Cationic metal-specific structures adopted by the poly(dG) region and the direct repeats in the chicken adult β^A globin gene promoter

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ABSTRACT

Naturally occurring contiguous deoxyguanine residues and their surrounding sequences in the chicken adult β^A globin gene promoter were analyzed for their inherent potential to adopt non-B DNA structures in supercoiled plasmid DNA. In particular, cationic effects on structure were studied by treating the supercoiled plasmid DNA harboring the chicken adult β^A globin 5' flanking sequence with an unpaired DNA base-specific probe, chloroacetaldehyde in the presence of either Mg^{++} , Cu^{++} , Zn^{++} , Ca^{++} or Co^{++} ions. The chloroacetaldehyde-reactive bases were mapped at a single base resolution by a chemical cleavage method that specifically cleaves DNA at the chloroacetaldehyde modified sites. These experiments revealed that while Mg^{++} and Ca^{++} ions induce a dG·dG·dC triple helix structure at the contiguous dG residues, Zn^{++} , Cu^{++} and Co^{++} ions induce yet another structure at the direct repeats immediately 5' of the dG residues. When Mg^{++} and Zn^{++} ions are both present, Zn^{++} inhibits the dG·dG·dC triplex at the contiguous dG residues and induces a particular non-B DNA structure at the adjacent direct repeats. The specific induction of non-B DNA structures by metal ions at the two adjacent sequences within the promoter region may be of biological significance.

INTRODUCTION

Poly(dG)-poly(dC) sequences have unusual structural properties dependent on the level of supercoiling, pH, and the presence or absence of Mg^{++} ions. We recently showed that in supercoiled plasmid DNA, the poly(dG)-poly(dC) sequence folds sharply into halves from the center of the tract forming a triple helix structure, either dG·dG·dC in the presence of Mg^{++} ions or dC⁺·dG·dC in the absence of the Mg^{++} ions (1). The latter protonated triplex was first reported as H-form DNA (2) and studied by a number of groups (1,3-8). Upon forming either of these triplex structures, a poly(dG)-poly(dC) sequence induces its neighboring DNA to be unpaired in a unidirectional and sequence independent manner (9).

A contiguous dG sequence of 16 base pairs (bp) exists in the promoter region of the chicken adult β^A globin gene. This stretch of dG residues exhibits hypersensitivity to S1 nuclease (10,11) and is reactive with the chemical probe, bromoacetaldehyde, which is specific for unpaired DNA bases (12) in supercoiled plasmid DNA. This sequence adopts two different chromatin structures in vivo: in cells that do not express the β -globin gene, this dG stretch is not associated with nucleosomes and is accessible to nuclease (13) whereas in mature erythrocytes which express the β -globin gene, the dG stretch is protected from nuclease attack (14). Lewis *et al.* (15) reported that a DNA binding protein (BGPI) exists that specifically binds to the dG stretch. BGPI requires Zn^{++} for DNA-binding activity. This protein is restricted to erythrocyte lineage and is most abundant in mature erythrocytes.

In this paper the inherent potential for adopting specific non-B DNA conformations was examined for a naturally occurring poly(dG)-poly(dC) sequence and its neighboring sequences with particular emphasis on the effect of cationic metals. It was of interest to find that Zn^{++} ions play a dominant role in determining the structure adopted by the poly(dG)-poly(dC) and its adjacent direct repeat sequences. These results raise the possibility for interplay between the inherent property of the DNA to assume a metal-specific conformation and the sequence-specific binding protein upon a protein-DNA complex formation.

MATERIALS AND METHODS

Chemicals

Chloroacetaldehyde (CAA) was purchased from Fluka and double-distilled (boiling point, 78–80°C) before use. Hydrazine, formic acid and piperidine were purchased from Sigma.

Plasmid DNA.

Supercoiled plasmid, pBSTN1, which contains the region from –303 to +167 of the chicken adult β^A globin gene in pUC8 was kindly provided by Dr. B. Emerson. Supercoiled plasmid DNAs, pCATCG14 and pCATCG18, respectively, contain 14 or 18 base pairs of poly(dG)-poly(dC) sequence inserted into the Sma I/Sac I site of the pUC13 plasmid, which also contains the bacterial chloroamphenicol acetyltransferase gene inserted into the Hind III site. All supercoiled plasmid DNAs used in this study have bacterial superhelical density.

Chemical modification of DNA and cleavage reactions.

The method is described in detail in Kohwi and Kohwi-Shigematsu, (1): briefly, thirty micrograms of supercoiled plasmid DNA were incubated with 4 μl of CAA in the 100 μl reaction buffer containing 25 mM Tris-Maleic acid (pH7) and in the absence or presence of various cationic metals as described in the Figure legends. The control DNA was treated similarly, except that no CAA was added. After the DNA was purified, 2 μg of DNA was cleaved with a restriction enzyme and then radiolabeled either at the 5' end with T4 kinase or at the 3' end with the Klenow fragment of DNA polymerase I. The labeled DNA was then digested with the second restriction enzyme so that the resulting end-labeled fragment contains the 5' flanking sequence of the chicken adult β^A globin gene. The labeled fragment was isolated from a 6% native polyacrylamide gel. The DNA was then treated with either hydrazine or formic acid and subsequently cleaved with piperidine as described by Maxam and Gilbert (16).

Figure 1. Effects of various metal ions on the non-B DNA structures adopted by the 5' flanking sequence of the chicken adult β^A globin gene.

(A) Supercoiled plasmid, pBSTN1, was treated with 0 μl (control, lane b, i, p and v) or with 4 μl of chloroacetaldehyde either in the absence of (lanes a, h, o and u) or in the presence of 2mM of metal ions (Mg^{++} , lanes c, j, q and w; Ca^{++} , lanes d and k; Zn^{++} , lanes e, l, r and x; Co^{++} , lanes f, m, s and y; Cu^{++} , lanes g, n, t and z) in 25 mM Tris-Maleic acid (pH 7.0). The CAA modified DNA was digested by Hind III, labeled with $\alpha^{32}\text{P}$ -ATP and Kinase at the 5' end (top strand) or with $\alpha^{32}\text{P}$ -dCTP and klenow at the 3' end (bottom strand). The labeled DNA was then digested with EcoRI. The Hind III-EcoRI fragments were isolated from a native polyacrylamide gel and were subjected to reaction with either hydrazine (HZ) or formic acid (FA). The DNAs were then cleaved by piperidine reaction.

(B) Summary to the chemical cleavage patterns for CAA modified DNA.

($\uparrow$) indicates the CAA modified sites in the presence of Mg^{++} and Ca^{++} ions.

($\downarrow$) indicates the CAA modified sites in the presence of Zn^{++} , Co^{++} and Cu^{++} ions.

RESULTS

An unusual structural property of a 16 bp tract of poly(dG)-poly(dC) sequence in the 5' flanking region of the chicken adult β^A globin gene was studied. This was analyzed by treating the supercoiled plasmid DNA pBSTN1 (17) containing the -303 to +167 fragment with chloroacetaldehyde (CAA) which reacts specifically with N1 and N⁶ positions of adenine, and N3 and N⁴ positions of cytosine (18, 19) when these sites are not involved with hydrogen bonding with their complementary bases (20-22). CAA also reacts with guanine residues to a limited extent (23). The sites of modification with CAA, representing unpaired bases, were determined by a combination of reactions with either hydrazine or formic acid, followed by cleavage with piperidine as previously published (1).

This method is very sensitive and allows precise mapping of the CAA modified sites on a sequencing gel.

The Chemical Cleavage Method to Map the CAA Modified Sites.

Briefly, the principle of this method is that the bases that are modified with CAA have an altered electronic structure and thus react differently with hydrazine, dimethylsulfate, or formic acid from the corresponding unmodified bases. Kohwi and Kohwi-Shigematsu (1) reported that hydrazine which normally reacts only with cytosine residues also reacts with CAA modified adenine and guanine residues. Dimethylsulfate which is specific for guanine residues also reacts with CAA modified adenine and cytosine residues. Formic acid which reacts specifically with adenine and guanine residues also reacts with CAA modified cytosine residues. These differences in reactivity between CAA modified and unmodified DNA bases with one of these chemicals then leads to differential susceptibility to piperidine which is subsequently used to cleave the DNA. The CAA modified bases can then be identified by the appearance of unexpected or new bands within the chemical sequence ladder.

Effects of various metal ions on the DNA structure adopted by the chicken adult β^A globin gene promoter region.

We previously showed that Mg^{++} ions had a dramatic effect on the structure adopted by a synthetic poly(dG)-poly(dC) sequence in supercoiled plasmid DNA. The present study examined the dependency of the structure adopted by the naturally occurring poly(dG)-poly(dC) sequence of the chicken adult β^A globin gene on various metal ions in addition to Mg^{++} ions. The CAA treatment with the supercoiled plasmid was performed at pH7 in the presence of one of several cationic metals, Mg^{++} , Ca^{++} , Zn^{++} , Co^{++} , and Cu^{++} , at a concentration of 2mM. The CAA modified sites on both strands were then identified using the chemical cleavage method described above with isolated DNA fragments (Hind III-EcoRI) labeled at either the 3' or 5' end of the Hind III site. When these chemically cleaved DNA fragments were separated on a denaturing polyacrylamide gel, it was revealed that even the 16 bp poly(dG)-poly(dC) sequence within the chicken adult β^A globin gene promoter adopted the dG·dG·dC triplex structure in the presence of 2mM Mg^{++} ions (Fig. 1). For the bottom strand, cytosine residues of the 5' half of the contiguous cytosine stretch were reactive with CAA, while the 3' half of the stretch was virtually unreactive with CAA (Fig. 1, lanes c and j). In contrast, the top strand containing contiguous guanine residues revealed CAA reactivity limited to specific guanine residues located at the center of the stretch (Fig. 1, lanes q and w). In addition to the DNA bases within the poly(dG)-poly(dC) tract being CAA reactive, the neighboring sequence 3' to the contiguous dG sequence was also reactive with CAA. This pattern of CAA reactivity, in the presence of Mg^{++} , is consistent with the dG·dG·dC triplex structure as previously determined

Figure 2. Effects of various metal ions on the non-B DNA structure adopted by the 14 and 18 contiguous dG residues.

Supercoiled plasmid DNA, pCATCG14 and pCATCG18, harboring the poly(dG)-poly(dC) tracts of 14 bp (lanes a to j) and 18 bp (lanes k to t) were treated with 0 μ l (control; lanes a, f, k and p) or 2 μ l of CAA either in the absence of (lanes b, g, l and q) or in the presence of 2 mM metal ions (Mg^{++} , lanes c, h, m and r; Zn^{++} , lanes d, i, n and s; Cu^{++} , lanes e, j, o and t) in 25 mM Tris-Maleic acid (pH 7.0). The CAA modified DNA was digested by Hind III and labeled with α - ^{32}P -dCTP and klenow at the 3' end. The labeled DNA was then digested with BglII. The Hind III-BglII fragments were isolated from a native acrylamide gel and were subjected to reaction with either hydrazine (HZ) or formic acid (FA). The DNAs were then cleaved by the piperidine reaction.

with a synthetic 30 base pair poly(dG)-poly(dC) sequence (1). The same CAA reaction pattern was also observed when Ca^{++} ions were present in the reaction buffer (lane d and k, Fig. 1., the CAA reactivity at the center of the dG tract is not shown). In addition to Mg^{++} and Ca^{++} ions, exactly the same CAA reaction pattern was found with Mn^{++} ions as well (data not shown).

This CAA reaction pattern, on the other hand, was not observed in the presence of Zn^{++} , Co^{++} , or Cu^{++} ions. No CAA reactivity was detected within the poly(dG)-poly(dC) tract. Instead, CAA reactivity was detected in the neighboring region 5' to the guanine tract as judged by the appearance of the new bands at two cytosine residues (lanes l, m and n, Fig.1) on the bottom strand and two cytosine (lanes x, y and z, Fig.1) and two guanine residues (lanes r, s and t, Fig.1) on the top strand. The CAA reactive sites are summarized in Fig. 1B.

The 5' region of the dG tract contains two direct repeats of the sequence AGAGCTG. The CAA reactive sites occur at the same bases for each repeat. It is likely, therefore, that the non-B DNA structure adopted exclusively in the presence of Zn^{++} , Co^{++} , and Cu^{++} results from these direct repeat sequences. Alternatively, the possibility exists that in the presence of Zn^{++} , Co^{++} and Cu^{++} a short tract of poly(dG)-poly(dC) sequence is capable of inducing an altered structure upon the 5' neighboring sequences.

Effects of various metal ions on the non-B DNA structure adopted by the synthetic short stretches of poly(dG)-poly(dC) sequences.

To distinguish between these two alternatives, a poly(dG)-poly(dC) tract of either 14 or 18 base pairs was inserted in pUC 13 vector sequences (pCATCG14 and pCATCG18 respectively) and the CAA reaction patterns for the bottom strand were compared. In the presence of Zn^{++} or Cu^{++} , neither the 14 nor the 18 base pair poly(dG)-poly(dC) tract exhibited CAA reactivity in the neighboring region 5' of the dG stretch as judged by the lack of new bands in this region (Fig. 2, lanes d, e, i, j, n, o, s and t). Thus, the CAA-reactive structure adopted by DNA sequence 5' to the dG stretch of the chicken adult β^A globin gene promoter is a consequence of the specific sequence and is not driven by the neighboring dG stretch. These data indicate that specific types of metal ions induce a particular type of non-B DNA structure exclusively formed by the poly(dG)poly(dC) tract or by the direct repeat sequences immediately preceding this tract.

When supercoiled plasmids DNA harboring short tracts of poly(dG)-poly(dC) were also examined, an 18 base pair tract in the presence of Mg^{++} ions at pH7 exhibits the typical CAA reaction pattern consistent with the dG·dG·dC triplex structure as observed for the 16 base pair dG stretch of the chicken β^A globin promoter (Fig. 2, lanes m and r). Furthermore, this structure was not induced by the presence of Zn^{++} or Cu^{++} ions (Fig. 2, lanes m and r). In contrast, a 14 base pair dG tract was not capable of forming the triplex under these same conditions (Fig. 2, lanes c and h). For both 14 and 18 base pair dG tracts, in the presence of Zn^{++} or Cu^{++} ions, one additional CAA reactive base was detected at the immediately 3' of the dG tract (Fig. 2, lanes i, j, s and t) which was not seen for the plasmid in the presence of Mg^{++} ions. Such reactivity was not observed for the corresponding site for the dG stretch in the chicken adult β^A globin gene promoter. *Non-B DNA structures adopted by the chicken adult β^A globin promoter sequence when both Mg^{++} and Zn^{++} ions are present.*

Since certain metal ions (Mg^{++} , Ca^{++} and Mn^{++}) induce a dG·dG·dC triplex structure and other metal ions (Zn^{++} , Cu^{++} , Co^{++}) induce a non-B DNA structure at the direct repeat sequence, it was of interest to determine which effect is dominant when

Figure 3. The non-B DNA structures adopted by the chicken adult β^A globin gene 5' flanking sequence when both Mg^{++} and Zn^{++} ions are present.

A) Supercoiled plasmid, pBSTN1, was treated with 0 μ l (control, lane a) or 4 μ l of CAA in the presence of various relative concentrations of metal ions (b: Mg^{++} , 2mM and Zn^{++} , 0.5mM; c: Mg^{++} , 2mM and Zn^{++} , 0.2mM; d: Mg^{++} , 2mM and Zn^{++} , 0.1mM; e: Mg^{++} , 2mM and Zn^{++} , 0.05mM; f: Mg^{++} , 2mM and Zn^{++} , 0.02mM; g: Zn^{++} , 0.5mM; h: Zn^{++} , 0.2mM; i: Zn^{++} , 0.1mM; j: Zn^{++} , 0.05mM; k: Zn^{++} , 0.02mM) in 25mM Tris-Maleic acid (pH 7.0). The DNAs were then treated as described in the legend of Figure 1.

B) Supercoiled plasmid, pCATCG18, was treated with 0 μ l (control, lane a) or 2 μ l of CAA in the presence of various relative concentrations of metal ions (b: Mg^{++} , 2mM and Zn^{++} , 2mM; c: Mg^{++} , 2mM and Zn^{++} , 0.5mM; d: Mg^{++} , 2mM and Zn^{++} , 0.2mM) in 25mM Tris-Maleic acid (pH 7.0). The DNAs were then treated as described in the legend of Figure 1.

both types of metal ions were present simultaneously. To address this question, DNA was modified with CAA in the presence of both Mg^{++} and Zn^{++} ions at various relative concentrations. First, DNA modification with CAA was performed in the presence of 2mM Mg^{++} and either 0.5, 0.2, 0.1, 0.05, or 0.02mM Zn^{++} . In the presence of 0.5mM Zn^{++} ions, the effect of Mg^{++} ions were totally abolished such that only the non-B DNA structure at the direct repeat was formed (Fig. 3, lane b). When the Zn^{++} concentration was reduced to 0.2mM, both the dG·dG·dC triplex and the non-B DNA structure formed

by the direct repeats were detected as judged by the CAA reaction pattern (Fig. lane c). As the concentration of Zn^{++} ions was further reduced to 0.1mM or less, only the typical CAA reaction pattern for the dG·dG·dC triplex structure in response to the Mg^{++} was detected (Fig. 3, lane d, e and f). When the CAA reaction was performed solely in the presence of Zn^{++} ions at concentrations of 0.5, 0.2, 0.1, 0.05 or 0.02mM, the corresponding CAA reaction pattern as well as the intensities of the new bands were more or less the same as when 2mM Mg^{++} ions were also present (Fig. 3, lanes g–k). This result indicates that Zn^{++} ions influence the structure of the direct repeat sequence regardless of the presence of 2mM Mg^{++} ions. Furthermore, 0.5mM Zn^{++} causes a switch from the dG·dG·dC triplex to the non-B DNA structure at the direct repeat even in the presence of 2mM Mg^{++} ions. There are at least two possible explanations for this phenomenon. One is that Zn^{++} inhibits Mg^{++} interaction with the dG stretch so that the sequence can no longer assume a triplex structure. The other explanation is that Zn^{++} induces the non-B DNA structure at the direct repeats of any given plasmid DNA molecule which would result in reduction of torsional stress of the plasmid DNA. This would subsequently inhibit the Mg^{++} dependent triplex formation at the dG stretch.

To distinguish between these two alternatives, CAA reaction was performed with the pCATCG18 plasmid in the presence of both Mg^{++} and Zn^{++} at various relative concentrations. The result showed (shown in Fig. 3B) that the intensity of new bands resulting from CAA reactivity of cytosine residues over the 5' half of contiguous dC stretch is reduced as the concentration of Zn^{++} is increased relative to that of Mg^{++} . This strongly suggests that Zn^{++} does interfere with the interaction between the poly(dG)-poly(dC) tract and Mg^{++} and thus inhibits the sequence from forming the Mg^{++} dependent dG·dG·dC triplex structure.

It is also noteworthy that at physiological pH, 0.2mM Zn^{++} is sufficient to induce the CAA reactive structure at the direct repeat while 0.5mM Mg^{++} is still insufficient for inducing the dG·dG·dC triplex structure at the dG stretch(data not shown).

DISCUSSION

This paper describes the effects of cationic metals on the unusual structure adopted by the dG stretch and neighboring DNA of the chicken adult β^A globin gene promoter in supercoiled DNA. The results show that Mg^{++} , Ca^{++} and Mn^{++} ions have similar effects in inducing the 16 contiguous dG residues in the promoter sequence to adopt a dG·dG·dC triplex at pH7. This structure accompanies the base unpairing of the neighboring region 3' of the dG tract. In contrast, Zn^{++} , Co^{++} or Cu^{++} ions have an entirely different effect: these ions do not induce a dG·dG·dC triplex but instead induce the direct repeat sequence immediately 5' to the dG tract to adopt yet another type of non-B DNA structure.

The CAA reaction pattern obtained for the direct repeat sequence is inconsistent with the DNA sliding model or a slipped structure which was previously suggested by others (24,25). Simple melting out of the sequence does not account for the structure either. No CAA reaction was observed with any of the adenine or some of the cytosine residues within the direct repeat sequence. If these residues were unpaired, they should have reacted with CAA. This result indicates that these residues are hydrogen bonded with their complimentary bases. The CAA reactivity with only certain bases in each repeat indicates a specific non-B DNA structure. In order to propose the exact structure adopted by the direct repeat sequence, independent study on other direct repeat sequences should be performed

employing CAA as well as other chemical and enzymatic methods.

It is known that protonation stabilizes unusual DNA structures (1–12). Metal ions also play an important role in inducing particular DNA conformations. Certain metal ions promote transition of right (B) to left-handed (Z) DNA (26, 27), and a straight to a bent DNA form (28). In both cases, the efficacy in the order of $\text{Ni}^{++} > \text{Co}^{++} > \text{Mn}^{++} > \text{Mg}^{++}$ in promoting the structural transition was observed which was similar to the order for metal complex stability (29–31). For the case of the salt dependent cruciform extrusion, on the other hand, the order $\text{Mg}^{++} > \text{Mn}^{++} > \text{Co}^{++} > \text{Ni}^{++}$, Cu^{++} was reported for optimal rates of extrusion, suggesting that this follows the affinity of these metals for phosphate binding rather than base binding (32).

For the promoter sequence of the chicken adult β^A globin gene, the structural transition to the non-B DNA structure adopted by the direct repeat sequence was promoted in the presence of Co^{++} , Zn^{++} and Cu^{++} and not with Mn^{++} , Mg^{++} or Ca^{++} . This is consistent with the observations for the B to Z and straight to bent DNA transitions. The opposite order of effectiveness of metal ions in promoting the $\text{dG} \cdot \text{dG} \cdot \text{dC}$ triplex structure formation was in agreement with the case for the salt dependent cruciform extrusion (32). Within the chicken adult β^A globin gene promoter, a given type of metal ions specifically induce either the $\text{dG} \cdot \text{dG} \cdot \text{dC}$ triplex structure at the dG stretch or the non-B DNA structure at the direct repeats at a physiological pH. When both Mg^{++} and Zn^{++} are present, the $\text{dG} \cdot \text{dG} \cdot \text{dC}$ triplex formation is inhibited provided that the Zn^{++} concentration is sufficient.

Much information needs to be accumulated for the better understanding of the biological significance of these metal specific structures adopted by the DNA sequences. Since BGP1, which specifically binds to the dG stretch requires Zn^{++} ions for binding (15), it is possible that this behavior of DNA is important for recognition by sequence-specific binding proteins. Although it is highly speculative, the dG stretch which is nucleosome free in cells in which the globin gene is inactive may be assuming the triplex. When the cells differentiate, the dG stretch may ultimately need to be double-stranded for a stable complex formation with BGP1. The Zn^{++} ions that are presumably brought in close proximity to the helix by the binding protein may be able to switch the structure adopted by the dG stretch from the triplex to the double stranded form. The Zn^{++} induced non-B DNA structure formation at the adjacent direct repeat may facilitate this switch. Future research will address this question.

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