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In Z-DNA the sequence G-C-G-C is neither methylated by *Hha* I methyltransferase nor cleaved by *Hha* I restriction endonuclease

(DNA conformation/*in vitro* methylation/supercoiled plasmid/left-handed DNA)

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ABSTRACT **Plasmids carrying 24- or 32-base-pair inserts of alternating (dG-dC) residues were used to analyze the level of methylation of the G-C-G-C sites by *Hha* I DNA methyltransferase and their cleavage by *Hha* I endonuclease in the B-DNA or Z-DNA conformation. In supercoiled plasmids in which the inserts formed Z-DNA, the extent of methylation at the insert G-C-G-C sites was dramatically lower than the level of methylation at the G-C-G-C sites located outside the insert in the same plasmid. Similarly, cleavage by *Hha* I endonuclease was sharply lowered when the insert was in the Z-DNA form. In the relaxed plasmid, all its G-C-G-C sites were methylated to the same extent and the unmethylated sites were readily cleaved. After treatment with the methylase, the supercoiled plasmid was linearized and then digested with *Hha* I restriction endonuclease. This exposed unmethylated G-C-G-C sites from the insert that had been protected against cleavage in the Z conformation. A chemical reaction was used to study the distribution of the unmethylated cytosine residues. No accumulation of unmethylated cytosine residues was found anywhere along the entire 32-base-pair insert, which is consistent with a cooperative B–Z transition.**

There is considerable interest in the polymorphism of DNA (for discussion, see ref. 1). It is known to exist in both right-handed and left-handed conformations; the latter is more prevalent in DNA that is torsionally strained because of negative supercoiling (2–5). Many enzymes act on DNA and it is interesting to know the DNA conformation when it is acting as a substrate. We have addressed this question for the DNA sequence G-C-G-C, which is recognized by both the restriction endonuclease *Hha* I and the *Hha* I methyltransferase (6). The methyltransferase is known to add a methyl group to the cytosine position 5 at the central C position. The *Hha* I restriction endonuclease recognizes and cleaves this sequence when unmethylated. However, sequences with alternating purines and pyrimidines, especially alternating guanine and cytosine residues, form Z-DNA readily (7, 8). We have varied the substrate conformation by using a plasmid that has an insert of alternating guanine and cytosine residues, either 24 or 32 base pairs (bp) long. Previous work has shown that this insert is in the form of Z-DNA at the negative superhelical densities found inside bacterial cells (4, 9). By increasing the negative superhelical density *in vitro*, the equilibrium for the insert can be pushed more strongly in the direction of Z-DNA. On the other hand, relaxing the plasmid results in the loss of Z-DNA in the insert. We have used these three systems of relaxed, native, and supercoiled plasmids as substrates for both the *Hha* I methyltransferase and the *Hha* I restriction endonuclease. Our results have been interpreted to indicate that neither of these two enzymes acts on the appropriate sequence of nucleotides when it is in the Z-DNA conformation but readily acts on the same sequence when it is in the B-DNA conformation. Further, the evidence is compatible with the entire segment existing as either B-DNA or Z-DNA when this segment is in dynamic equilibrium between these two conformations.

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MATERIALS AND METHODS

Plasmid and DNA Preparation. Plasmid pLP32 has been described (3) and plasmid pDHg16 was a gift of D. E. Pulleyblank (10). The plasmids were supercoiled or relaxed by topoisomerase I and the superhelical densities of the resultant plasmids were determined as described (3, 4, 9).

***In Vitro* Methylation of the Plasmid DNA.** DNA methyltransferase *Hha* I and restriction endonucleases were obtained from New England Biolabs. Plasmid DNA at different superhelical densities was incubated at 50 μg/ml in 50 mM Tris·HCl, pH 7.5/10 mM EDTA/5 mM 2-mercaptoethanol/80 μM *S*-adenosylmethionine containing 1 unit of *Hha* I methyltransferase per 1 μg of DNA at 37°C. At various times after initiation of the reaction, DNA methylation was terminated by incubation at 65°C for 20 min. DNA was analyzed by restriction endonuclease cleavage and electrophoresis on horizontal 1% agarose slab gels.

Sequencing of the Cytosine Residues in the Methylated and Unmethylated Plasmids. Methylated or unmethylated plasmids were cleaved with *Hpa* II restriction endonuclease and the fragments were ^{32}P labeled at their 5′ termini by using [γ-^{32}P]ATP (New England Nuclear; >3000 Ci/mmol; 1 Ci = 37 GBq) and polynucleotide kinase as described (11). Subsequently, the DNA fragments were cleaved with restriction endonucleases *Eco*RV and *Bgl* I to generate a fragment 234 nucleotides (nt) long. This fragment contained the $(C\text{-}G)_{16}$ insert, was labeled at one end, and could be separated from the rest of the labeled fragments easily. The labeled fragments were separated on a 5% polyacrylamide gel, and the 234-nt-long fragment was cut out and thermoeluted according to published protocols. The Maxam and Gilbert sequencing method was used (11). The reaction products were analyzed on a 20% polyacrylamide sequence analysis gel.

RESULTS

Methylation of G-C-G-C Sites in a B or a Z Conformation. The C-G insert generates a series of overlapping recognition sites (5′ G-C-G-C 3′) for the *Hha* I methyltransferase and for its restriction endonuclease that allows us to study whether DNA in a Z or a B conformation can serve as an equally good substrate for methylation or nuclease activity. The *Hha* I methyltransferase recognizes the G-C-G-C sequence and methylates the internal cytosine in this site (6). Plasmids pLP32 and pDHg16 contain 15 or 11 methylation sites in the C-G insert and 32 or 12 methylation sites outside the insert, respectively. The distribution of the G-C-G-C sites on the two plasmids is shown in Fig. 1 (12).

Negative superhelical coiling induces the Z-conformation in the C-G insert of the plasmid (2–4). The presence of G-C-

Abbreviations: bp, base pair(s); nt, nucleotide(s).

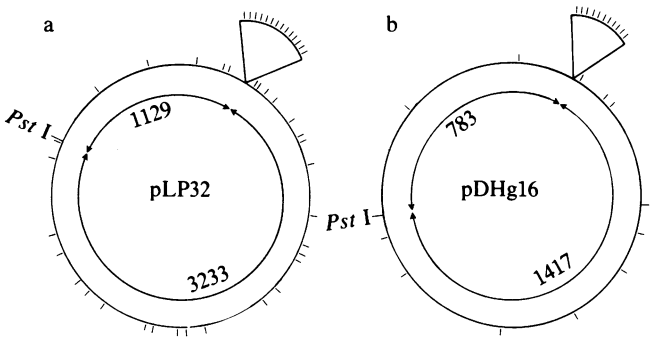


FIG. 1. Distribution of *Hha* I sites on plasmids pLP32 and pDHg16 (3, 10, 12). (*a*) pLP32 is 4.3 kilobases long and contains a 32-bp insert of alternating dG-dC residues at the *Bam*HI site. (*b*) pDHg16 is 2.1 kilobases long and contains a 24-bp insert of alternating dG-dC residues at the *Hin*dIII site. The dashes mark the positions of the G-C-G-C sites along the molecule. pLP32 and pDHg16 contain 15 or 11 sites within the insert and 31 and 12 sites outside the insert, respectively. Cleavage of pLP32 at the insertion site and at the *Pst* I site generated fragments slightly larger than 3233 and 1129 nt long. Cleavage of pDHg16 at the insertion site and at the *Pst* I site generated fragments slightly larger than 1417 and 783 nt long.

G-C sites in the Z-DNA insert as well as in the other region of the plasmid provides an internal control for possible preferential methylation patterns of the different regions. Restriction endonuclease *Hha* I recognizes and cleaves the sequence G-C-G-C but cannot cleave this site when the internal cytosine is methylated (6). This enzyme was used to determine the level of methylation of the G-C-G-C sites in the different plasmids.

Plasmid pLP32 was supercoiled (S in Fig. 2) to a linking difference of −40 in the presence of the intercalator ethidium. The native superhelical density (N in Fig. 2) had a linking difference of −19, while the third plasmid was relaxed (R in Fig. 2). The plasmids at the different superhelical densities were then methylated using the *Hha* I methyltransferase for

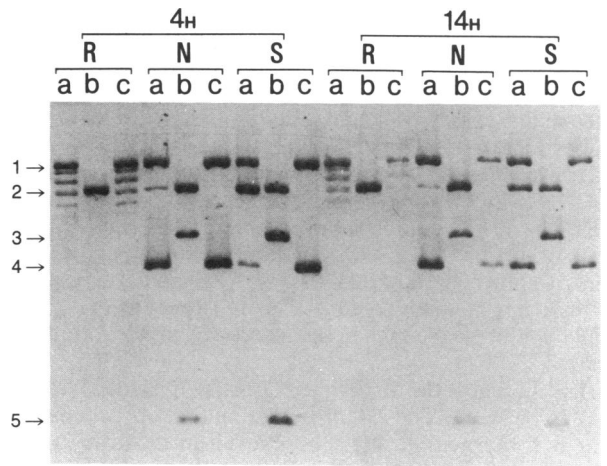


FIG. 2. Patterns of methylation of plasmid pLP32. pLP32 DNA was relaxed (R), left in the native superhelical density (N), or highly supercoiled (S). DNA at the three different superhelical densities was methylated for 2, 3, 4, and 14 hr using the *Hha* I methyltransferase. To analyze the level of DNA methylation in the different DNA probes, the methylated DNA was cleaved with the *Hha* I restriction endonuclease (lanes a) or restriction endonucleases *Hha* I and *Pst* I (lanes b) or was left uncleaved (lanes c). Subsequently, the DNA was analyzed by electrophoresis on horizontal 1% agarose slab gels. Arrows: 1, nicked plasmid; 2, linearized plasmid; 3, 3233-nt-long fragment; 4, supercoiled plasmid; 5, 1129-nt-long fragment. [Since the exact *Hha* I cleavage site within the $(C\text{-}G)_{16}$ insert is not known, the actual lengths of the 3233- and 1129-nt fragments could be slightly larger.]

20–90 min, 2, 3, 4, and 14 hr. To determine the level of methylation, the methylated plasmids were cleaved with restriction endonuclease *Hha* I (lanes a in Fig. 2) or were left uncleaved (lanes c in Fig. 2). The products were then analyzed on an agarose slab gel that was stained with ethidium bromine. The predominant bands seen in the methylated and uncleaved plasmids (lanes c in Fig. 2) represent the nicked and the supercoiled forms of the plasmids. In the relaxed plasmid [R (lanes c) in Fig. 2], a range of relaxed topoisomers can be seen.

Cleavage of the plasmids after a short time of methylation (results not shown for the short time periods) generated a range of bands in many different sizes, indicating that many of the G-C-G-C sites in each molecule were still unmethylated. As the time of methylation was increased, most of the restriction fragments disappeared and gave rise to the nicked linear and supercoiled forms of the plasmids.

There is a significant difference in the methylation pattern of the relaxed and the supercoiled forms of the plasmid. In the relaxed plasmid, 2 hr of methylation are sufficient to methylate all the G-C-G-C sites in the plasmid. In the supercoiled forms, methylation for as long as 14 hr cannot protect all the G-C-G-C sites and, on cleavage with restriction endonuclease *Hha* I, a certain percentage of the DNA molecules could still be linearized. The appearance of a linear form means that one site, or several sites near each other, were not methylated.

To locate the unmethylated sites in the DNA molecules, the plasmids were cleaved with restriction endonucleases *Hha* I and *Pst* I simultaneously (lanes b in Fig. 2). *Pst* I cleaves pLP32 at a single site at nt 3608 (12) (Fig. 1*a*). The $(C\text{-}G)_{16}$ segment was inserted at nucleotide 375 (*Bam*HI site) (3). If the unmethylated sites in the supercoiled plasmids were located at the C-G insert, cleavage with *Hha* I/*Pst* I should generate two fragments. Depending on the cleavage site in the 32-nt insert, one fragment should be a little more than 3233 nt long and the other slightly more than 1129 nt long (Fig. 1*a*). After 3 hr or more of methylation, cleavage of the supercoiled plasmids with restriction enzymes *Hha* I and *Pst* I (lanes b in Fig. 2) generated three fragments. The high molecular weight fragment is the linear form of the DNA plasmid, which was created by a single *Pst* I cleavage and it represents the DNA population that was fully methylated at all *Hha* I sites. The two smaller fragments are of the expected size from cleavage at the *Pst* I site as well as at one (or more) *Hha* I site in the insert. These results show that after a long period of methylation the only sites that could not be methylated were located in the Z-DNA segment.

A similar experiment was carried out with plasmid pDHg16 (Fig. 3). This plasmid, which is 2.1 kilobases long, contains a segment of $(C\text{-}G)_{12}$ (10). The plasmid was relaxed (R), was left at the native superhelical density (N), or was highly supercoiled (S). Supercoiling was carried out under the same conditions as for pLP32, but the linking number was not determined. The plasmids at the different superhelical densities were methylated for different time periods using *Hha* I methyltransferase and then analyzed by cleavage with restriction endonuclease *Hha* I (lanes a in Fig. 3). Here too the difference between the relaxed and the supercoiled plasmids is obvious. The relaxed plasmids could be completely methylated, whereas even after 14 hr of methylation the supercoiled plasmid could be linearized by cleaving with the *Hha* I restriction endonuclease. To locate the unmethylated sites, the plasmids were simultaneously cleaved with restriction enzymes *Pst* I and *Hha* I (lanes b in Fig. 3). Three fragments were found. The fragment in the linear form represents the completely methylated plasmid molecules that were cleaved only by the *Pst* I restriction endonuclease. The two other fragments, about 1417 and 783 nt long, indicate that in addition to the cleavage at the *Pst* I site, the super-

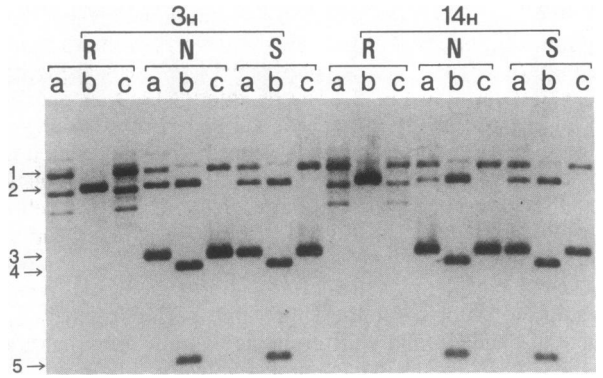


FIG. 3. Patterns of methylation of plasmid pDHg16. pDHg16 DNA was relaxed (R), left at the native superhelical density (N), or highly supercoiled (S). DNA at these three different superhelical densities was methylated for 3 and 14 hr by using the *Hha* I methyltransferase. The level of DNA methylation was determined by cleaving with the restriction endonuclease *Hha* I (lanes a) or *Hha* I and *Pst* I (lanes b) or left uncleaved (lanes c). The DNA was analyzed by electrophoresis on a horizontal 1% agarose slab gel. The nicked, linear, and supercoiled forms of the plasmids as well as the 1417- and 783-nt-long fragments are indicated by arrows 1–5, respectively. [Since the exact *Hha* I cleavage site within the $(C\text{-}G)_{12}$ insert is not known, the actual lengths of the 1417- and 783-nt-long fragments generated could be slightly larger.]

coiled plasmids were cleaved by the *Hha* I restriction endonuclease at the C-G insert (see Fig. 1*b*).

These results indicate that supercoiling of both plasmids, which induced a Z-DNA conformation at the C-G insert, sharply reduced the ability of the *Hha* I methyltransferase to methylate its recognition sites within the insert. In contrast, the ability to methylate the G-C-G-C sites located outside the Z-DNA insert was not affected by the supercoiling of the plasmids.

It should be noted, however, that even at the high superhelical density with supercoiling of −40, a considerable percentage of the plasmid molecules was completely methylated in the Z-DNA segment. That suggests a dynamic structure in which there is an equilibrium between the Z and the B conformations. This would, after a long period of methylation, produce complete methylation of the insert. The degree of methylation is less with higher degrees of negative supercoiling, as reported below, which is consonant with a shift of the equilibrium toward Z-DNA.

Quantitative Measures of Methylation and Cleavage. The conversion of B-DNA to Z-DNA involves a dramatic change in the DNA structure. It is likely that hydrolytic enzymes that recognize a specific sequence would also distinguish between the two forms. We extended our study to ask whether restriction endonuclease *Hha* I would also recognize the difference between the B and the Z form.

After 14 hr of methylation, the supercoiled pLP32 and pDHg16 plasmids still contained unmethylated G-C-G-C sites in the Z-DNA segment. This could be measured as *Pst* I cleaves both plasmids at a single site, generating a linear molecule in which the C-G insert is no longer in a Z-DNA conformation. The supercoiled plasmids pLP32 and pDHg16 were methylated for 14 hr with the *Hha* I methyltransferase and then cleaved with the *Hha* I restriction endonuclease (lanes a in Figs. 2 and 3) or with *Hha* I and *Pst* I simultaneously (lanes b in Figs. 2 and 3). We measured the extent of cleavage at the G-C-G-C restriction sites in the circular and linear forms of the plasmids. Using a Joyce–Loebl densitometer, we scanned photographs of the gels and then calculated the percentage of the DNA in the various cleaved fractions. The plasmid preparation had some nicked material that we assume was fully methylated after 14 hr of incubation. The amount of nicked DNA was estimated by quantitative absorption measurements of the a lanes after 14 hr of incubation (Figs. 2 and 3) and are given in Table 1. The linear material in the b lanes represents the fully methylated plasmids cut by *Pst* I, and they are also given in Table 1. The latter group arises from nicked plasmids as well as from fully methylated supercoiled plasmids. The data in Table 1 allow us to determine the latter quantity. We found that the extent of methylation is considerably reduced in the supercoiled plasmids (S) compared with the plasmids at native superhelical density (N).

We can also make measurements regarding the extent of *Hha* I cleavage in these same plasmids. *Hha* I cleavage occurs only at unmethylated sites in the insert after 14 hr of methylation. The two restriction fragments in the b lanes (Table 1) arise from molecules that were cleaved by *Pst* I and also had an unmethylated site in the insert. Thus the percentage of molecules cleaved by *Hha* I when the insert is in the Z conformation can be calculated (Table 1). To interpret these numbers, we need to know the number of target sites for *Hha* I cleavage in the insert when it is in the Z conformation. Unfortunately, that number is unavailable. Instead, we know that there are more molecules with unmethylated sites in the insert in the supercoiled plasmids (S) compared with the normal (N). The data in Table 1 show, for example, that only 4% of the pLP32 plasmids are fully methylated in the supercoiled state (S) compared with 22% of the normal (N) plasmids. This means that there will be many more potential sites for *Hha* I cleavage in the supercoiled plasmids compared with the normal. This is reflected in the fact that a higher percentage of sites are cleaved in the supercoiled plasmid compared with the normal. Because of this difference in target size, we cannot determine the effect of supercoiling *per se* on *Hha* I cleavage.

However, the ability of *Hha* I to cleave an unmethylated G-C-G-C site was considerably reduced when the DNA segment was in a Z conformation. For example, the results obtained with pLP32 and pDHg16 at the native superhelical density showed that 84% of the DNA molecules that contain at least one unmethylated G-C-G-C site at the G-C insert could *not* be cleaved when the insert was in the Z conformation.

Distribution of the Methylated Cytosine Residues Within the Z-DNA Segment. The Z-DNA segment that borders on DNA in a B conformation might be uneven in its structure, and there might be specific sites that are less accessible to the DNA methyltransferase. Some experiments suggest a structural perturbation near the B–Z junction (14). The location of

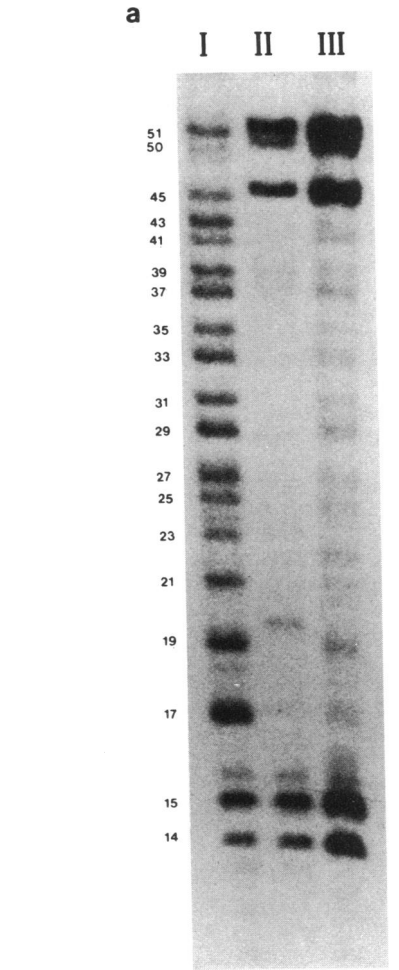
Table 1. Percentage of DNA in various forms after 14 hr of incubation

	Linear DNA*	Nicked DNA†	100% methylated supercoiled DNA	Linear DNA†	Restriction fragments*	Cleaved *Hha* I sites in Z conformation
pLP32-N	62	40	22	6	38	16
pLP32-S	37	33	4	28	63	44
pDHg16-N	37	18	19	10	63	16
pDHg16-S	27	19	8	16	72	22

*b lanes.
†a lanes.

the unmethylated G-C-G-C sites within the insert might reflect the nature of the Z-DNA structure. We took advantage of the fact that a cytosine residue that is methylated at position 5 is less reactive to hydrazine so that a band corresponding to that base does not appear in the piperidine cleavage pattern (15, 16).

A highly supercoiled and a relaxed plasmid pLP32 were methylated for 14 hr with the *Hha* I methyltransferase. An unmethylated pLP32 plasmid at the native superhelical density was used as control. The plasmids were cleaved with the restriction endonuclease *Hpa* II and were ^{32}P labeled by using polynucleotide kinase. The fragments were then cleaved with restriction endonucleases *Eco*RV and *Bgl* I to generate a 234-bp fragment that contained the C-G insert and was labeled at one end.



b

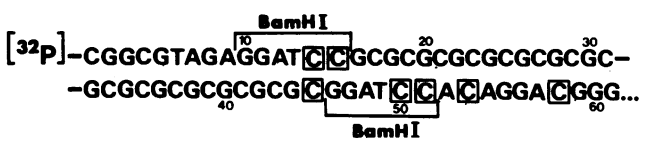


FIG. 4. Sequencing of the cytosine residues in methylated and unmethylated plasmids. (*a*) A highly supercoiled plasmid pLP32 (lane III) and a relaxed pLP32 (lane II) were methylated for 14 hr with *Hha* I methyltransferase. Plasmid pLP32 at the native superhelical density (lane I) was left unmethylated. Using the appropriate restriction enzymes and an end-labeling reaction, we prepared fragments 234-nt-long containing the $(G\text{-}C)_{16}$ insert and labeled at the 5′ end from the different plasmids. The Maxam and Gilbert sequencing reaction for the cytosine residues was carried out. The reaction products were analyzed on a 20% polyacrylamide sequencing gel. (*b*) Sequence contained in the labeled 5′ end. The cytosine residues outlined by squares are residues that are not within a methylation site of *Hha* I methylase and therefore could not be methylated. These show up in all three lanes in *a*. Numbers along the sequence ladder indicate positions of the cytosine residues in the sequence shown in *b*.

The fragments were separated on a 5% polyacrylamide gel and the fragment containing the C-G insert was eluted and sequenced. A portion of the sequence containing the labeled 5′ end of the fragment is shown in Fig. 4. The cytosine residues surrounded by squares are not part of a recognition site for the *Hha* I methyltransferase and therefore they can serve as an internal control for the level of methylation. The results of the sequencing reaction for the cytosine residues are also shown in Fig. 4, which shows the portion between residues 14 and 51 of the sequence. Unmethylated plasmid pLP32 (Fig. 4*a*, lane I) shows an even level of label along all the cytosine residues, inside and outside the C-G insert. The relaxed plasmid methylated with the *Hha* I methyltransferase showed a clear difference between the unmethylated cytosine residues outside the insert (including the last cytosine residue within the insert, which could not be methylated by the *Hha* I methyltransferase) and the methylated residues in the C-G insert (Fig. 4*a*, lane II). This low level of label of the methylated cytosine residues represents the background level of the reaction. A low and even level of labeling was found in the C-G insert of the methylated supercoiled plasmid (Fig. 4*a*, lane III). Since 63% of the supercoiled plasmid molecules contain at least one unmethylated G-C-G-C site within the insert (Table 1), the presence of specific unmethylated sites within the insert should have shown up as distinct highly labeled bands. The level of labeling in the C-G insert suggests that few cytosine residues in each plasmid molecule were left unmethylated. The distribution of the labeled bands indicated that there were no "hot spots" that could not be methylated in the Z conformation, but rather a random-location pattern of unmethylated G-C-G-C sites was found in the Z-DNA insert of the different plasmid molecules.

DISCUSSION

The structural transition of right-handed B-DNA to left-handed Z-DNA is energetically favored in a negatively supercoiled circular double helix (2–5). This has been shown through the use of specific anti-Z-DNA antibodies that bind to the Z-DNA in the plasmids and has been visualized by two-dimensional gel electrophoresis in which one dimension is carried out with an intercalating molecule that unwinds the plasmid and converts the Z-DNA to B-DNA, resulting in a change in mobility. These methods have been used to show that both the pBR322 plasmid carrying an insert of 32 bp (pLP32) of alternating dG-dC residues (3) and the pDHg16 derivative of pBR322 carrying a 24-bp alternating dG-dC insert (10) have the Z-DNA conformation at bacterial negative superhelical densities. Crosslinking experiments with the anti-Z-DNA antibody showed that the antibodies combine directly with the $(C\text{-}G)_n$ insert in supercoiled pLP32 (4).

The present experiments show that negatively supercoiled plasmids at typical *in vivo* bacterial negative superhelical densities have a deficit of methylation relative to the relaxed plasmid and that this deficit decreases steadily on incubation with *Hha* I methyltransferase. The methylation deficit is even greater with the more highly negatively supercoiled plasmid. These results have been interpreted to indicate that Z-DNA is not a substrate for this enzyme. It is clear that the rate of cleavage by *Hha* I is sharply decreased in the C-G insert when it is in the Z conformation. However, the dependence of the cleavage rate on the level of negative supercoiling could not be determined in these experiments.

The experiments with the cytosine sequencing reaction show an interesting uniformity in the level of undermethyla-

tion, which spans the entire insert without any accumulation of undermethylation at any point. This suggests that there is an equilibrium at all times between the B- and Z-DNA forms and that the position of that equilibrium is influenced by the level of negative supercoiling. Higher levels of negative supercoiling appear to favor a higher level of Z-DNA and this is reflected in a lower level of methylation. However, the uniform distribution of unmethylated sites throughout the insert suggest that the conversion from Z-DNA to B-DNA (and vice versa) is one that occurs uniformly through the entire segment without favoring an accumulation of one structural form or the other at one point of the insert. This is consistent with observations about the cooperative nature of the transition from B-DNA to Z-DNA or vice versa. Cooperativity implies that the entire segment converts within a very short time period from one form to the other.

Behe and Felsenfeld (17) have shown that the equilibrium between B-DNA and Z-DNA in the linear molecule poly(dG-dC) is affected considerably by methylation of the cytosine residues. Poly(dG-dC) exists largely as B-DNA at physiological salt concentrations. However, poly(dG-m^5dC) exists almost entirely as Z-DNA at the same salt concentration (17). The methyl group thus strongly influences the equilibrium in favor of Z-DNA. The structural basis of this preference for Z-DNA was revealed in the crystal structure analysis of $(m^5dC\text{-}dG)_3$, in which the methyl group was found to fill a small hydrophobic depression on the surface of the molecule and thereby exclude water molecules from that region (18). The Z-DNA molecule thus appears to be stabilized by hydrophobic bonding on the surface that excludes the entry of destabilizing water molecules. More recently, Klysik *et al.* (19) have shown that the tendency for methylation to stabilize Z-DNA can also be demonstrated in a plasmid, since a lower negative superhelical density is required to stabilize a methylated insert of alternating dG-dC in the Z conformation than would be necessary if the insert were not methylated.

The results of the present investigation present an interesting paradox: as the *Hha* I methyltransferase begins to work on the plasmid and methylate the insert, the insert has a stronger tendency to convert to the Z-DNA conformation at bacterial negative superhelical densities. Favoring the Z-DNA conformation means that the methyltransferase is inhibited and is able to methylate less of the insert. Thus, the structural transformation induced by the methylation reaction acts in a negative way to decelerate the rate at which the reaction can proceed on other residues. This is a good example of negative feedback, which is often found as a component of a control system.

These experiments have been carried out with a prokaryotic methyltransferase and an endonuclease that act biologically to restrict the entrance of foreign DNA into the prokaryotic host cell. In eukaryotes, there exists another kind of methyltransferase that acts to put methyl groups on the same position 5 of cytosine in C-G sequences. In eukaryotes, methylation in that position has been associated with gene inactivation; that is, it inhibits transcription of neighboring genes (20). We do not know whether the characteristics of the prokaryotic methyltransferase are related in any way to those of the eukaryotic methyltransferase; however, the reactions have some elements in common because they both use the same *S*-adenosylmethionine substrate and they methylate the same site on the cytosine residue. The prokaryotic enzyme, like the eukaryotic enzyme, will fully methylate a C-G sequence which is hemimethylated on only one strand. It is possible that the eukaryotic enzymes may have similar properties and if so they will also be subject to the negative feedback characteristics found in controlling the extent of methylation that occurs in this prokaryotic system. However, it should be pointed out that the experiments reported here have as a substrate a long insert with alternating cytosine and guanine residues. Segments such as this do not occur in eukaryotic systems, although there are segments with long sequences of alternating purine and pyrimidine residues that can form Z-DNA. However, it remains to be seen whether similar considerations govern the eukaryotic methylases *in vivo*.

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