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Conformational Properties of B-Z Junctions in DNA†

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ABSTRACT: The structural consequences of specific base sequences in DNA can exert a strong influence on the function of DNA. It has previously been reported that the presence of multiple B-Z conformational junctions in constructed DNA oligomers results in unusually *enhanced* electrophoretic gel mobilities of these oligomers [Winkle, S. A., & Sheardy, R. D. (1990) *Biochemistry 29*, 6514-6521]. In order to investigate this phenomenon further, we designed and synthesized several DNA oligomers capable of pure Z or B-Z junction formation for polyacrylamide gel electrophoresis studies. The results indicate that both pure Z-DNA and polymorphic B-Z-DNA oligomers exhibit unusual gel migratory properties. The results of gel mobility studies in the absence and presence of cobalt hexamine indicate that a B-Z junction corresponds to a stiff bend of the helix axis, with two or more conformers accessible at the junction site. This is a different bend and mechanism than that in oligo(A) tracts.

The conformation and dynamical properties of a segment of DNA are strongly dependent upon both its sequence and its environment. One approach to the study of sequence and environmental influences on the biophysical properties of nucleic acids is through rational design of model systems based upon short (i.e., 16-80 bp) DNA oligomers. Many unusual DNA structures have been revealed in this way, including left-handed conformations (Wang et al., 1979; Gessner et al., 1985), bent DNA (Wu & Crothers, 1984; Hagerman, 1984, 1985, 1986), branched DNA structures (Ma et al., 1986;

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BZ-I		$(C^*GC^*G)_2(ACTG)_2$ $(GC^*GC^*)_2(TGAC)_2$
I	$Z_{40}B_{40}$ME:	$(C^*GC^*G)_{10}(ACTG)_{10}$ $(GC^*GC^*)_{10}(TGAC)_{10}$
II	$Z_{40}B_{40}$UNME:	$(CGCG)_{10}(ACTG)_{10}$ $(GCGC)_{10}(TGAC)_{10}$
III	$B_{20}Z_{40}B_{20}$ME:	$(CAGT)_5(C^*GC^*G)_{10}(ACTG)_5$ $(GTCA)_5(GC^*GC^*)_{10}(TGAC)_5$
IV	$Z_{20}B_{60}$ME:	$(C^*GC^*G)_5(ACTG)_{15}$ $(GC^*GC^*)_5(TGAC)_{15}$
V	$Z_{60}B_{20}$ME:	$(C^*GC^*G)_{15}(ACTG)_5$ $(GC^*GC^*)_{15}(TGAC)_5$
a	Z_{20}ME:	$(C^*GC^*G)_5$ $(GC^*GC^*)_5$
b	Z_{40}ME:	$(C^*GC^*G)_{10}$ $(GC^*GC^*)_{10}$
c	Z_{60}ME:	$(C^*GC^*G)_{15}$ $(GC^*GC^*)_{15}$

FIGURE 1: DNA oligomers discussed in the report. The design, synthesis, and characterization of all component strands are detailed in the main text. Here, C* is 5-methylcytidine.

Seeman et al., 1989), and B–Z conformational junctions (Sheardy, 1988; Sheardy & Winkle, 1989). Although the biological roles of Z-DNA and B–Z junctions remain unclear at this time, these structures are interesting from a physical chemical point of view (Jovin & Soumpasis, 1987; Soumpasis, 1988; Doktycz et al., 1990; Suh et al., 1991; Lu et al., 1991).

The B–Z junction forming molecule BZ-I (Figure 1) has been found to display unusual properties under low salt conditions (when the molecule is fully right-handed) as well as high salt conditions (when the molecule contains the B–Z junction). Binding isotherms obtained from the interaction of ethidium bromide to BZ-I suggest that the molecule exists in two distinct forms under both low and high salt conditions. Furthermore, the binding of ethidium is *enhanced* at 4.5 M NaCl relative to low salt conditions (Suh et al., 1991). Treatment of BZ-I with MPE-Fe(II) results in a highly asymmetric cleavage of the two strands under both low and high salt conditions (Lu et al., 1991), indicating that the two strands are not equivalent. Finally, the imino proton resonance for the G base of the G-C base pair at position 8 is upfield shifted from the other G base iminos in the NMR spectrum of the duplex under low salt conditions (Sheardy & Winkle, 1989).

It has previously been suggested that B–Z junctions might bend the helical axis (Lavery, 1988) and that they might have unusual flexural properties (Sheardy & Winkle, 1989; Winkle & Sheardy, 1990). The opposite case has also been argued: according to Porschke et al. (1987), B–Z junctions are neither bent nor flexible. One experiment that has been used to detect DNA bending is the observation of retarded electrophoretic migration through polyacrylamide gels (Koo et al., 1986, 1990; Hagerman, 1984, 1985, 1986; Cacchione et al., 1989; Rice & Crothers, 1989; Drak & Crothers, 1991). These studies examined the migration of DNA oligomers constructed to contain single or multiple bends: the more bends, the greater the relative retardation in mobility (up to about 200 base pairs in length). The phasing of static bends is critical to the extent of gel retardation. Maximal retardation of the oligomers occurs when the bends are in phase resulting in minimum end-to-end distance (Wu & Crothers, 1984; Slater & Noolandi, 1986; Drak & Crothers, 1991).

In order to determine if B–Z junctions bend the helix axis, DNA oligomers were constructed to contain multiple phased B–Z junctions. Surprisingly, these oligomers displayed dramatically *enhanced* migration through polyacrylamide gels both under low salt conditions and in the presence of cobalt hexamine (Winkle & Sheardy, 1990). This behavior is different from those observed with curved DNAs. Several potential factors could influence the mobilities of these polyjunction molecules: (1) increased flexibility at the junction region; (2) anisotropic bending of the junction (i.e., consecutive junctions are bent in opposite directions resulting in a zigzag arrangement of the oligomer); (3) lower affinity for the gel matrix as a consequence of the sequence; or (4) any combination of these effects.

Determining the contribution of a single junction to the electrophoretic mobility of B–Z junction forming molecules could help discriminate among the possibilities. To this end, we designed and synthesized several new DNA oligomers for gel electrophoretic studies. Certain constraints were considered in the design of these molecules. Each oligomer must contain a Z-forming segment abutting a non-Z-forming segment. The Z-forming segments are primarily composed of contiguous (5medC-dG) repeats: modification of C bases greatly facilitates salt-induced B to Z transitions (Behe & Felsenfeld, 1981). Since a single bend in a short oligomer may not result in an appreciably detectable retardation, DNA oligomers of 80 bases were chosen. The position of the B–Z junction, and hence the potential bend, was varied to change the end-to-end distance for the oligomers. As a control, one nonmethylated analogue was synthesized to test the effect of the cytidine methyl group in the Z-forming segment. As additional controls, pure Z-forming oligomers of varying lengths were also synthesized.

The oligomers synthesized and studied in this report are shown in Figure 1. Oligomer I is simple an "extended" version of BZ-I, with a Z-forming segment of 40 bases and a non-Z-forming segment of 40 bases. Oligomer II is the unmethylated sequence analogue of I. Oligomer III contains a B–Z–B conformational block sequence motif with 20, 40, and 20 bases, respectively. Oligomers IV and V are 80-mers with Z-forming blocks of 20 and 60 bases, respectively, rather than the 40 bases in I. Finally, oligomers a, b, and c are pure Z-forming oligomers of 20, 40, and 60 bases, respectively.

The results of CD and PAGE studies confirm the unusual structure and dynamics of B–Z junctions that have been previously noted (Winkle & Sheardy, 1990; Suh et al., 1991; Lu et al., 1991). The observed altered gel mobilities of these oligomers are consistent with bending at the conformational interfaces present in these molecules under conditions of high salt.

Materials and Methods

Synthesis and Purification of Oligonucleotides. Individual strands were synthesized on an Applied Biosystems 380B DNA synthesizer using the phosphoramidite chemistry of Caruthers (1982). Purification was effected by preparative polyacrylamide gel electrophoresis under denaturing conditions. In particular, different gel percentages were used for the different lengths of the oligomers: the 60-mer and all 80-mers were run through 6% gels, the 40-mer was run through a 12% gel, and the 20-mer was run through a 20% gel. The band corresponding to the desired oligomer was cut from the gel and

soaked in buffer (0.5 M NH_4Ac, 1 mM EDTA) overnight. The oligomer was desalted and isolated by precipitation with ice-cold ethanol (2 times).

Annealing Reactions. For the non-self-complementary duplexes, equal molar amounts of the individual strands were mixed at 1 μM concentration in a mixture of 50 mM Tris-HCl, pH 7.0, and 100 mM NaCl, with or without 100 μM $Co(NH_3)_6^{3+}$ in a total volume of 10 μL. Solutions of the self-complementary duplexes were generated in the same buffers and at the same total DNA concentration. An Eppendorf tube containing each solution was immersed in boiling water for three minutes, cooled slowly to room temperature, and finally chilled to 4 °C.

CD Studies. Circular dichroism spectra were recorded with an AVIV Model 60DS CD spectropolarimeter at 4 °C and at 25 °C. DNA samples ([DNA] = 1.5 μM) were prepared in a solution of 50 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, and 100 mM NaCl, with or without 100 μM $Co(NH_3)_6^{3+}$.

Gel Electrophoresis. Native gels (19:1 monomer/BIS) were run at 8 V/cm. The electrophoretic plates were jacketed and cooled with circulating water to provide a running temperature of 4 ± 1 °C in the gel throughout the electrophoresis. The buffer system contained 40 mM Tris, 20 mM acetic acid, pH 8.1, and 1 mM EDTA or the same buffer with 100 μM $Co(NH_3)_6^{3+}$. No dyes were added in these runs. The gels were stained in 9:11 formamide/water containing 0.01% Stainsall dye (Aldrich). Permanent records of the gels were made with Kodak EDP electrophoresis duplicating paper. Due to the inability of Stainsall to stain pure Z-forming oligomers a, b, and c in the presence of $Co(NH_3)_6^{3+}$, these oligomers were end-labeled using [γ-^{32}P]ATP and T4 kinase. Detection of the resultant bands was via autoradiography. For a DNA size marker, a *Hpa*II digest of pBR322 plasmid DNA was also run for each electrophoresis.

Plots of ln L (length of fragment in base pairs) vs relative gel mobility were constructed for the bands arising from the *Hpa*II digest of pBR322. These plots yielded straight lines in both the absence and presence of trivalent cobalt. From these plots, L_{app} (apparent length) for each oligomer could be extrapolated and compared to L_{act} (actual length) (Hagerman, 1984, 1985, 1986; Drak & Crothers, 1991). A retardation factor (R_T for low salt or R_T' for high salt) is then evaluated (for example):

$$R_T = L_{app}/L_{act}$$

From these values, a retardation ratio can be evaluated as R_T'/R_T. Since we are interested in the relative mobility of each oligomer in the presence of $Co(NH_3)_6^{3+}$ to that in the absence of $Co(NH_3)_6^{3+}$, the retardation ratio thus obtained is a more meaningful way to look at the observed mobilities in the presence of the trivalent cobalt.

The dependence of oligomer mobility on total gel composition is referred to as a Ferguson plot (Rodbard & Chrambach, 1977). The simplest behavior is that in which the log of the mobility is linearly related to the gel composition:

$$\log M = \log M_0 - K_R T$$

where M is the mobility at gel composition T (percentage of acrylamide), K_R is the retardation coefficient, and M_0 is the free mobility of the DNA oligomer. In many cases, linear plots are not observed; we observe reasonably linear plots for the species studies here.

The constant K_R is a measure of the friction constant of the migrating species and qualitatively is related to the shape of the species. M_0 is thought to vary with the charge density of the migrating species. Thus, changes in the slopes and/or y-intercepts of Ferguson plots evoked by changes in gel conditions indicate changes in shapes and/or charge densities of the migrating species.

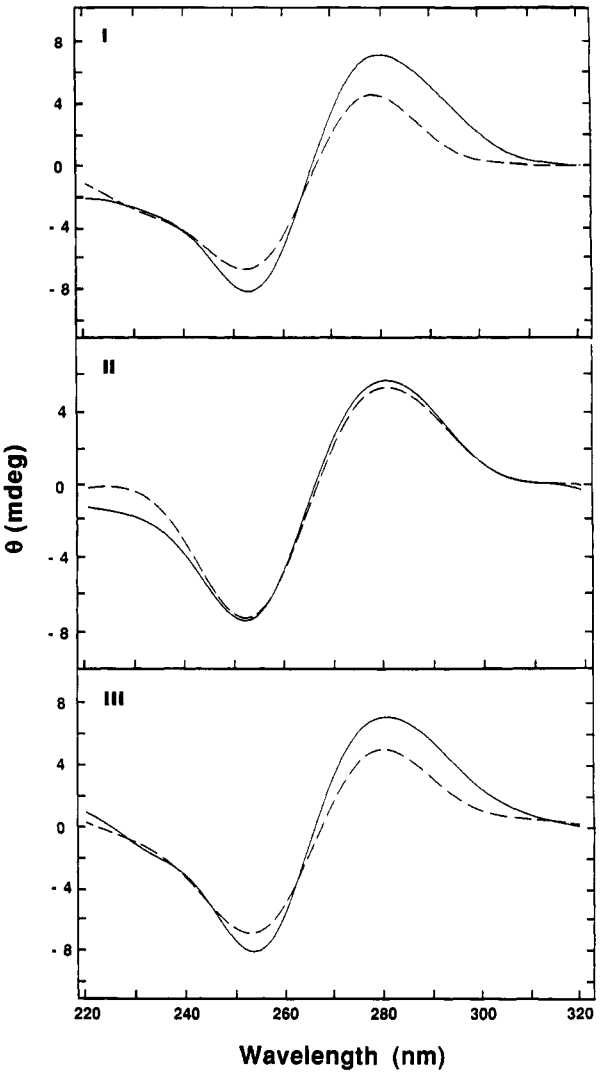

FIGURE 2: CD spectra of DNA oligomers I, II, and III in Tris-HCl buffer at low salt (100 mM NaCl, solid line) and high salt (100 mM NaCl and 100 μM $Co(NH_3)_6^{3+}$, dashed line) at 25 °C. CD spectra were also run at 4 °C; no differences in the resultant spectra were observed.

RESULTS

The CD spectra of I, II, and III in the absence and presence of $Co(NH_3)_6^{3+}$ (low salt and high salt), respectively, are shown in Figure 2. While both I and III undergo salt-induced conformational changes indicated by the observed differences in their low and high salt spectra, II apparently does not undergo a salt-induced conformational change. In all cases, each low salt spectrum is characterized by a peak at 280 nm and a trough at 255 nm. The high salt spectrum of I or III is characterized by a shallow trough at 295 nm, a peak at 280 nm, and a trough at 255 nm. These spectral characteristics are similar to those observed for the high salt spectrum of BZ-I (Sheardy, 1988; Sheardy & Winkle, 1989). The high salt spectrum of II, on the other hand, is very similar to its low salt spectrum. Although the high salt spectra of I and III are similar to that of BZ-I, as well as similar to each other, it should be noted that they are not identical to that of BZ-I. The high salt spectrum of BZ-I has a much deeper trough at 295 nm and a much shallower trough at 255 nm. However, Na^+ was used to drive the conformational transition of BZ-I while $Co(NH_3)_6^{3+}$ was used to drive the conformational transitions for I–III. We have observed that the shape of the

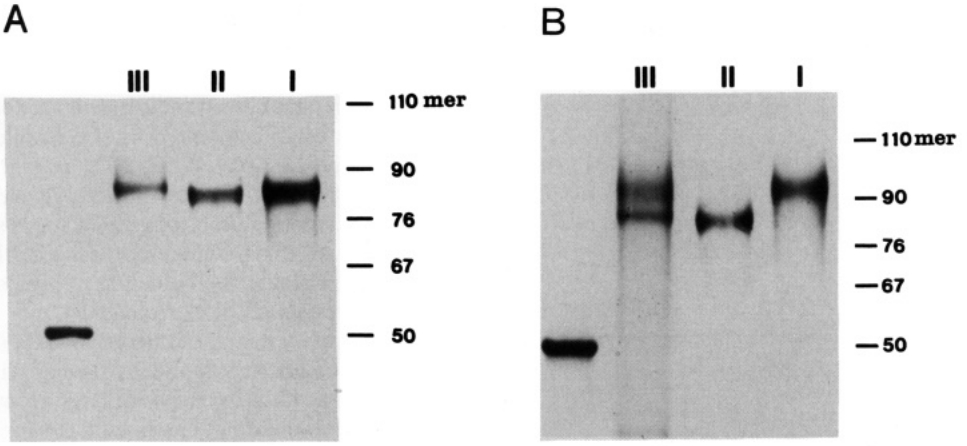

FIGURE 3: Gel electrophoresis analysis of DNA oligomers I–III (6 μg of DNA/lane) under native conditions. Shown is a photograph of a 10% polyacrylamide gel run in the absence of cobalt hexamine (A) and in the presence of 100 μM $Co(NH_3)_6^{3+}$ (B). The marker bands are from the *Hpa*II digest of pBR322 DNA. The native gel was stained as detailed in Materials and Methods.

Table I: Retardation Factors and Retardation Ratios for DNA Oligomers of This Study[a]

oligomer		R_T	R_T'	R_T'/R_T
I	Z40B40ME	1.03	1.175	1.14
II	Z40B40UNME	1.01	1.05	1.04
III	B20Z40B20ME	1.05	1.075	1.02
III′			1.16	1.11
IV	Z20B60ME	1.03	1.13	1.11
V	Z60B20ME	1.03	1.22	1.18
a	Z20ME	1.00	1.53	1.53
b	Z40ME	1.04	1.47	1.41
b′		0.875	1.31	1.49
c	Z60ME	1.08	1.45	1.34
c′		0.97	1.40	1.44

[a] All values are ±0.005. The retardation factors were calculated as $R_T = L_{app}/L_{act}$ (R_T' is the retardation factor in the presence of cobalt hexamine). The retardation ratio is defined as R_T'/R_T. The retardation factors calculated for b′ and c′ are based upon an L_{act} of 20 and 30 bases, respectively (since these species are hairpinned).

Table II: Summary of Ferguson Analysis for DNA Oligomers of This Study[a]

	−log M (× 10^2)		K_R (× 10^3)	
oligomer	−	+	−	+
I	4.7	8.5	5.2	4.4
II	4.4	5.4	5.2	4.0
III	5.0	5.9	5.3	4.1
III′		8.2		4.4
IV	6.0	6.8	3.4	3.8
V	6.0	8.3	3.4	3.8
a	0.71	3.4	2.3	5.3
b	3.7	10	11	10
c	7.8	17	15	11

[a] Mobilities of these oligomers in the absence (−) or presence (+) of 100 μM $Co(NH_3)_6^{3+}$ were determined as a function of the percentage of acrylamide as described in Materials and Methods. Reported values are within ±5%.

CD spectra of junction forming molecules under conditions of high salt depends upon the cation used (R. D. Sheardy, unpublished results). This dependence of the CD on cation could result from different types of Z structure in Na^+ and in $Co(NH_3)_6^{3+}$. The electrophoretic results discussed indicate that the transitions observed by CD for I and III are complete. Therefore, I and III must contain B–Z junctions under conditions of high $Co(NH_3)_6^{3+}$, while II apparently does not. Methylation of the cytidine bases in the Z-forming segments of I and III facilitates the B to Z transition (Behe & Felsenfeld, 1981).

The results of the electrophoresis of I–III in a 10% native polyacrylamide gel and in the absence of $Co(NH_3)_6^{3+}$ are shown in Figure 3A. As can be seen, all oligomers essentially run as 80-mers, although the methylated versions are slightly retarded. Retardation factors (R_T) for these oligomers are given in Table I. Ferguson plots for these oligomers were constructed, and the resultant linear parameters are presented in Table II. Examination of the Ferguson data indicates that all oligomers have nearly identical frictional coefficients (i.e., K_R) and similar charge densities (i.e., log M_0) as they pass through the gel (Rodbard & Chrambach, 1971).

The shift in conformation in I and III that occurs in the presence of $Co(NH_3)_6^{3+}$ is accompanied by a corresponding change in their gel mobilities (Figure 3B). Specifically, in the presence of $Co(NH_3)_6^{3+}$, I is highly retarded in the gel and II is only slightly retarded in the gel while, interestingly, III is split into a highly retarded band (III′) and an essentially nonretarded band (III). The retardation factors (R_T') and the ratios of R_T'/R_T are given in Table I. As noted above, the retardation ratio is a more meaningful way to evaluate the effect of $Co(NH_3)_6^{3+}$ on the gel mobilities (i.e., this ratio gives the retardation in the presence of cobalt relative to the retardation in the absence of the cobalt). As can be seen, the order of retardation is I ~ III′ > II > III. Examination of the Ferguson data (Table II) indicates that $Co(NH_3)_6^{3+}$ induces slight decreases in the slopes and more dramatic decreases in the y-intercepts for those species displaying the greatest degree of retardation. For the nonretarded species, only slight decreases in their y-intercepts are noted. Thus, the retardations observed may be a direct result of decreased charge densities. This possibility will be addressed later.

Figure 3B indicates that only one species is present for I and II, while two species are present for III. Since the high salt CD spectra of I and III are nearly identical, they presumably have similar conformational compositions. Thus, the splitting of III is not due to incomplete conformational transition: it must be due to two structural isomers. At 25 °C or 40 °C, only a single band of intermediate retardation is detected for III in the presence of cobalt hexamine (data not shown).

In view of the relationship between DNA bending and retardation of mobility [for example, Hagerman (1984)], the results presented above may also be interpreted in terms of B–Z junction bending. To test this hypothesis, the electrophoretic mobilities of IV and V in the absence and presence of $Co(NH_3)_6^{3+}$ were determined. In principle, both of these molecules contain a single B–Z junction in high salt that differs in position with respect to the ends of the duplex relative to that of I. Whereas I has its junction at position 40, IV has its junction at position 20 and V has its junction at position

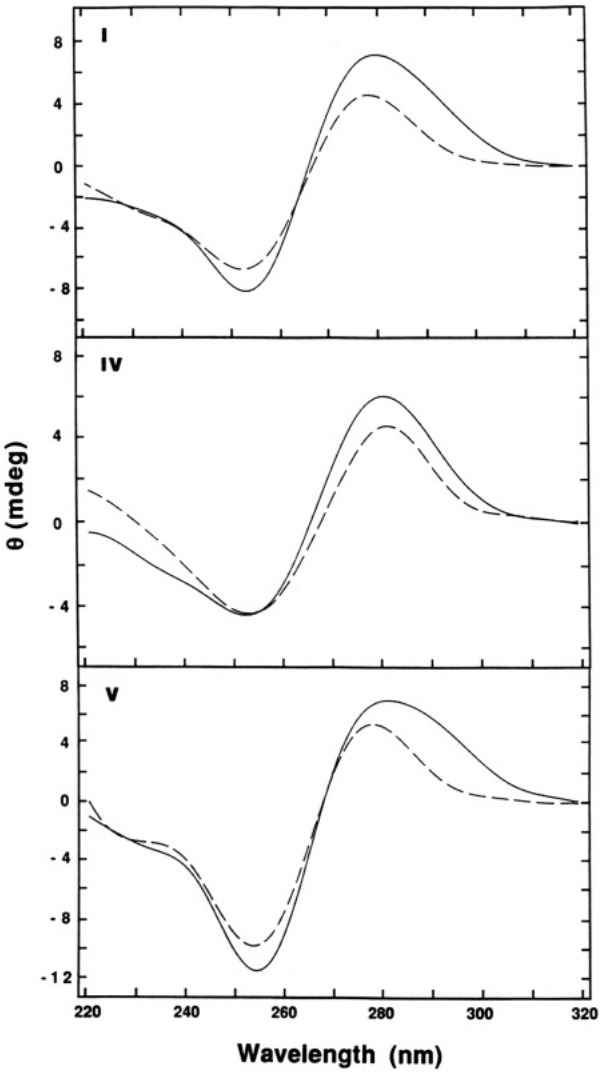

FIGURE 4: The CD spectra for DNA oligomers I, IV, and V in Tris-HCl buffer at low salt (100 mM NaCl, solid line) and high salt (100 mM NaCl and 100 μM $Co(NH_3)_6^{3+}$, dashed line) at 25 °C.

60. Since the observed mobilities of bent oligomers depend upon the location of the bend with respect to the ends (Drak & Crothers, 1991), and in the case that a B–Z junction represents a static bend, then I, IV, and V should display different retardations in the presence of $Co(NH_3)_6^{3+}$.

The comparative CD spectra of I, IV, and V in low and high salt are shown in Figure 4. As can be seen, each of these oligomers undergoes a salt-induced conformational change. Noting the $Co(NH_3)_6^{3+}$-induced differences in ellipticities at 295 nm [$\Delta\theta_{295} = \theta_{295}(Co(NH_3)_6^{3+}) - \theta_{295}$(no cobalt)] for each oligomer qualitatively indicates completed transitions (i.e., the amount of Z-form conformation should increase in the following order: V > I > VI; $\Delta\theta_{295}$ also increases in that order). Furthermore, as discussed below, gel electrophoresis of these oligomers in high salt indicates a single species present under these conditions. Therefore, each of these oligomers must contain a single B–Z junction at high salt.

The results of the electrophoresis of I, IV, and V are shown in Figure 5A,B, and the Ferguson data are given in Table II. All oligomers run identically through the polyacryalmide in low salt at all gel concentrations. However, anomalous behavior for these oligomers is noted for the gels run in $Co(NH_3)_6^{3+}$. The order of retardation is V > I > IV. In other words, the extent of retardation varies monotonically with the extent of Z-form present in the oligomer and not with the *position* of the B–Z junction. The Ferguson data suggest that decreases in the charge densities of these oligomers in the presence of cobalt may also contribute significantly to the retardations observed.

It appears then that the observed retarded mobility of junction containing molecules may simply be due to the presence of Z-form DNA per se and not necessarily due to bending at the junction. In order to calibrate the effect of Z-form DNA on gel mobilities, we synthesized a series of pure Z-forming oligomers of various lengths. These oligomers have the general sequence $(5medC\text{-}dG)_n$ where n is 10, 20, or 30. The CD spectra of these oligomers in the presence of $Co(NH_3)_6^{3+}$ show that each oligomer assumes a fully left-handed conformation (data not shown).

Polyacrylamide gel electrophoresis of these oligomers in low salt indicates some unusual behavior. Under low salt conditions (Figure 6A), the 20-mer a runs as a single species with a mobility of a true B-DNA 20-mer. However, the 40-mer b and the 60-mer c display anomalous behavior. First, both b and c are slightly retarded in the gel, with c more retarded than b. Second, both oligomers give rise to two bands in the gel. The mobilities of these additional bands are consistent with a potential hairpin conformation in these oligomers.

In the presence of $Co(NH_3)_6^{3+}$, all species are dramatically retarded in mobility (see Figure 6B). Again, a factor contributing to the relative retardations is the decrease in charge densities of the oligomers in the presence of trivalent cobalt. Furthermore, the relative extent of retardation, as estimated by the retardation ratios (R_T'/R_T) listed in Table I, decreases

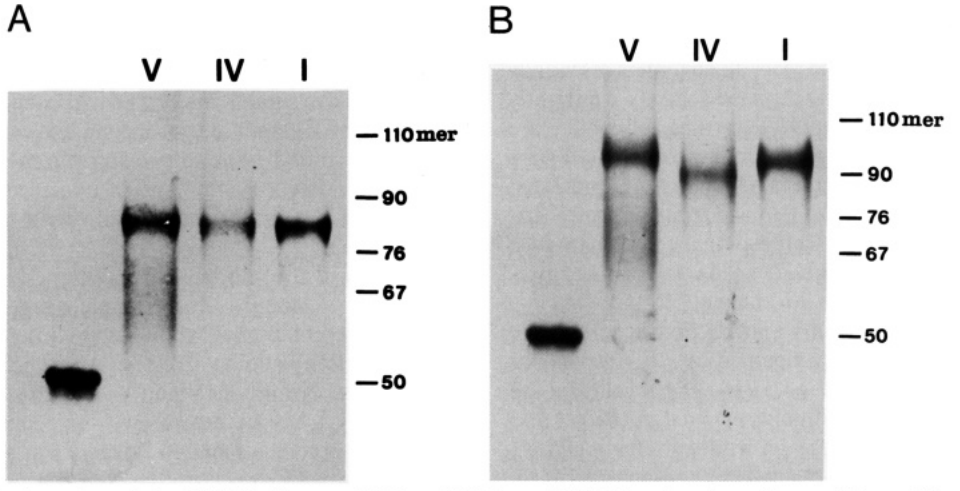

FIGURE 5: Gel electrophoresis analysis of DNA oligomers I, IV, and V (6 μg of DNA/lane) under native conditions. Shown is a photograph of a 10% gel run in the absence of cobalt hexamine (A) and in the presence of 100 μM $Co(NH_3)_6^{3+}$ (B).

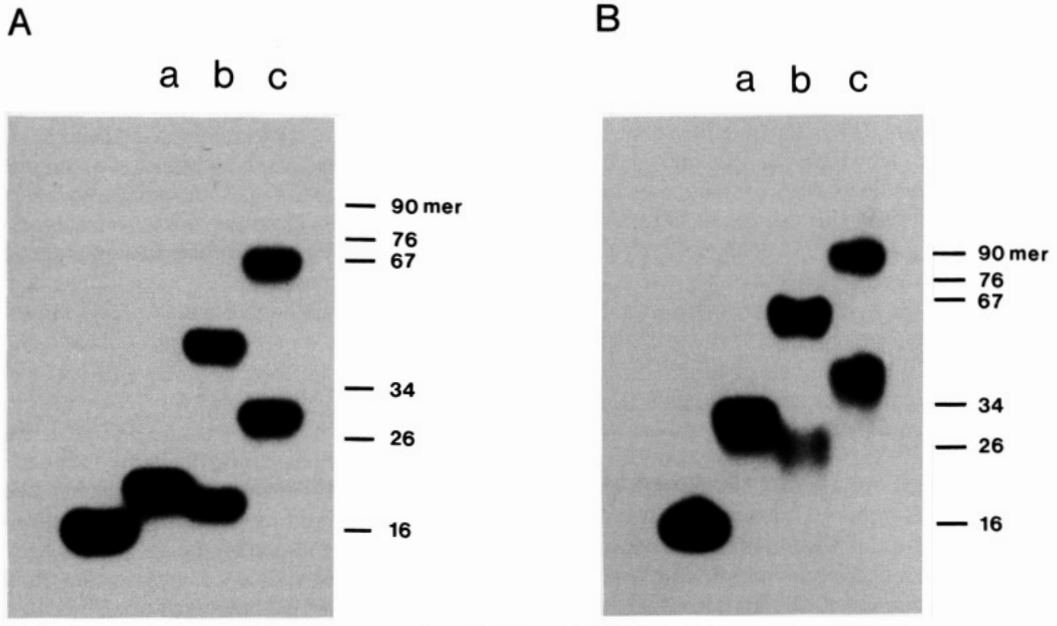

FIGURE 6: Gel electrophoresis analysis of DNA oligomers a, b, and c (6 μg of DNA/lane) under native conditions. Shown is an autoradiogram of a 10% gel run in the absence of cobalt hexamine (A) and in the presence of 100 μM $Co(NH_3)_6^{3+}$ (B).

with increasing Z-form. This is completely opposite from what is observed in the 80-mers I, III, IV, and V. A plot of ln L (actual length) vs R_T'/R_T for a, b, and c yields a straight line from which a retardation ratio for a pure Z-DNA 80-mer can be extrapolated. The value obtained (1.29) is still much higher than the greatest retardation ratio for junction forming 80-mers (i.e., 1.18 for V).

Discussion

The factors that influence the migration of a DNA oligomer through polyacryalmide gels are of fundamental interest (Calladine et al., 1991). Anomalous gel migration of DNA oligomers has typically been associated with unusual structure (Hagerman, 1984, 1985, 1986; Wu & Crothers, 1984; Koo et al., 1986, 1990; Cacchione et al., 1989; Rice & Crothers, 1989). For example, the anomalously slow migration observed in multimers of (GA_4T_4C) has been suggested to arise from bending at the A–T step which would result in overall curvature of the DNA multimers (Hagerman, 1984, 1985, 1986). Slow migration has also been associated with bending induced by a bulge defect (Rice & Crothers, 1989). On the other hand, anomalously fast electrophoretic mobility of DNA oligomers has been observed with DNAs of high GC content (Anderson, 1986) and oligomers containing multiple potential B–Z junctions (Winkle & Sheardy, 1990).

Is the Observed Retardation a Result of Enhanced Gel Affinity? It has been suggested that the retardation observed for the multimers of (GA_4T_4C) might be due to enhanced affinity of the DNA oligomers for the gel matrix (Jernigan et al., 1987; Song & Schurr, 1990). The results presented in Figure 3 suggest that affinity is not a significant factor in the case under study. In the absence of $Co(NH_3)_6^{3+}$, the methylated oligomers I and III are only slightly more retarded in the gel relative to the unmethylated version. The presence of the methyl groups on the Z-forming segment certainly will increase the overall hydrophobicity of the DNA oligomer. Thus, the observed slight retardations of I and III may be due to slightly enhanced affinities for the gel matrix as a result of the increased hydrophobicities of those oligomers.

In the presence of $Co(NH_3)_6^{3+}$, I is highly retarded in the gel, II is slightly retarded in the gel, and III is split into a highly retarded band and an essentially nonretarded band. If the observed retardation is simply a function of enhanced affinity of the oligomer for the gel matrix in the presence of $Co(NH_3)_6^{3+}$, one would predict that I and III would have identical mobilities since they both have the same number of dG-5medC base pairs. Since this is not observed, the retardation of I and III must arise from some other source.

In determining the source(s) of retardation, one must consider several possibilities: (1) enhanced contour length of the oligomers in the presence of $Co(NH_3)_6^{3+}$; (2) changes in flexibilities of the oligomers; (3) curvature of the oligomers; (4) decreased charge densities of the oligomers; and (5) bending of the helix axis. As discussed above, retardation in the gel due to increased affinity does not explain our observations.

Are the Observed Retardations Due to Changes in Contour Lengths and/or Flexibilities of the Oligomers in the Presence of Cobalt Hexamine? Sedimentation studies of poly(dG-dC) in high salt indicate that Z-form DNA is both stiffer and longer than B-form DNA (Chaires, 1985). In the presence of $Co(NH_3)_6^{3+}$, oligomers a, b, and c are dramatically retarded with retardation ratios of 1.53, 1.41, and 1.34, respectively. Using the values of 3.52 Å for a G–C step and 4.13 Å for a C–G step in Z-DNA, one can calculate an approximate length for these oligomers in the presence of the Z-form inducing agent $Co(NH_3)_6^{3+}$. The lengths thus obtained are 73 Å, 150 Å, and 226 Å, respectively, for a, b, and c. The observed mobilities are consistent with B-form DNA oligomers of 103 Å, 198 Å, and 292 Å, respectively (these lengths are calculated from the apparent lengths of the oligomers in base pairs times 3.36 Å/step). It is apparent that these oligomers are moving through the gel as though they are longer than they actually are.

It has been suggested that when a B-DNA segment is flanked by any different structural motifs (e.g., A, Z, or B′), there is an increase in overall flexibility provided by bending and enhanced fluctuations at the conformational interfaces (Reich et al., 1991). It is therefore not unlikely that B–Z junctions have some degree of enhanced conformational flexibility which may influence overall flexibility. However the existence of two "isomers" in III at 4 °C places limits on this flexibility (see below). The observation that only a single band is observed for III at higher temperatures does however indicate some degree of flexibility at these temperatures.

Are These Oligomers Curved? The enhanced retardation

may also be due to curvature of the DNA oligomers. Porschke has suggested that $Co(NH_3)_6^{3+}$ induces strong bending in DNA oligomers (1986). Furthermore, for most curved DNA, the retardation increases with fragment length (McNamara et al., 1990; Rice & Crothers, 1989; Slater & Noolandi, 1986; Drak & Crothers, 1991). However, for oligomers a, b, and c, the extent of retardation decreases with oligomer length. Although we cannot rigorously rule out cobalt hexamine induced curvature of these oligomers as a source of retardation, this trend is opposite to that predicted above.

How Important Is the Decrease in Charge Density? It has been shown that $Co(NH_3)_6^{3+}$ binds specifically to G bases in Z-DNA through hydrogen bonding between three of the coordinated ammonia molecules and hydrogen bond acceptor sites on the guanine base and phosphate one residue away on the 5′ side (Gessner et al., 1985). Considerable neutralization of the phosphate backbone must arise due to the high charge of the cobalt ion. This is evidenced here by the decreased charge densities observed for all oligomers in the presence of $Co(NH_3)_6^{3+}$. However, if a decreased charge density was the sole source for the retardation observed, then one would predict the order for retardation ratios would parallel the number of G bases: c > b > a. The order is just the opposite; hence, decreases in charge densities must play only a minor role, and differences in the friction constants themselves are suggested.

It seems that a single factor is not the sole source of the retardation observed. Increased length, decreased charge density, and possibly curvature all contribute. The trend in retardation ratios, however, is opposite to that predicted if any one, or a combination of these factors, is operative. Furthermore, the reduced flexibility of the Z conformation may also play a role in the retarded mobilities displayed by these oligomers.

Are B–Z Junctions Bent? The initial goal of this project was to determine via gel electrophoretic studies of B–Z junctions cause bending of the helix axis. At first, the retardation of oligomers, I, IV, and V, seen in Figure 5B, might suggest bending. However, the relative order and observed mobilities for the pure Z-forming oligomers a, b, and c complicate the interpretation.

The original rationale for the design of I, IV, and V was based upon the observations of Drak and Crothers (1991). By shifting the position of the potential bend, one would also alter the observation retardation. A second factor must be considered: the relative length of Z-DNA vs that of B-DNA. Even though the B–Z junction is in the middle of I in terms of sequence, the resultant Z and B arms are not of equal lengths. Therefore, the potential bend is not in the middle of I, lengthwise. In fact, the end-to-end lengths of these oligomers, regardless of whether these oligomers are bent or not, increase in the following order: V > I > IV. This order corresponds to the order of their observed gel retardations. Thus, the retardation ratios obtained for these oligomers may only be due to length and, possibly, flexibility effects.

We believe that the answer to whether B–Z junction molecules are bent or not lies in the observation of two bands for oligomer III in the presence of $Co(NH_3)_6^{3+}$ at low temperatures. In the absence of $Co(NH_3)_6^{3+}$, this oligomer runs as a single species—a verification of product purity. In the presence of $Co(NH_3)_6^{3+}$, this oligomer undergoes a conformational transition consistent with the formation of a B–Z junction. However, this oligomer now migrates as two distinct species in the polyacrylamide gel. One of these species, designated as III in Tables I and II, is only slightly retarded in the gel relative to its mobility in the absence of cobalt hexamine. The other species (III′) is retarded to almost the same degree as I. Examination of the densities of the bands in Figure 3B indicates that III is the favored species (ca. 65%). The presence of two bands could also be interpreted in terms of an incomplete conformational transition. The observation that each of these bands is distinct, with no smearing between the bands, argues against this possibility. Therefore, the two bands must arise from two distinct structural conformations of the resultant high salt form in slow exchange on the time scale of the experiment. As noted above, only one band is observed for III at higher temperatures suggestive of a faster exchange between possible conformers.

Bending of the DNA oligomer must be invoked to account for the two distinct bands for III in the presence of $Co(NH_3)_6^{3+}$. Since I (with a single B–Z junction) and III (with two B–Z junctions) have the same number of consecutive dG-5medC base pairs (i.e., their Z-forming domains are the same lengths) and have the same CD spectrum in cobalt hexamine, their respective flexibilities, contour lengths, and charge densities should be very similar in the absence of bending. Thus, if B–Z junctions are not bent, I and III would have identical mobilities in the presence of $Co(NH_3)_6^{3+}$. This is not observed.

Why are two bands present at 4 °C? We suggest the presence of two dominant bent conformations, not a flexible ball joint. In one isomer of III, both bends are in phase resulting in a "cis" conformation of the oligomer. In the other, the bends are out of phase resulting in a "trans" conformation of the oligomer. Consistent with the observation of Drak and Crothers (1991), we assign the more retarded band to the cis conformation (III′) and the less retarded band to the trans conformation (III). Although we cannot predict the magnitude of the angle from these data, we can calculate the end-to-end distance for a variety of assumed angles. In all cases, the calculated end-to-end distance for the cis isomer is similar to that for the end-to-end distance of I at the corresponding angle. Furthermore, the end-to-end distance for the trans isomer is similar to that of an unbent oligomer. On the basis of these end-to-end distances, our assignments are consistent with the observed mobilities. If the end-to-end distance for the trans isomer is close to that of an unbent molecule, we can assume that a single B–Z junction represents a slight bend.

The above argument places limits on how flexible a B–Z junction might be. For example, a B–Z junction cannot act as a flexible ball joint, since otherwise III could not have two distinct bands. Winkle and Sheardy (1990) measured the mobility of polymers of B–Z and Z–B junctions, spaced 10 base pairs apart. Both in low salt and in the presence of cobalt hexamine, these multiple junction oligomers show *enhanced* electrophoretic mobilities relative to B-DNA standards. At the time, this behavior was interpreted as due to formation of an accordion-like structure, enhanced flexibility of the B–Z junction, or both. We can now suggest that the results of both experiments are consistent with the idea that a B–Z junction is slightly flexible and tends to form a limited set of two or more moderately bent conformers. About each "valence angle", we imagine a range of configurations might exist. This behavior is different from static bending, for example. The periodicity observed in the retardation ratios seen in polyjunction molecules might be consistent with this interpretation (Winkle & Sheardy, 1990).

A surprising aspect of these results is that single and polyjunction molecules display enhanced mobilities even under conditions of low salt. We have also observed distinctive drug binding properties in junction forming molecules in both low

and high salt (Suh et al., 1991; Lu et al., 1991). Furthermore, BZ-I is preferentially cleaved by DNase I, under low salt conditions, in the middle of the molecule at the site of the junction under high salt conditions (J. B. Chaires and R. D. Sheardy, unpublished results). It is therefore apparent that these B–Z junction forming molecules have unusual structure and properties under all conformational conditions. We suggest that the segment that ultimately forms the junction could be classified as a B–B′ conformational junction. The observation that cobalt hexamine further enhances the observed mobilities of the polyjunction molecules suggests that B–Z junctions have bending and flexibility properties different from those of B–B′ junctions.

Conclusions

The results reported here indicate that Z-DNA itself and polymorphic B–Z sequences display anomalous polyacrylamide gel mobilities. Several factors have been considered in the analysis of the gel migration data. The observed mobilities for these DNA chains are the result of both retardation and enhancement factors. We attribute the anomalous gel migration observed for Z-DNA and B–Z junction DNA in the presence of $Co(NH_3)_6^{3+}$ to the following: (1) increased hydrodynamic length of the oligomer; (2) decreased overall flexibility of the oligomer; (3) a decrease in overall charge density of the oligomer; and (4) bending to product two or more conformers in the specific case of the B–Z junctions.

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