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Magnesium ion-dependent triple-helix structure formed by homopurine-homopyrimidine sequences in supercoiled plasmid DNA

(chloroacetaldehyde/chemical cleavage/poly(dG)·poly(dC) sequence/G·G·C triplet/C$^+$·G·C triplet)

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ABSTRACT **DNA can be chemically cleaved at the site of chloroacetaldehyde-modified residues by the chemicals used for Maxam–Gilbert sequencing reactions. Use of this technique facilitates fine structural analysis of unpaired DNA bases in DNA with non-B-DNA structure. This method was used to study the non-B-DNA structure adopted by the poly(dG)·poly(dC) sequence under torsional stress at various ionic conditions. In the presence of 2 mM Mg^{2+}, the 5′ half of the deoxycytosine tract is very reactive to chloroacetaldehyde, while the 3′ half is virtually unreactive. In the poly(dG) tract, chloroacetaldehyde reaction is restricted to the center guanine residues. In the absence of Mg^{2+}, however, it is the 5′ half of the deoxyguanine tract that is reactive to chloroacetaldehyde, while the 3′ half is unreactive. And chloroacetaldehyde reaction is restricted to the center cytosine residues in the poly(dC) stretch. These results strongly suggest that the poly(dG)·poly(dC) sequence is folded into halves from the center of the sequence to form a tetra-stranded-like structure. Such a structure contains either a triplex consisting of poly(dG)·poly(dG)·poly(dC) strands in the presence of Mg^{2+} or a triplex consisting of poly(dC)·poly(dG)·poly(dC) strands in the absence of Mg^{2+}. The fourth strand, not involved in triplex formation, is closely associated with the triplex and is positioned in such a way that DNA bases are exposed and freely accessible to the chloroacetaldehyde reaction.**

The torsional stress that results from negative supercoiling is relieved through the formation of non-B-form DNA structures. Under torsional stress, non-B-DNA structures are preferentially formed by certain DNA sequences. Homopurine/homopyrimidine sequences are one type of such DNA sequences known to adopt non-B-DNA structure in supercoiled DNA. Long stretches of homopurine/homopyrimidine are often found in putative regulatory regions of eukaryotic genes. The anomalous sensitivity of these homopurine/homopyrimidine sequences to S1 nuclease, when studied in supercoiled DNA, has been reported by a number of groups (1–9).

We previously devised a method that used bromoacetaldehyde and chloroacetaldehyde to detect and analyze the altered DNA conformation of chromatin *in vivo* and in supercoiled DNA (10–12). Both bromoacetaldehyde and its less potent analog chloroacetaldehyde react specifically at the N-1 and N-6 positions of adenine and the N-3 and N-4 positions of cytosine residues (13–16), when these bases are not hydrogen bonded (17, 18). Although less reactive, the N-1 and N-2 positions of guanine residues also react with chloroacetaldehyde (19). Using bromoacetaldehyde as the probe and then digesting the bromoacetaldehyde-modified sites with S1 nuclease after cleaving the DNA with a restriction enzyme, we have shown that the 16 contiguous guanine residues at −180 base pairs (bp) from the mRNA cap site of the chicken adult β^A-globin gene constitute one of the major sites detected by bromoacetaldehyde in supercoiled DNA but not in linear DNA (10). Furthermore, by fine-structure mapping on the bromoacetaldehyde-modified sites of supercoiled plasmid DNA harboring two sets of poly(dG)·poly(dC) sequences, we have shown that poly(dG)·poly(dC) sequences in supercoiled plasmid DNA at bacterial superhelical density are capable of inducing their neighboring sequences to adopt altered DNA conformations (11). Bromoacetaldehyde-reactive sites were detected not only at the center of each poly(dG)·poly(dC) stretch, but also at neighboring sequences located 3′ of the deoxyguanine residues in the same DNA molecule. Such reactivity was observed when the poly(dG)·poly(dC) segment was adjacent to a variety of neighboring sequences.

To understand this phenomenon, the precise nature of non-B-DNA structure adopted by homopurine/homopyrimidine was studied. We report here a method to detect the chloroacetaldehyde-modified bases that do not use S1 nuclease. This alternative method permits the detection of a single base modified with chloroacetaldehyde. We found that chloroacetaldehyde-modified residues are different from unmodified residues in their reactivity to chemicals used for Maxam–Gilbert reactions (20). Not only new unexpected cleavage due to chloroacetaldehyde modification but also enhancement in cleavage at the site of chloroacetaldehyde-modification was observed.

Previous studies by others have shown that a triple-stranded structure can be formed between a linear homopurine/homopyrimidine duplex and a corresponding homopyrimidine strand (21–23). Based on data obtained by two-dimensional gel electrophoresis, Lyamichev *et al.* (24) have proposed a triple strand as the structure adopted by homopurine/homopyrimidine sequences in supercoiled plasmid DNA. Using a similar technique, Mirkin *et al.* (25) have reported evidence consistent with the model suggested by Lyamichev *et al.* There is no evidence yet available that is based on a chemical analysis that demonstrates a triple-helical structure in supercoiled plasmid DNA.

With the use of this alternative means of chemically cleaving the bromoacetaldehyde- or chloroacetaldehyde-modified sites, we report here evidence that non-B-DNA structure adopted by poly(dG)·poly(dC) sequences contains either a poly(dG)·poly(dG)·poly(dC) triple helix in supercoiled plasmid DNA or a poly(dC)·poly(dG)·poly(dC) triple helix, depending on the presence or absence of Mg^{2+}.

MATERIALS AND METHODS

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

Plasmid DNA. Supercoiled pCδ104 plasmid (11) contains a mouse immunoglobulin C_δ sequence inserted at the *Pst* I site

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by the G-C tailing method. The supercoiled pCATCG30 plasmid has a 30-bp poly(dG)·poly(dC) sequence inserted into the *Sma* I/*Sac* I site of the pUC13 plasmid, which also contains the bacterial chloramphenicol acetyltransferase gene inserted into the *Hin*dIII site.

Chemical Modification of DNA. Fifty micrograms of supercoiled plasmid DNA was incubated with various amounts of chloroacetaldehyde per 100-μl reaction volume for 1 hr at 37°C under the conditions described in the figure legends. The control DNA was treated similarly, except no chloroacetaldehyde 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, distal from the suspected chloroacetaldehyde-modified sites. The DNA fragment containing the chloroacetaldehyde-modified sites was isolated from a 6% native polyacrylamide gel. The DNA was then treated with either hydrazine, dimethyl sulfate, or formic acid and subsequently treated with piperidine as described by Maxam and Gilbert (20), except after the piperidine reaction DNA was purified by successive ethanol precipitations.

RESULTS AND DISCUSSION

The supercoiled pCδ104 plasmid at bacterial superhelical density was chosen for study so the results obtained with the chemical cleavage method could be compared with previous results obtained by the S1 nuclease method (11). Chloroacetaldehyde was used in the present experiment, because we previously demonstrated that both chloroacetaldehyde and bromoacetaldehyde detect identical sites in supercoiled plasmid DNA, as judged by single base resolution (12). In addition, we have confirmed the identical reaction pattern of chloroacetaldehyde and bromoacetaldehyde for the plasmids used in this study (data not shown). Chloroacetaldehyde treatment was first performed on pCδ104 plasmid at pH 5 in the presence of 2 mM $MgCl_2$ (Fig. 1), the conditions under which our previous experiments were carried out (11). In the chemical cleavage method, the chloroacetaldehyde-modified DNA was first digested with *Hin*dIII, end-labeled with Klenow or kinase to study both strands, and digested for the second time with *Pvu* I. The 351-bp fragment, *Hin*dIII/*Pvu* I, was isolated and treated with hydrazine, dimethyl sulfate, or formic acid, followed by piperidine treatment. The chloroacetaldehyde-modified fragments migrated slower than unmodified fragments.

Denaturing polyacrylamide gel analysis of these DNA fragments (Fig. 1 *Left*) revealed that, for each chloroacetaldehyde-modified DNA treated with a different chemical, unexpected bands with different levels of intensity were observed that were absent in the control with unmodified DNA. Hydrazine/piperidine treatment, which normally cleaves at unmodified cytosine residues, also cleaved DNA at chloroacetaldehyde-modified adenine and some guanine bases to give rise to another band (Fig. 1 *Left*, lanes e, f, g, and h) and showed enhanced cleavage at chloroacetaldehyde-modified cytosine bases (lanes p, q, and r). Dimethyl sulfate/piperidine treatment, which cleaves DNA at unmodified guanine bases, also cleaved DNA at chloroacetaldehyde-modified cytosine and adenine bases (lanes m, n, and o). Formic acid/piperidine treatment, which cleaves DNA at unmodified adenine and guanine bases, also cleaved at chloroacetaldehyde-modified cytosine bases (lanes s and t) and showed enhanced cleavage when adenine and guanine bases were chloroacetaldehyde-modified (lanes i, j, k, and l). Many fewer guanine residues as compared to adenine and

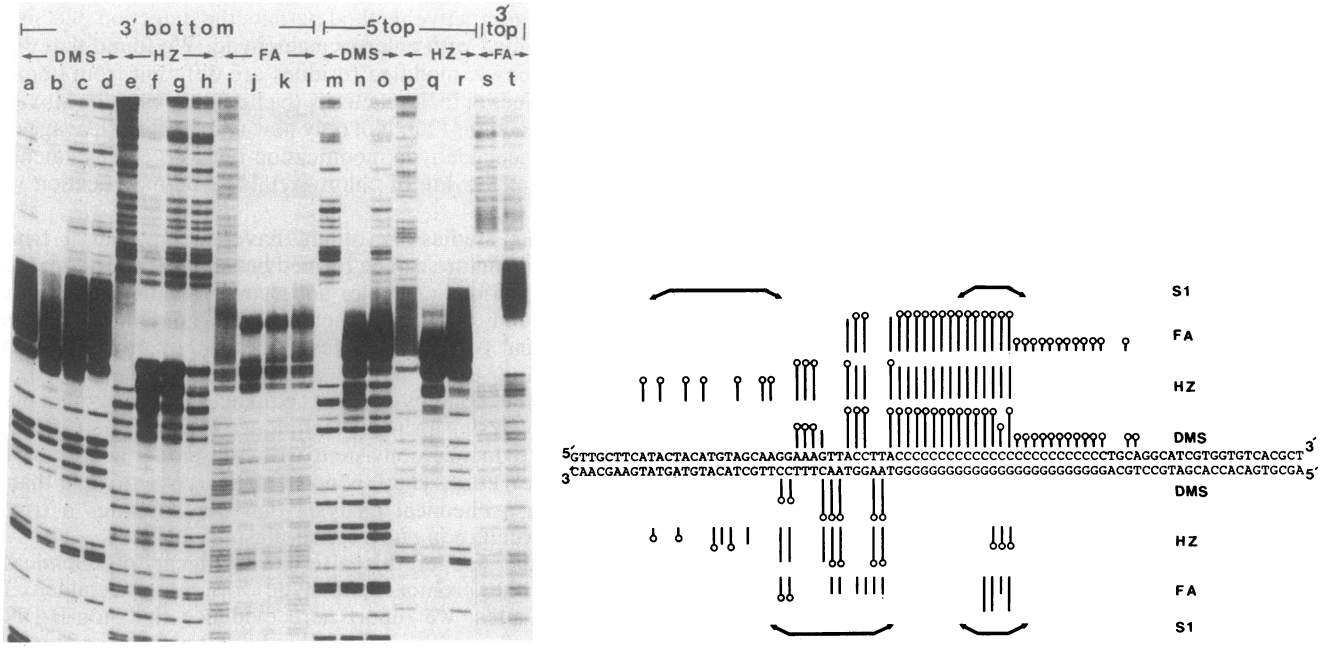


FIG. 1. Fine-mapping analysis of Maxam–Gilbert chemical cleavage. (*Left*) Determination of the chloroacetaldehyde-modified nucleotides: the end-labeled 351-bp DNA fragments (*Hin*dIII/*Pvu* I) derived from either chloroacetaldehyde-modified or unmodified supercoiled pCδ104 plasmid were isolated from a native acrylamide gel and subjected to Maxam–Gilbert chemical reaction. Lanes: a–l, DNA was labeled at the 3′ end of the *Hin*dIII site (bottom strand); m–r, DNA was labeled at the 5′ end of the *Hin*dIII site (top strand); s and t, DNA was labeled at the 3′ end of the *Pvu* I site (top strand) with T4 DNA polymerase. DNA not treated with chloroacetaldehyde (controls) (lanes a, e, i, m, p, and s); DNA treated with 5 μl of chloroacetaldehyde (lanes b, f, j, n, and q); DNA treated with 2 μl of chloroacetaldehyde (lanes c, g, k, o, r, and t); DNA treated with 1 μl of chloroacetaldehyde (lanes d, h, and l). All chloroacetaldehyde reactions were performed in 50 mM NaOAc buffer (pH 5) in the presence of 2 mM Mg^{2+}. DMS, dimethylsulfate; HZ, hydrazine; FA, formic acid. (*Right*) Summary of the chemical cleavage pattern for chloroacetaldehyde-modified DNA. DNA bases that show enhanced cleavage are indicated by vertical lines, relative degree of enhancement is indicated by different lengths. ○, Cleavage (bands in addition to those normally expected) due to chloroacetaldehyde modification at that site. ↙↘, S1 nuclease cleavage pattern of chloroacetaldehyde-modified DNA from previous studies (11). S1, S1 nuclease; FA, formic acid; HZ, hydrazine; DMS, dimethyl sulfate.

cytosine residues were detected as chloroacetaldehyde-modified. This reflected the reaction specificities of chloroacetaldehyde.

A side-by-side comparison of the cleavage patterns of chloroacetaldehyde-modified DNA treated with each of the different chemicals (Fig. 1 *Left*) revealed that specific chloroacetaldehyde-modified DNA sites, cleaved by one of these chemicals to give another band, were usually much more reactive to alternative chemical treatment, resulting in bands of greater intensity. No additional cleavage was observed at any thymidine residues, which are known to be chloroacetaldehyde unreactive. These results are summarized in Fig. 1 (*Right*). The sites with enhanced reactivity are marked by vertical lines of different lengths, expressing various levels of enhancement, and additional bands are indicated by open circles on top of vertical lines.

Analysis of chloroacetaldehyde-reactive bases by the chemical cleavage method within the poly(dG)·poly(dC) stretch provided an unexpected result. Although our previous data obtained with S1 nuclease to cleave chloroacetaldehyde-modified DNA showed cleavage at the center of the poly(dG)·poly(dC) stretch on both strands, the chloroacetaldehyde reactivity pattern determined by the chemical cleavage method for the top strand containing cytosine residues was drastically different from that of the bottom strand containing guanine residues. For the top strand, cytosine residues of the 5′ half of the contiguous cytosine stretch were reactive to chloroacetaldehyde, while the 3′ half of the stretch was virtually unreactive to chloroacetaldehyde. In contrast, the bottom strand containing contiguous guanine residues revealed chloroacetaldehyde reactivity limited to specific guanine residues located at the center of the stretch.

Chloroacetaldehyde reaction with the neighboring sequences was also analyzed. As determined by the S1 nuclease procedure (11), the chemical cleavage method revealed that the neighboring sequences 3′ of the contiguous guanine residues were very reactive to chloroacetaldehyde, whereas little reactivity was observed for the 5′ neighboring sequences (summarized in Fig. 1 *Right*). The data derived from the chemical cleavage method suggest that the non-B-DNA structure assumed by the poly(dG)·poly(dC) sequence involves folding of the sequence at the center, forming a poly(dG)·poly(dG)·poly(dC) triple helix derived from the 5′ and 3′ halves of the contiguous guanine sequence and the 3′ half of the contiguous cytosine sequence. The 5′ half of the cytosine strand remained closely associated with the triple helix; however, the N-3 and N-4 positions of cytosine bases are not involved in hydrogen bonding and are freely accessible to chloroacetaldehyde reaction. We think the fourth strand, the 5′ half of the contiguous cytosines, is closely associated with the rest of the strands, since S1 nuclease digestion of chloroacetaldehyde-modified DNA was limited only to the center of the contiguous cytosine sequence where a presumably local single-stranded structure exists upon folding of DNA. Thus, the structure is likely to be tetrastranded, containing the triple helix as the core structure. This non-B-DNA structure is induced by negative supercoiling of plasmid DNA. We confirmed this by treating linear plasmid DNA with 5 μl of chloroacetaldehyde per 100-μl reaction volume and then treating it with the chemicals used for the Maxam–Gilbert reaction. The sequencing gel pattern was identical to that of unmodified DNA (data not shown).

Based on pH-dependent structural transition studies on two-dimensional polyacrylamide gel electrophoresis, Lyamichev *et al.* (24) and Mirkin *et al.* (25) proposed a model triplex containing polypyrimidine/polypurine/polypyrimidine strands when the cytosine residues of one of the pyrimidine strands are protonated. A similar triplex was also suggested based on chemical reaction patterns of homopurine–homopyrimidine tracts in supercoiled plasmid DNA (B. Johnston, personal communication; ref. 26). Again, the reaction occurred only under acidic conditions.

Although the polypyrimidine/polypurine/polypyrimidine triplex in supercoiled DNA is consistent with their data, our experiment with supercoiled pCδ104 plasmid indicated a polypurine/polypurine/polypyrimidine (G·G·C) triplex instead. To understand this discrepancy, we used a plasmid construct (pCATCG30) containing a 30-bp poly(dG)·poly(dC) tract and studied in detail the effects of different ionic conditions on triplex formation. The pCATCG30 is useful for such study because convenient restriction enzymes are located close to the poly(dG)·poly(dC) stretch, enabling sequencing gel analysis at much higher resolution in the poly(dG)·poly(dC) region than the pCδ104 plasmid.

Chloroacetaldehyde at Very Low Concentrations Still Detects the Triplex Structure. Using supercoiled pCATCG30 plasmid, we first asked whether chloroacetaldehyde indeed detects the structure that existed prior to the chloroacetaldehyde reaction or whether the reaction at one site subsequently induced such a structure. The reaction pattern at various chloroacetaldehyde concentrations is shown in Fig. 2. When doubly distilled chloroacetaldehyde in the range of 0.1–5 μl was used per 100-μl reaction volume, even the lowest concentration in which a single nucleotide is estimated to be modified per plasmid molecule by chloroquine gel analysis (ref. 10 and references therein) gave rise to the typical cleavage pattern, suggesting a G·G·C triplex (lanes b and g). These results show that chloroacetaldehyde is indeed detecting the structure and not inducing such a structure.

Mg^{2+} Dependency. Next, we tested the effect of Mg^{2+} on the formation of triplex structure. At pH 5, in the presence of

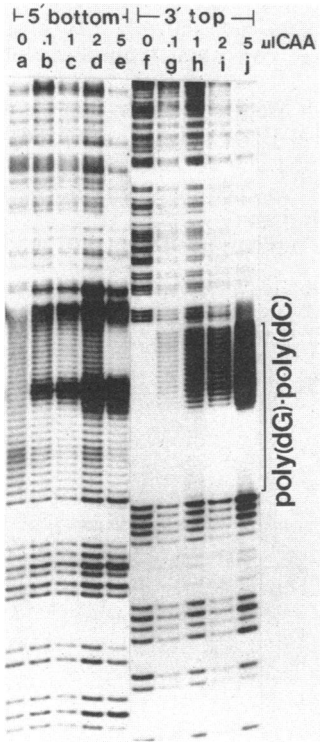


FIG. 2. Detection of the triple helix at various chloroacetaldehyde concentrations. The supercoiled plasmid pCATCG30 was treated with chloroacetaldehyde at 0, 0.1, 1, 2, and 5 μl of chloroacetaldehyde per 100-μl reaction volume in 50 mM NaOAc (pH 5) in the presence of 2 mM $MgCl_2$. The end-labeled 242-bp *Ban* I/*Hin*dIII DNA fragment containing chloroacetaldehyde-modified bases was then subjected to a Maxam–Gilbert chemical reaction with formic acid. Lanes: a–e, DNA was labeled at the 5′ end of the *Hin*dIII site (bottom strand); f–j, DNA was labeled at the 3′ end of the *Hin*dIII site (top strand).

2 mM Mg^{2+}, the pCATCG30 plasmid also revealed a chloroacetaldehyde reaction pattern consistent with that of the pCδ104 plasmid (Fig. 3 *Left*, lanes c, f, i, l, o, and r). In the absence of Mg^{2+}, however, even though the basic patterns of reaction were similar to those found in the presence of Mg^{2+} ions, it was the deoxyguanine tract that was reactive over half of its length rather than the deoxycytosine tract (lane e). The data obtained with DNA labeled at the 5′ end of the *Hin*dIII site were confirmed by a similar experiment with a DNA fragment labeled at the 3′ end of the *Ban* I site (data not shown). For the deoxycytosine tract, the chloroacetaldehyde reaction was detected at the center cytosine residues (lanes h, k, n, and q) in the absence of Mg^{2+}, in contrast to the center guanine residues in the deoxyguanine tract in the presence of Mg^{2+}. This shows that the structure detected in the absence of Mg^{2+} is a triplex containing poly(dC)·poly(dG)·poly(dC) strands. These data (summarized in Fig. 3 *Right*) demonstrate that the triplex formed by the poly(dG)·poly(dC) sequence in supercoiled plasmid DNA has dynamic properties, and a specific triplex structure, either G·G·C or C·G·C, is formed depending on the presence or absence of Mg^{2+} (2 mM).

pH Dependency. We next examined pH dependency for the formation of each type of triplex. As shown in Fig. 4, chloroacetaldehyde reaction was limited to the center cytosine residues of the poly(dC) tract, indicating that the C·G·C triplex was seen only up to pH 5.5 and suggesting that one cytosine strand of the C·G·C triplex was protonated. In contrast to this, the chloroacetaldehyde reaction over the half of the cytosine residues of the poly(dC) tract indicated that a G·G·C triplex was unchanged for pH 5.0–7.5 (this is also true for pH 8; data not shown). These results were confirmed by studying the chemical cleavage patterns for the deoxyguanine tract as well (data not shown). The probable hydrogen bonding schemes consistent with these observations are shown in Fig. 5.

The Proposed Models for Non-B-DNA Structures. The models for non-B-DNA structures that are the closest approximation to the actual structure based on our data are shown in Fig. 5. In each of these models, the structure is tetrastranded in nature with a triple helix as the core structure. (The tetrastranded-structure model is preferred because of the results obtained with S1 nuclease as discussed earlier.) In the presence of Mg^{2+}, it forms a G·G·C triplex, while in the absence of Mg^{2+} it forms a C^{+}·G·C triplex. The tetrastranded-like structure with a triplex as the core most likely explains the unidirectional conformational effect exerted by the poly(dG)·poly(dC) sequence upon its neighboring regions as observed (11). The remaining guanine strand or cytosine strand that did not form a triplex seems positioned so that DNA residues are exposed and unpaired. This DNA strand, the fourth strand of the tetrastranded structure, and its complementary strand that forms a triple helix must change their mutual orientation in space so they can once again be base-paired in the neighboring region of the poly(dG)·poly(dC) tract. This results in a local unwinding of the DNA helix in the neighboring region. Thus, under conditions (the absence of Mg^{2+}) allowing the 3′ half of the

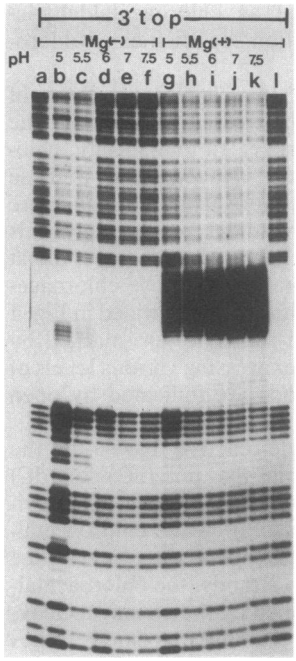
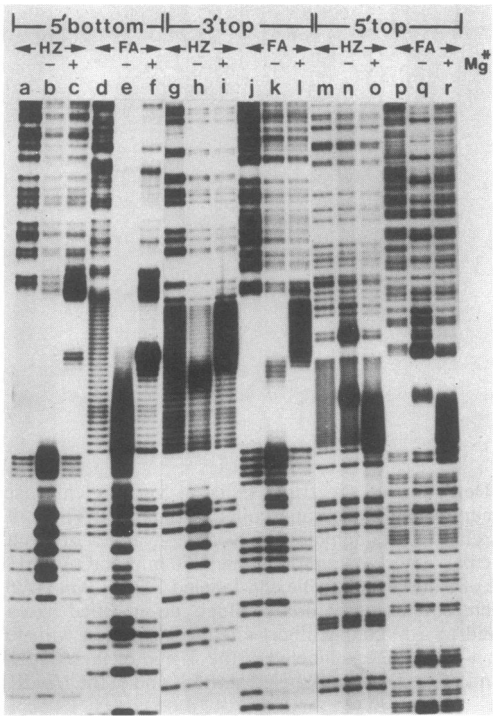


FIG. 4. pH dependency on triple-helical structure. Supercoiled plasmid pCATCG30 was treated with 2 μl of chloroacetaldehyde in 50 mM Tris maleic acid buffer at pH 5, 5.5, 6, 7, and 7.5 in the presence or absence of 2 mM $MgCl_2$. The *Ban* I/*Hin*dIII DNA fragment labeled at the 3′ end of the *Hin*dIII site (top strand) was then isolated and subjected to Maxam–Gilbert chemical reaction with formic acid. Lanes a and l, chloroacetaldehyde-untreated control DNA. pH had no effect on control DNA.



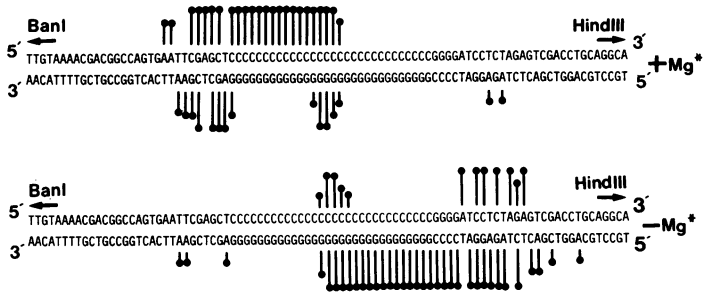


FIG. 3. Effect of Mg^{2+} on triple-helical structures. (*Left*) Determination of chloroacetaldehyde-modified nucleotides. Supercoiled pCATCG30 plasmid was treated with 0 (control) or 2 μl of chloroacetaldehyde either in the presence or absence of 2 mM $MgCl_2$ and 50 mM NaOAc (pH 5). DNA fragments indicated below were isolated from a native acrylamide gel and subjected to a Maxam–Gilbert chemical reaction. Lanes: a–f, DNA was labeled at the 5′ end of the *Hin*dIII site (bottom strand, *Ban* I/*Hin*dIII fragment); g–l, DNA was labeled at the 3′ end of the *Hin*dIII site (top strand, *Ban* I/*Hin*dIII fragment); m–r, DNA was labeled at the 5′ end of the *Ban* I site (top strand, 800-bp *Ban* I/*Nco* I fragment). Lanes a, d, g, j, m, and p, controls without chloroacetaldehyde; +, the presence of 2 mM $MgCl_2$; −, the absence of $MgCl_2$. HZ, hydrazine; FA, formic acid. (*Right*) Summary to the chemical cleavage patterns for chloroacetaldehyde-modified DNA. •, Chloroacetaldehyde-modified sites. (*Upper*) In the presence of $MgCl_2$; (*Lower*) in the absence of $MgCl_2$.

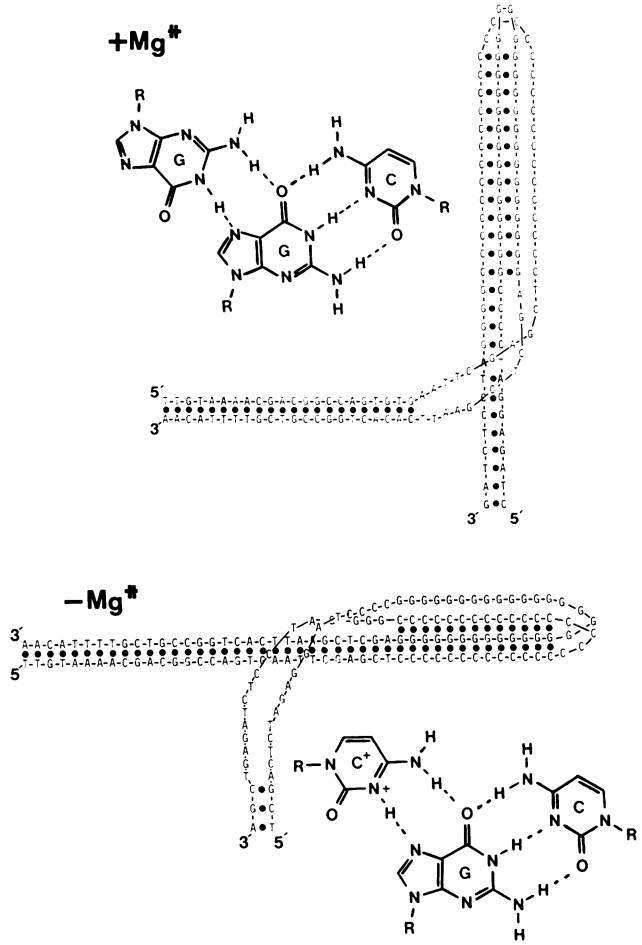


FIG. 5. Probable structure adopted by the poly(dG)·poly(dC) sequence in supercoiled plasmid DNA and the corresponding hydrogen-bonding scheme for the base triplet in the presence (*Upper*) or absence (*Lower*) of 2 mM Mg^{2+}. •, Hydrogen bonds in the non-B-DNA structure; - - -, hydrogen bonds in the triplets.

deoxyguanine tract to become unpaired, the neighboring sequence 3′ of the deoxyguanine tract has the major conformational alteration. On the other hand, under conditions (the presence of Mg^{2+}) allowing the 5′ half of the deoxycytosine tract to become unpaired, the neighboring sequence 5′ of the deoxycytosine tract also has this conformational change. The unidirectional conformational effect exerted by the poly(dG)·poly(dC) sequence on its neighboring sequence, which was previously detected to occur only on the 3′ neighboring sequence of the guanine stretch in the presence of Mg^{2+}, is now reversed in its directionality by simply deleting Mg^{2+} from the chloroacetaldehyde reaction mixture. This is illustrated in the model in Fig. 5.

The models presented here are not necessarily the simplest, because we propose that the DNA region at one junction of the poly(dG)·poly(dC) sequence and neighboring sequences crosses over the other junction in space. We present these models in an attempt to explain, at least in part, the asymmetrical digestion pattern of S1 nuclease as opposed to the symmetrical reactivity of chloroacetaldehyde in the neighboring region (Fig. 1 *Right*). Each of the two DNA strands of neighboring sequences that are unpaired in the models are in stereochemically different environments: one strand exposed outward is more accessible to S1 nuclease, while the other strand is more protected from digestion. Unlike S1 nuclease, chloroacetaldehyde, which is a very low molecular weight chemical, does not experience such steric hindrance.

Theoretically, there exist other isomers that may be energetically different from what we show here. For example, in the presence of Mg^{2+}, the alternative isomeric form for the G·G·C triplex is the triplex involving the 3′ half of the deoxycytosine tract instead of the 5′ half of the deoxycytosine tract. Similarly, in the absence of Mg^{2+}, the alternative isomeric form for the C^+·G·C triplex is the triplex involving the 5′ half of the deoxyguanine tract instead of the 3′ half. Although we do not know why one isomer is preferred over the other, the present chemical analysis permitted us to tell unambiguously which of these isomers truly represents the actual structure.

The formation of the G·G·C base triplet as depicted in Fig. 5 does not require the protonation of cytosine residues. Linear poly(dG)·poly(dG)·poly(dC) seems to form stably in solution at neutral pH as judged by acid titration analysis (27). This is also true for another linear polypurine/polypurine/polypyrimidine triplex consisting of an A·A·U triplet (28). The G·G·C triple helix in supercoiled plasmid DNA that we detected is also formed at neutral pH as well as acidic pH when Mg^{2+} is present. Since the G·G·C triplex forms in supercoiled DNA under physiological conditions, it is likely that at some steps during active chromatin assembly, such a polypurine/polypurine/polypyrimidine triplex is formed *in vivo* by the homopurine/homopyrimidine stretch if the DNA regions containing such sequences are locally under torsional stress.

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