# Cationic Metals Promote Sequence-Directed DNA Bending†

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ABSTRACT: A DNA segment of ~200 base pairs (bp) from *Crithidia fasciculata* kinetoplast minicircles was previously shown by electron microscopy (EM) to bend into a small circle due to its unique nucleotide sequence containing repeated blocks of 4–6 A's. When this segment was flanked by 207 bp of plasmid DNA on one side and 460 bp on the other, the resulting 890-bp DNA was found to appear either relatively straight or extremely bent as visualized by EM. The bend was located one-third the distance from one end. The fraction of molecules with the most extreme bend increased from ~2% to 50–60% following incubation of the DNA with increasing concentrations of $Zn^{2+}$, $Co^{2+}$, $Ba^{2+}$, and $Mn^{2+}$. These observations suggest that sequence-directed bending in DNA is an inducible and not a static phenomenon. Possible roles of transitions between the bent and straight conformations in the control of gene expression are discussed.

Sequence-directed DNA bending refers to the ability of certain nucleotide arrangements to confer a preferred three-dimensional trajectory on the DNA. The first evidence for these bends came from studies on junctions between A and B form DNAs (Selsing et al., 1979). The first naturally occurring bent DNA was found in the kinetoplast minicircles of trypanosomes and their relatives (Marini et al., 1982; Wu & Crothers, 1984). Sequence-directed bends have now been detected in the origins of replication of λ phage (Zahn & Blattner, 1985) and SV40 DNA (Ryder et al., 1986) and in yeast ARS segments (Snyder et al., 1986).

The bends in the kinetoplast minicircle DNA appear to arise from the presence of blocks of 4–6 A's along one strand of the DNA repeated in phase with the helix (Marini et al., 1982; Wu & Crothers, 1984). Several models have been proposed as to how these blocks of A's produce a bend in the DNA (Hagerman, 1984, 1985; Trifonov, 1985; Koo et al., 1986; Ulanovsky & Trifonov, 1987). The bent trypanosome DNAs have been studied by gel electrophoretic methods and in a recent study (Griffith et al., 1986a) by electron microscopy (EM).[1] A highly bent DNA of about 200 bp from the kinetoplast minicircles of *Crithidia fasciculata* had been identified, which contains 18 blocks of 4–6 A's, 16 of which are on one strand and in phase with the helix (Kitchin et al., 1986). Direct visualization of the isolated bent (223-bp) DNA by EM revealed a nearly equal mixture of relatively straight fragments and fragments that were bent into nearly perfect planar circles (Griffith et al., 1986a). Subsequent examination of this 223-bp DNA embedded in larger DNAs, however, suggested that the fraction of bent molecules seen by EM was highest when the DNA was prepared for EM in the presence of cationic metals. This was not surprising since metals such as $Mg^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ba^{2+}$, and $Mn^{2+}$ have been shown to potentiate the right- to left-handed transition in DNA (Zacharias et al., 1980; van de Sande et al., 1982).

To systematically probe the effects of cationic metals on the conformation of a bent helix segment, we have used an 890-bp DNA in which the 223-bp bent DNA described above is flanked by plasmid DNA, 207 bp on one side and 460 bp on the other. When the 223-bp segment is in a highly bent conformation, the two plasmid arms cross over themselves forming a looped molecule in which a ~200-bp circle is present one-third the distance from one end. Such structures can be counted in fields of molecules examined in the EM, providing a rapid means of quantitating the effects of different treatments of the DNA on the fraction of molecules in the most highly bent state.

## Materials and Methods

*DNA.* The 890-bp DNA was isolated from the plasmid pPK201/CAT (Kitchin et al., 1986) by cleavage with *Sph*I and *Pvu*II. The fragment was purified by electrophoresis on 5% acrylamide gels that were stained with 0.5% methylene blue. The 890-bp band was excised, and the DNA was eluted overnight from the acrylamide, ethanol precipitated, and dissolved in 10 mM Tris-HCl and 0.1 mM EDTA (pH 7.5). The 223-bp DNA was isolated from pPK201/CAT by *Bam*HI cleavage and purified in the same manner.

*Electron Microscopy.* The DNA at 1 μg/mL in a solution of 0.01 M Tris-HCl (pH 7.5) was incubated with various concentrations of metals at 55 °C for 5 min and 37 °C for 5 min and then cooled to room temperature. For visualization by direct-mounting EM, 50 μL of this solution was briefly mixed with a buffer containing 2 mM spermidine and 0.15 mM NaCl and immediately applied to a carbon-coated EM-supporting grid, which was then washed, dehydrated, air-dried, and rotary shadow-cast with tungsten as described (Griffith & Christiansen, 1978). For examination by drop-spreading EM (Delain & Brack, 1974), following incubation with metals as above, cytochrome *c* protein was added to 7.5 μg/mL, and a 50-μL drop of this mixture was placed on a sheet of parafilm for 30 s. A parlodion-covered copper grid was touched to the surface of the drop and then dehydrated in 70% ethanol for 30 s. The grids were rotary shadow-cast with platinum. The samples were examined in a Philips EM400 instrument operated at 20 kV.

## Results

When the 890-bp DNA was examined directly by EM, molecules could be found that appeared relatively straight or that contained a steep bend or loop within the molecule, and many more appeared looped following treatment with metal

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[1] Abbreviations: bp, base pair(s); EM, electron microscopy; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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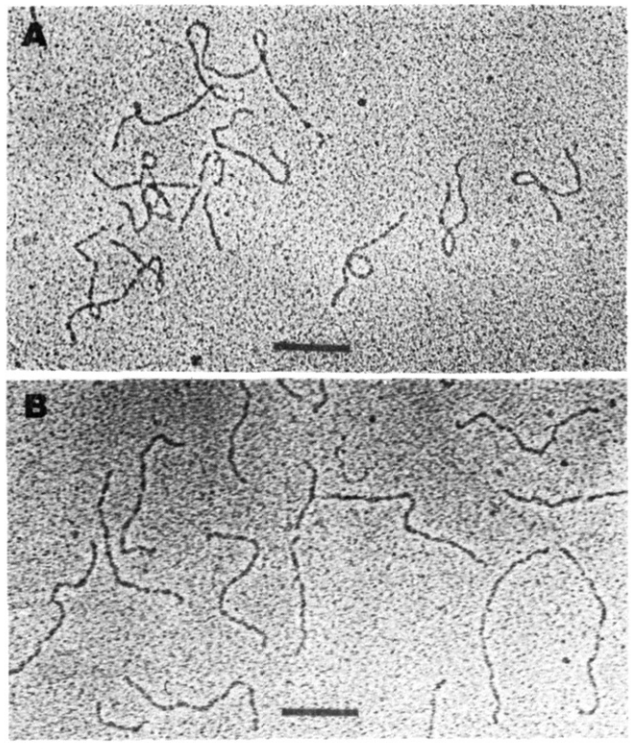


FIGURE 1: Visualization of a DNA fragment containing a bent helix segment in straight and metal-induced bent conformations. The 890-bp fragment was incubated in 10 mM Tris-HCl, pH 7.5, with 5 mM $ZnCl_2$ (A) or with 5 $\mu$M $ZnCl_2$ (B) and mounted directly onto thin carbon films, washed, dehydrated, and rotary shadow-cast with tungsten (Griffith & Christiansen, 1978). Bar equals 0.1 $\mu$m.

ions (Figure 1). From DNA sequence analysis, the center of the bent helix segment is located 36% from the nearest end of the fragment. In the micrographs (Figure 1) the center of the loop or bend measured 36 ± 4% ($n$ = 30) from the nearest end.

When the 890-bp DNA was incubated in 10 mM Tris, pH 7.5, and 0.1 mM EDTA, the percentage of looped molecules numbered 2–3% of the total molecules present. However, when the 890-bp DNA was incubated with various cationic metals, the percentage of looped molecules increased to as much as 60% of the total (Figures 1 and 2). The fraction of looped molecules was dependent on both the type and concentration of cationic metal: $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, and $Zn^{2+}$ ions were maximally effective in inducing loops at concentrations of 5–10 mM; $Mn^{2+}$, hexaamminecobalt, and $Ba^{2+}$ at 50–100 mM; and $Mg^{2+}$ and $Ca^{2+}$ at 0.5–1.0 M. For $Mg^{2+}$ and $Ca^{2+}$, the maximum fraction of looped molecules was less than that observed with the other cations tested. In these experiments (Figure 2) the DNA was incubated with the metal for 5 min at 55 °C, followed by 5 min at 37 °C, and cooled to 25 °C before mounting onto the carbon supports. When the samples were incubated with optimal concentrations of metals for 5 min at only 37, 25, or 4 °C before mounting, no significant difference in the number of looped molecules as compared to the data in Figure 2 was observed (data not shown). When the 223-bp DNA was incubated with concentrations of $Co^{2+}$, $Mn^{2+}$, and $Ba^{2+}$ found to be optimal for looping of the 890-bp DNA, the fraction of circles was found to increase by 30% as compared to controls (data not shown).

The curves in Figure 2 describe the state of the most highly bent fraction of molecules. To further examine the form of each molecule, fields of 890-bp fragments were photographed and all of the molecules placed into one of three classes: (1) looped molecules, (2) molecules that were not looped but

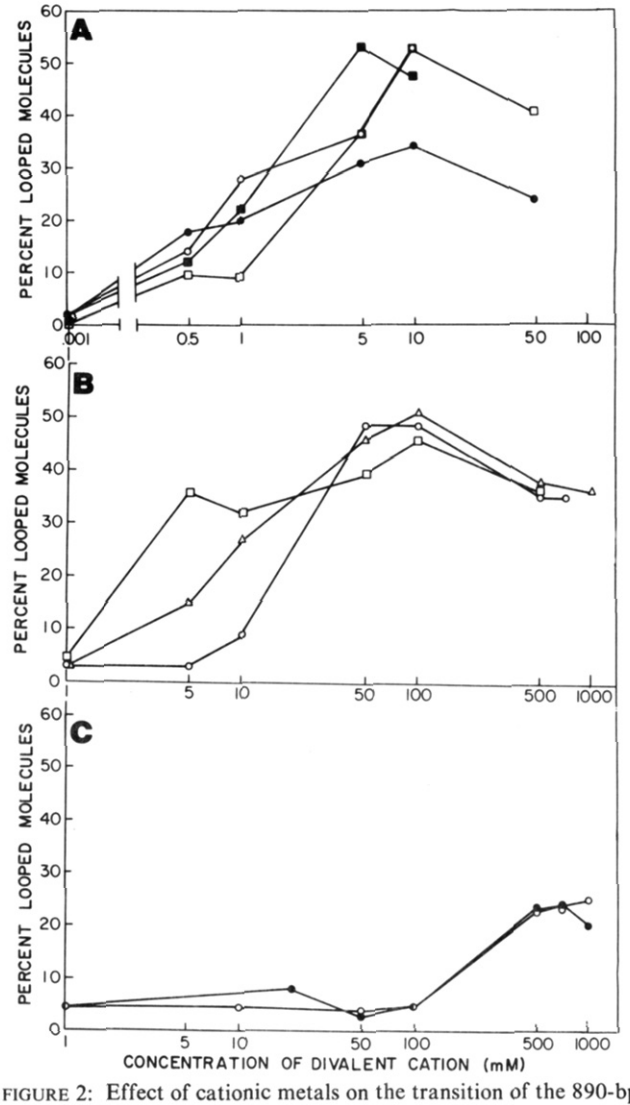


FIGURE 2: Effect of cationic metals on the transition of the 890-bp DNA from a straight to a bent form. The samples were prepared as in Figure 1 after incubation with different metal ions. At low magnification in the electron microscope, the fraction of the total molecules observed, in which the two plasmid arms crossed over themselves forming a loop about one-third the distance from one end of the molecule, was determined by scoring at least 200 molecules for each condition. (A) $CuSO_4$ (○), $CoCl_2$ (□), $NiCl_2$ (●), and $ZnCl_2$ (■); (B) $MnCl_2$ (△), hexaamminecobalt (□), and $BaCl_2$ (○); (C) $MgCl_2$ (●) and $CaCl_2$ (○).

contained a bend of 120° or more one-third the distance from one end, and (3) all remaining molecules. Following treatment of the 890-bp DNA with 5 $\mu$M $ZnCl_2$, 2% of the molecules were in the first class, 46% in the second, and 52% in the third ($n$ = 187). Parallel incubation with 5 mM $ZnCl_2$ yielded values of 43%, 37%, and 20%, respectively ($n$ = 482).

It was important to determine whether the EM-mounting steps, particularly the spermidine moiety of the mounting buffer, might cause a skewed fraction of molecules that appeared highly bent since spermidine has been shown to promote a change from right- to left-handed DNA conformations (Behe & Felsenfeld, 1981). In these experiments the DNA was mixed with the spermidine buffer immediately before mounting onto the carbon films, and the buffer was therefore in contact with the DNA for no more than 5 s before mounting. When the 890-bp DNA was incubated in the mounting buffer for extended times, a decrease was observed. The fraction of looped molecules was reduced from 50% at 5 s to 25% after 30 s of incubation in the buffer and fell to background (2%) after 5 min.

An alternate method of preparing DNA for EM is to complex it with a 100–200 Å thick coating of denatured cytochrome *c* protein. The protein coating might stiffen the DNA eliminating bending, or possibly the basic residues in the bound protein might induce DNA bending. Furthermore, the strong surface tension forces are known to minimize the overlaying of two DNA strands, and this effect should reduce the fraction of 890-bp molecules scored as being looped. In numerous experiments, when the 890-bp DNA was surface spread in the absence of metal ions, from 20% to 30% of the molecules were scored as being looped. Following incubation and spreading in the presence of optimal metal concentrations (as defined by the curves in Figure 2), the fraction of looped molecules increased to 40–80% of the total. Thus the essential features of the observations described above were confirmed by this alternate preparative method.

## Discussion

From these EM observations we conclude that the 223-bp bent helix segment alone or in a larger nonbent DNA exists in a continuum of conformations from straight to highly bent probably in rapid equilibrium in solution. Several cationic metals were found to strongly favor this DNA being in the most highly bent state.

Arguments derived largely from our previous study (Griffith et al., 1986a) support the conclusion that the EM procedure used here accurately sampled the state of the DNA in solution and, furthermore, that in solution the 223-bp DNA is in rapid equilibrium between relatively straight and highly bent conformations. The observations are as follows: When the isolated 223-bp fragment with the bent helix was examined by EM with no cationic metals, an equal mixture of relatively straight molecules and molecules that were bent into nearly perfect planar circles was found. This DNA migrated on 6% polyacrylamide gels with an apparent size of 850 bp. Treatment of the 223-bp DNA with distamycin eliminated the circular species as seen by EM and caused it to migrate on gels with an apparent size of ~220 bp (Griffith et al., 1986a; Griffith et al., unpublished observations). The 223-bp DNA was rapidly circularized by DNA ligase into a species appearing to be the same, as seen by EM, as the unligated circles. These ligated circles migrated on the 6% acrylamide gels approximately opposite a 1350-bp marker. We propose that the DNA can assume either a relatively straight form (migrating on these gels with an apparent size of 223 bp) or a circular form (1350 bp apparent size) and that the 850-bp position on these gels of the unligated DNA reflects a rapid equilibrium between the two (equally populated) states, as suggested by EM.

The anomalous migration of bent DNA segments on highly cross-linked acrylamide gels has served as a powerful tool for characterization of these molecules. Diekmann and Wang (1985) found that bent DNAs were further retarded when they were electrophoresed in the presence of 10 mM $Mg^{2+}$. By EM, this amount of $Mg^{2+}$ did not increase the fraction of most highly bent molecules, but may nonetheless have shifted the overall degree of bending in a significant way, which was not measured by our criteria.

We have repeatedly tried to examine the electrophoretic behavior of the 890-bp DNA in the presence of metal concentrations shown by EM to sharply increase the fraction of the most highly bent molecules (e.g., 50 mM $Ba^{2+}$ or 5 mM $Zn^{2+}$). However, to date, aggregation of the DNA and precipitation of the metal on the electrodes have precluded our obtaining useful results. Evidence supporting the conclusions cited here has been recently obtained in our studies of histone binding to bent DNA (to be described elsewhere). In these studies we find that the affinity of the 223-bp segment for nucleosome formation is 2–3-fold higher than that of the surrounding nonbent DNA and that this factor is increased to nearly 10-fold when the histone reconstitution is carried out in the presence of 50 mM $Ba^{2+}$.

The sequence nature of the plasmid DNA on either side of the 223-bp bent helix segment did not seem to be important in causing bending, as we have observed similar effects of the metal cations with the 223-bp bent segment embedded in other plasmid DNAs (unpublished observations) as well as with the 223-bp fragment alone. Direct measurement of the bent molecules determined that the middle of the loop corresponded to the center of the inserted 223-bp sequence. The presence of flanking DNA, however, appeared to reduce the fraction of highly bent molecules observed in the absence of cationic metals (as contrasted to the 223-bp DNA alone). Whereas this fraction was as low as 2% for the 890-bp DNA, it had been found to be as high as 50% for the isolated 223-bp DNA fragment (Griffith et al., 1986a). This is not surprising as sufficiently greater energy may be required to hold a large DNA into a tightly looped form as contrasted to a small fragment that would not be constrained by the inertia of large DNA arms.

The rapid movement of these DNA fragments between bent and straight conformations is in contrast to the right- to left-handed transition where the high activation energy tends to maintain the DNA in one conformation or the other. However, the curves obtained here showing the influence of metal ions on the conformation of this highly bent DNA are similar in shape and magnitude to curves obtained by other investigators for the induction of the right- to left-handed transition in DNA by these same metals (Zacharias et al., 1980; van de Sande et al., 1982).

Those authors have discussed the possible roles of these metals in the induction of the right- to left-handed transition, including the observation that the order of effectiveness of the metals was similar to the Irving–Williams order for metal complex stability (Irving & Williams, 1948) and that $Mn^{2+}$ and $Co^{2+}$ react with N7 of guanine as well as with the phosphate groups of DNA while $Mg^{2+}$ has a much lower affinity in this regard, perhaps reflecting the requirement for increased concentrations of $Mg^{2+}$ in inducing bending. This bent helix is not assuming a left-handed conformation, as experiments will be presented elsewhere showing that the straight to bent transition is accompanied by only a small angular rotation of the DNA helix. The reader is also referred to a recent study in which the same order of effectiveness for cationic metals was observed in the promotion of salt-dependent cruciform extrusion. In that study (Sullivan & Lilley, 1987) they suggest that this follows the affinity of these metals for phosphate binding rather than base binding. The strong effect of $Zn^{2+}$ in inducing bending may be of particular interest as several DNA binding proteins have been shown to contain $Zn^{2+}$ bound in regions that interact closely with the DNA helix (Miller et al., 1985).

Whereas the *C. fasciculata* bent DNA helix segment may serve in organizing the interlocked minicircle DNAs in the kinetoplast (Ray et al., 1986; Silver et al., 1986), other bent helix segments are being found in regions controlling DNA replication and transcription (Zahn & Blattner, 1985; Ryder et al., 1986; Snyder et al., 1986). Clues as to how an inducible switch in DNA conformation might function can be found in recent studies that demonstrate the importance of facilitating protein–protein interactions along DNA control elements.

Functional interactions between proteins were shown to be maintained even when the protein binding sites were separated as long as the sites remained on the same face of the helix (Hochschild & Ptashne, 1986; Takahashi et al., 1986; Griffith et al., 1986b). Rotation of the two sites away from each other inhibited these interactions. When the separations were great (50 bp), the DNA between the two sites was held into a loop by the binding of the proteins to the DNA and to each other (Ptashne, 1986; Griffith et al., 1986b). Models of DNA looping have assumed that, beyond providing the protein binding sites, the DNA plays a passive role. However, an inducible DNA bend situated between two protein binding sites could either facilitate or inhibit interactions between proteins. The net effect would depend on the starting position of the two sites, the angle of the induced bend, and the angular rotation of the two sites relative to each other following induction (or loss) of the bend. Angular rotation of the two sites may be a particularly important feature as it would be greatly affected by right- to left-handed transitions and the placement of blocks of A's at nonintegral turns of the helix. The result could be a three-dimensional bending and rotation of the DNA when the bends are lost or gained, thus creating the backbone of a complex molecular switch.

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