that for $N > 6$, the two ligation products arising from an even number of ligations comigrated as a single band through the gel. A typical autoradiogram is shown in Figure 3. Electrophoresis of the ligation products through denaturing polyacrylamide gels also resulted in a ladder of discreet bands indicative of ligation (data not shown).

Plots of ln L (L is fragment length) vs M (mobility) for the ligated oligomers under all experimental conditions resulted in linear plots from 64 base pairs to at least 293 base pairs (i.e., N = 3 to N = 15). The products arising from one and two ligations behave somewhat differently from the multiple ligation products. The N = 1 product has only 28 base pairs, and the N = 2 products have 44 and 48 base pairs. The eight-base dangling ends for these products may, thus, have

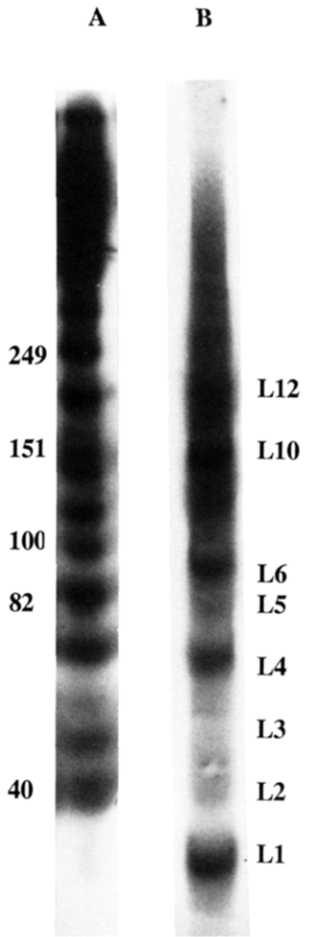

FIGURE 3: Autoradiogram of a typical electrophoresis experiment. For this particular experiment, lane A is the *Hin*fI digest of ϕX174 DNA, and lane B is the ligated oligomers. In all experiments, the distances traveled by the ligated oligomers were determined by comparison to the ϕX174 marker bands run on the same gel as described under Methods.

an effect on the mobility (giving rise to the nonlinearity of these points on the above plots) not manifested with the larger ligation products.

Data on the mobilities of the ligation products are plotted in the manner of Hagerman (1985, 1986). Plots of the ratio of apparent size/actual size (in base pairs) vs N (number of ligations) for the 6%, the 8%, and the 12% gel in the absence of cobalt are given in the upper left-hand panels of Figures 4, 5, and 6, respectively. For intermediate numbers of ligations (N = 2–14), the ligation fragments have apparent sizes which are smaller than the actual sizes, regardless of acrylamide percentage. This suggests that these fragments possess unusual structural features even when the Z-inducing agent (i.e., the cobalt) is absent. This contrasts with the observations made with other DNAs found to have anomalous gel mobilities. Generally, sequences showing unexpected mobilities have been found to run on gels as if they were larger, not smaller, in size than the actual sizes (Hagerman, 1986, 1986; Wu & Crothers, 1984; Koo et al., 1986; Zahn & Blattner, 1985; Cacchione et al., 1989). The explanation for the mobilities shown by such DNAs is that the fragments are bent which leads to the observed retarded mobility. Since our ligated fragments, containing potential B–Z and Z–B junctions, run smaller than they should, their structure(s) as manifested in acrylamide gels may be different from the "bent" structures proposed by these and other authors (Jernigan et al., 1987; Tung & Burks, 1987).

Previously, Hagerman (1984) had observed with his fragment sets that as the fragments become large (greater than ca. 20 repeating units) the deviations in mobilities lessen. An explanation offered by Tung and Burks (1987) is that deviations may diminish for fragments long enough to form a "complete superhelical circle". As shown in Figures 4–6, we observe that as the number of ligations increases the deviations from theoretical mobility also decrease. The number of ligations at which this deviation begins to lessen varies with acrylamide concentration, probably due to the change in pore size in the gel.

The relative mobility plots for the ligated oligomers in the presence of 50 μM $Co(NH_3)_6$ are shown in the lower left-hand panels of Figures 4–6. Circular dichroism data on oligomers 1A and 3A suggest that the $(C^*G)_4$ regions are in a Z-related conformation at high salt concentrations (see below). Comparison of these plots with the appropriate plots in the absence of cobalt indicates that the mobility behavior of the fragments is altered in the presence of cobalt. The exact nature of the altered mobility depends upon the acrylamide percentage and is illustrated in Figure 7.

The plots of the ratio of apparent mobility in the presence of 50 μM Co^{3+} to that in the absence of Co^{3+} vs N shown in Figure 7 reveal some interesting results. There is a pronounced periodicity (at $N < 12$) of that ratio in both 6% and 8% gels. This periodicity peaks at around 100 and 200 base pairs. There is a slight periodicity in 12% gels also peaking at around 100 and 200 base pairs. This periodicity probably reflects differences in the manner in which the cobalt-altered structures move through the gel due to differences in persistence length and/or differences in charge densities of the ligated oligomers in the presence and absence of Co^{3+}.

It should be emphasized, however, that the presence of 50 μM cobalt generally causes the ligation fragments to behave as if they are much smaller than their actual size—the mobility shifts suggest that at least certain fragments can behave as if they are only 40% as large as their actual size. Dimensions such as base pair separation distances obtained from X-ray studies (Wang et al., 1979) suggest that a DNA oligomer in a Z form might be longer than the same oligomer in a B form. Thus, we might have expected the conversion by 50 μM cobalt of the $(C^*G)_4$ regions into a Z form to have lengthened the ligation products, resulting in their running on gels as if they were larger, not smaller.

We have previously noted that, on the basis of ^{1}H NMR results, the base pairs involved in the B–Z junction of BZ1 have unusual properties (Sheardy & Winkle, 1989). The anomalous behavior manifested by the ligation products, which may possess multiple conformational junctions in the presence of cobalt, may be the result of structural peculiarities in the region of the junction. Manning (1988) has suggested that perturbations in DNA structure, such as those that might occur at B–Z junctions, could propagate for some number of base pairs. Further, such perturbations can give rise to an "elastic response" toward internal motions within the fragment. This, perhaps, could give rise to alterations in gel mobility.

The mobility behavior demonstrated by the ligation products in the absence of cobalt may also reflect unusual structural properties of the junction regions even when the oligomer is fully right-handed. Unpublished ^{1}H NMR results on BZ1 in our laboratory suggest that this may be so. The structure of the base pairs in the prejunction region may have a partially "collapsed" or "open" structure in comparison to the surrounding base pairs. In the presence of 50 μM cobalt, in which the C*G region is in a Z structure, BZ1 has a B–Z junction. Saturation-transfer experiments on BZ1 in high salt suggest that the base pairs in the junction have altered accessibility

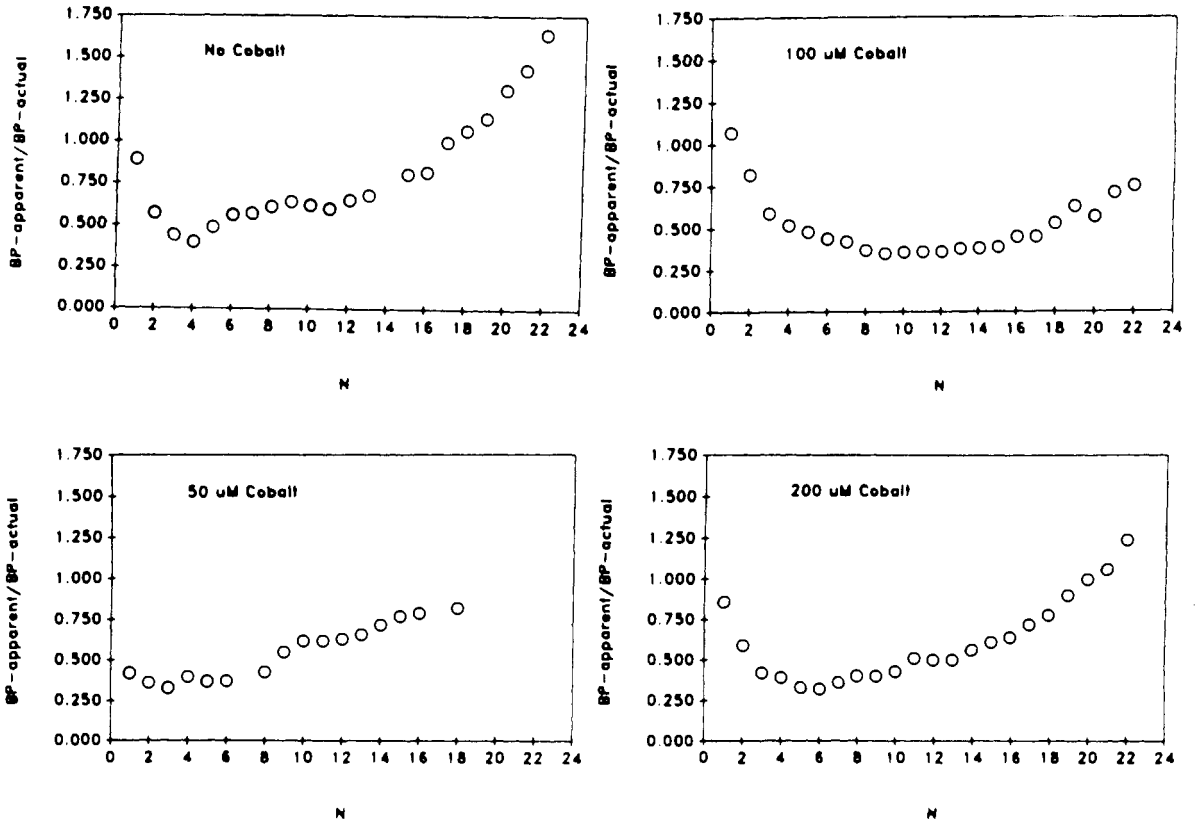

FIGURE 4: Plots of the ratio of the apparent number of base pairs to the actual number of base pairs (BP-apparent/BP-actual) vs number of ligations (N) from mobility data of the ligation products arising from 1A plus 3A through 6% acrylamide gels and at the various cobalt concentrations indicated.

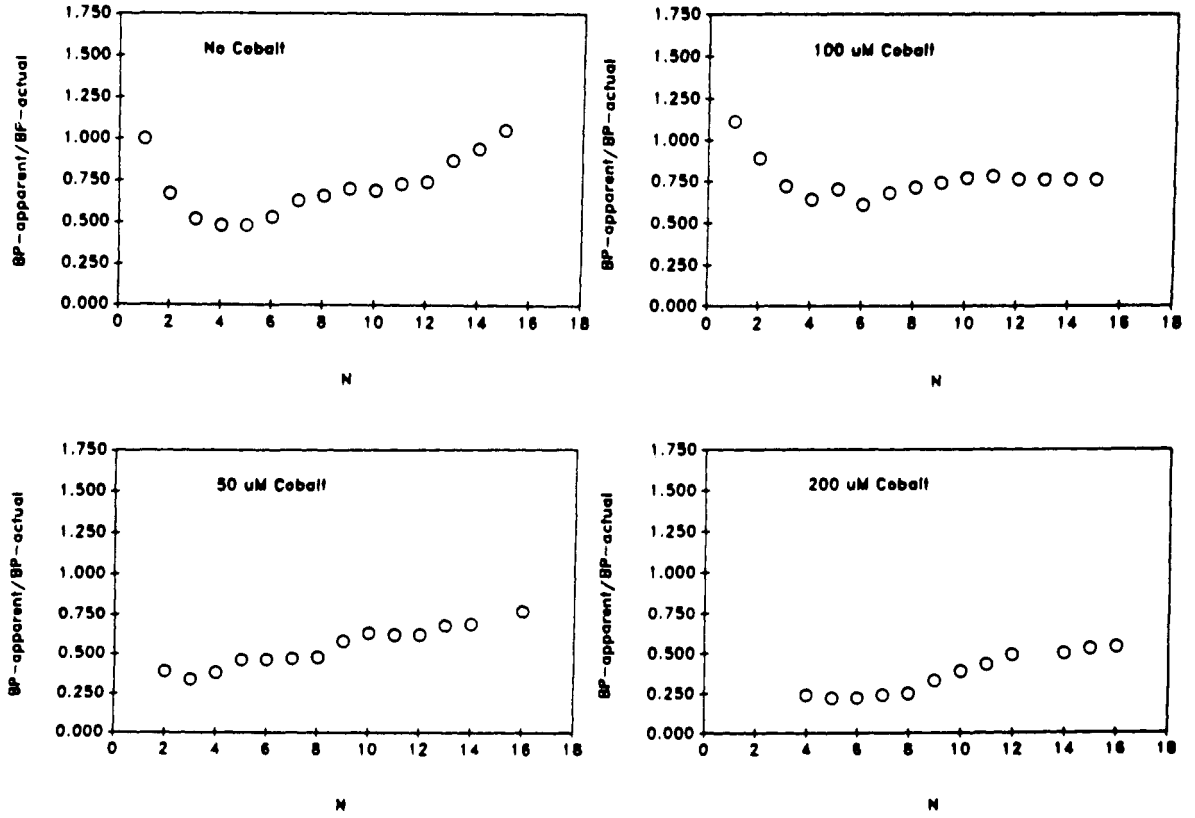

FIGURE 5: Plots of BP-apparent/BP-actual vs N from mobility data of the ligated oligomers through 8% acrylamide gels at the cobalt concentrations indicated.

to water, relative to the other base pairs (Sheardy & Winkle, 1989). The gel experiments described here support the concept that the junctions are in an altered, perhaps "collapsed/open", type of structure. In other words, the prejunction region may be predisposed to junction formation even in the absence of a Z-inducing agent. As noted above, previous studies (Hag-

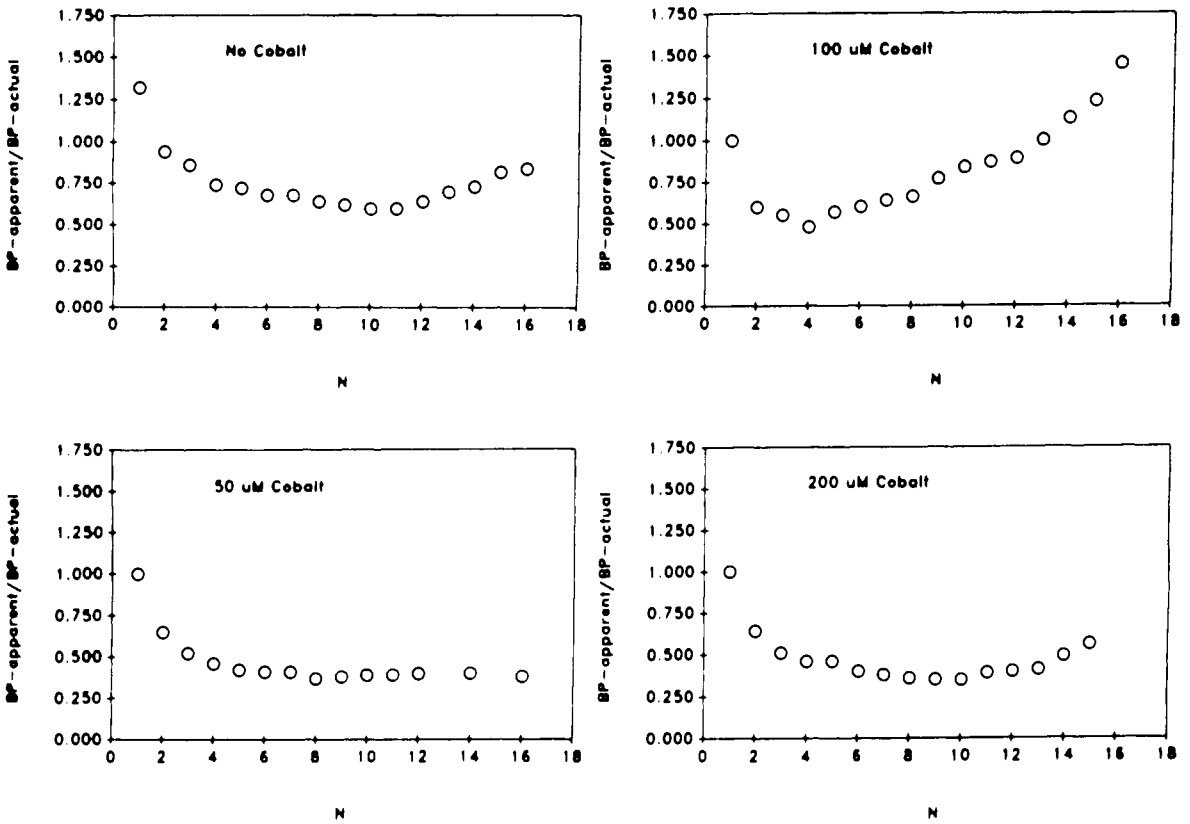

FIGURE 6: Plots of BP-apparent/BP-actual vs N from mobility data of the ligated oligomers through 12% acrylamide gels at the cobalt concentrations indicated.

erman, 1984, 1985, 1986; Wu & Crothers, 1984; Koo et al., 1986; Zahn & Blattner, 1985; Cacchione et al., 1989) suggested that the bending of DNA results in the DNA behaving on acrylamide gels as if it were larger, not smaller, than in reality. Since our ligation fragments run smaller, this may suggest that their mobilities are not caused by bending of the junctions, in agreement with Porschke et al. (1987).

As shown in the upper right-hand panels in Figures 4–6 for 100 μM cobalt and in the lower right-hand panels of Figures 4–6 for 200 μM cobalt, anomalies in gel migration occur at these higher cobalt concentrations as well as at 50 μM. However, interpretation of the results at high cobalt concentrations is probably complicated by factors such as the potential conversion of the Z regions into ψ-type DNA structures. Recent work by Thomas (1989) has suggested that this may occur at high cobalt concentrations.

In order to verify that the ligated oligomers contain multiple junctions at high salt, we carried out a series of preliminary CD studies. The low- and high-salt CD spectra of an unligated equimolar mixture of 1A and 3A are shown in Figure 8A. The low-salt spectrum is characterized by a peak at 284 nm and a trough at 254 nm. This spectrum is similar to those obtained for BZ1 and BZ3 (Sheardy, 1986; Sheardy, unpublished results) and indicates that the unligated concatomers arising from 1A and 3A form in right-handed double helices.

The high-salt spectrum for the unligated concatomers is characterized by a trough at 296 nm, a peak at 280 nm, and another trough at 248 nm. This high-salt spectrum has spectral characteristics of both left-handed DNA (the trough at 296 nm) and right-handed DNA (the trough of 248 nm) and thus indicates that the unligated concatomers possess regions of both left-handedness and right-handedness. It must be kept in mind that the unligated concatomers do not contain true B–Z junctions: the Z-forming blocks are connected to the B-forming blocks through only one sugar–phosphate backbone (i.e., the Z and B regions are noncontiguous).

The low-salt spectrum for the ligated oligomers (Figure 8B) is characterized by a peak at 282 nm and a trough at 252 nm indicative of the right-handed conformation for the ligated concatomers. The high-salt spectrum (Figure 8B) is characterized by a trough at 295 nm, a peak at 279 nm, and another trough at 253 nm. This spectrum is similar not only to that of the unligated concatomers at high salt but also to that of BZ1 at high salt. These concatomers must contain both regions of left-handed and right-handed conformations and therefore must possess multiple B–Z and Z–B junctions.

Although it is not possible to quantitatively assess the ratio of Z base pairs to B base pairs from the CD spectra of ligated oligomers in high salt, comparison of the high-salt spectra of the ligated oligomers to those of the unligated oligomers indicates similarities. Comparison of the changes in ellipticities at 295 and 252 nm in going from low salt to high salt for the unligated oligomers to the ligated oligomers suggests that the unligated oligomers have a higher content of both B and Z forms than the ligated oligomers. This is certainly reasonable since the ligated oligomers contain bases that are neither Z-like nor B-like (i.e., those that reside in the junction regions). Although the CD studies were carried out in the presence of Na^+ while the gel studies were carried out in the presence of Co^{3+}, the CD results presented here indicate that the ligated oligomers will undergo a salt-induced conformational change similar to that observed with BZ1. The complete detailed CD spectroscopic analysis of this system will be presented elsewhere.

The data we have presented here and elsewhere (Sheardy, 1986; Sheardy & Winkle, 1989; Doktycz et al., 1990) suggest that B–Z junctions have unusual structural and dynamic properties that could enhance gel migration. Other physical properties of these oligomers must also be considered to account for the enhanced mobility. It has previously been shown

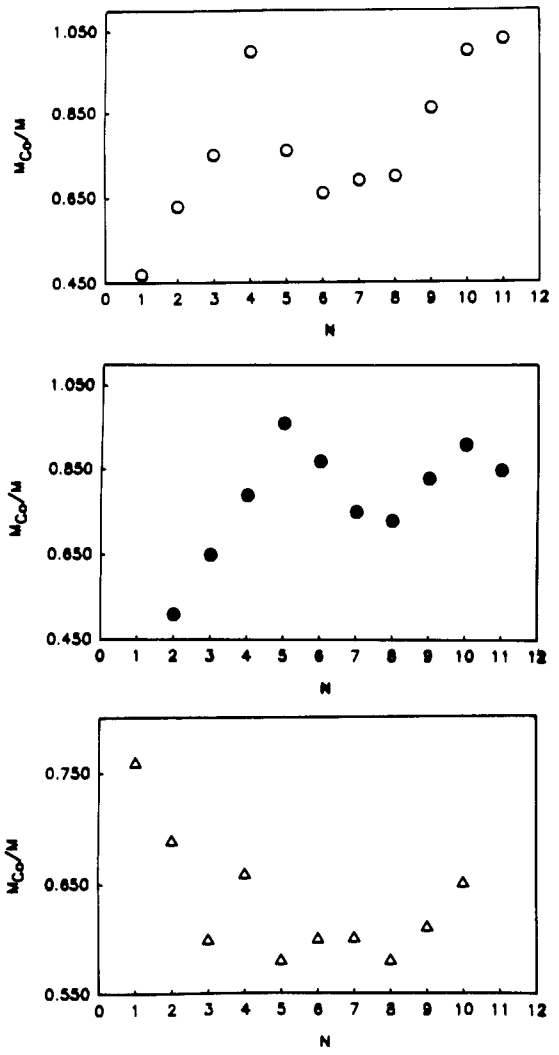

FIGURE 7: Plots of the ratio of the apparent mobility in the presence of 50 μM Co^{3+} to the apparent mobility in the absence of Co^{3+} as a function of the number of ligations (N). The upper panel represents data from 6% acrylamide gels, the middle panel is for the 8% gels, and the bottom panel is for the 12% gels. Notice that the y axis for the 12% gels is different than the other two.

that poly(dG-dC) is more rigid than calf thymus DNA (Thomas & Bloomfield, 1983). This rigidity has been correlated to the observed enhanced gel mobilities of natural and synthetic DNA polymers of high GC content (Anderson, 1986). Since our ligated oligomers are 65% to 70% GC-rich, they may exist as very rigid rods which could result in minimized frictional resistance to movement through the gel. However, invoking the stiffness of our ligated oligomers as the source of their enhanced mobilities may not solely account for the observed magnitude of the effect. Therefore, the structure/dynamics of the junction regions probably play a major role in the mobilities manifested by these ligated oligomers.

Conclusions

The electrophoresis studies reported here on ligated oligomers containing multiple copies of potential B–Z junctions (and Z–B junctions) suggest that these DNA molecules possess structural anomalies in the absence of Z-inducing agents. In the presence of 50 μM cobalt hexamine, the C*G regions of these oligomers switch to Z structures, creating conformational junctions. The oligomers in both the absence and the presence of cobalt behave on gels as if they were significantly smaller than their actual sizes, giving further indication that such conformational junctions have unusual structures. Under both

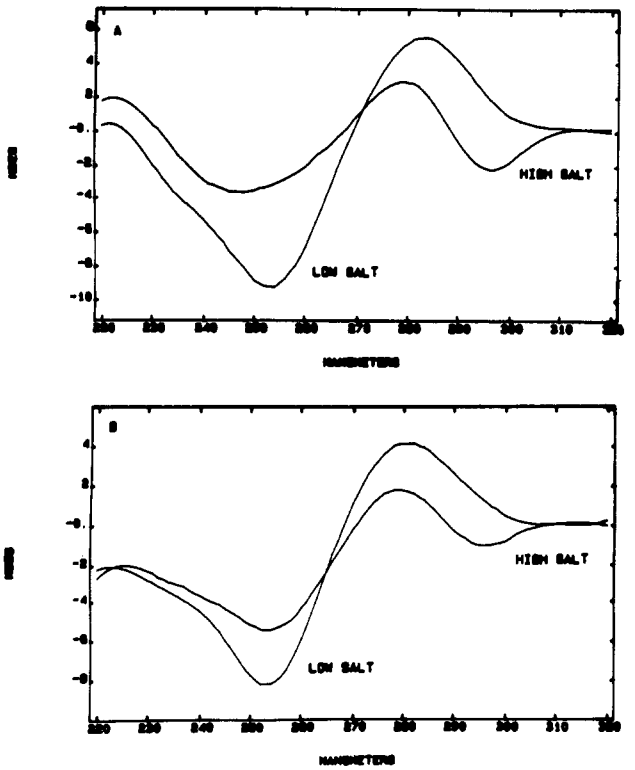

FIGURE 8: Circular dichroism (CD) spectra at 25 °C of (A) an unligated equimolar mixture of strands 1A and 3A in phosphate buffer at low (i.e., 100 mM NaCl) and high (i.e., 5 M NaCl) salt and (B) the ligated mixture of 1A and 3A in phosphate buffer at low and high salt.

conditions, the data indicate that the oligomers must have a periodic distortion down the chain which significantly contributes to the anomalous gel behavior. We are currently investigating the nature of this distortion by probing the ligated oligomers with a variety of small molecules and specific digesting enzymes.

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NMR Studies of the Interaction of Chromomycin A_3 with Small DNA Duplexes. Binding to GC-Containing Sequences†

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ABSTRACT: The interaction of chromomycin A_3 with the oligodeoxyribonucleotides **1**, d(ATGCAT), **2**, d(ATCGAT), **3**, d(TATGCATA), and **4**, d(ATAGCTAT), has been investigated by ^1H and ^{31}P NMR. In the presence of Mg^{2+}, chromomycin binds strongly to the three GC-containing oligomers **1**, **3**, and **4** but not to the CG-containing oligomer **2**. The proton chemical shift changes for **1** and **3** are similar, and these DNA duplexes appear to bind with a stoichiometry of 2 drugs:1 Mg^{2+}:1 duplex. The same stoichiometry of 2 drugs:1 duplex is confirmed with **4**; however, proton chemical shift changes differ. An overall C_2 symmetry is exhibited by the drug complex with **1**, **3**, and **4**. At a molar ratio of 2.0 (drugs:duplex), no free DNA proton NMR signals remain. Two-dimensional nuclear Overhauser exchange spectroscopy (NOESY) of the saturated chromomycin complex with **1** and **3** positions both chromomycinone hydroxyls and the E carbohydrates in the minor groove and provides evidence suggesting that the B carbohydrates lie on the major-groove side. This is supported by several dipolar coupling cross-peaks between the drug and the DNA duplex. Drug-induced conformational changes in duplex **1** are evaluated over a range of NOESY mixing times and found to possess some characteristics of both B-DNA and A-DNA, where the minor groove is wider and shallower. A widening of the minor groove is essential for the DNA duplex to accommodate two drug molecules. This current minor-groove model is a substantial revision of our earlier major-groove model [Keniry, M. A., Brown, S. C., Berman, E., & Shafer, R. H. (1987) *Biochemistry 26*, 1058–1067] and is in agreement with the model recently proposed by Gao and Patel [Gao, X., & Patel, D. J. (1989a) *Biochemistry 28*, 751–762].

Chromomycin is an antitumor antibiotic belonging to the aureolic acid group (see Figure 1; Thiem & Meyers, 1979). Along with the related analogues mithramycin and olivomycin, chromomycin is believed to complex with double-stranded DNA, thereby inhibiting DNA and RNA synthesis (Remers, 1979; Miyamoto et al., 1979; Kersten & Kersten, 1974; Berlin et al., 1966; Wakisaka et al., 1963; Slavek & Carter, 1975; Gause, 1965; Ward et al., 1965; Behr et al., 1969; Hartmann et al., 1968). The mode of drug binding to DNA is essential in elucidating the potent antitumor behavior of the aureolic acid group of drugs. In the case of chromomycin, a large variety of techniques has been applied to achieve this goal (Van Dyke & Dervan, 1983; Fox & Howarth, 1985; Hayasaka & Inoue, 1969; Dagleish et al., 1974; Cobreros et al., 1982; Behr et al., 1969; Nayak et al., 1973; Ward et al., 1965).

Early spectrophotometric studies demonstrated that the anionic chromomycin binds to DNA only in the presence of dicationic metals, e.g., Mg^{2+} and Mn^{2+}. The apparent binding constant ($K_{app} \sim 10^5$–10^6 M^{-1}) is similar to that of the neutrally charged intercalator actinomycin (Kersten et al., 1966; Behr et al., 1969; Horwitz & McGuire, 1978). This comparison led some researchers to believe that the chromomycinone chromophore also intercalated into DNA (Behr et al., 1969; Horwitz & McGuire, 1978). In the first NMR studies of chromomycin binding to DNA we observed additional evidence in support of intercalation, based on ^{13}C and ^1H NMR experiments of calf thymus DNA and poly(dG-dC) (Berman & Shafer, 1983; Berman et al., 1988). However, subsequent high-resolution NMR analysis of the oligonucleotide complex with chromomycin demonstrated that binding occurred via a nonintercalative binding mode, despite the observation of chemical shift effects typical of intercalation (Berman et al., 1985; Keniry et al., 1987).

Footprinting studies demonstrated that the DNA binding site consists of three or more base pairs and usually contains two contiguous guanine–cytosine base pairs. Although the requirement for guanine or a 2-aminopurine substitute has been established for tight binding with the aureolic acids, evidence for olivomycin binding to poly(dA-dT)·poly(dA-dT) suggests that a weaker binding interaction ($K_{app} < 10^5$ M^{-1})

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