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Flipping of cloned $d(pCpG)_n \cdot d(pCpG)_n$ DNA sequences from right- to left-handed helical structure by salt, Co(III), or negative supercoiling

(DNA supercoiling/B- to Z-DNA transition/left-handed DNA)

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ABSTRACT **Negative supercoiling of plasmid DNAs containing 24–42 base pairs of alternating d(pCpG) inserts is shown to cause the flipping of the helical hand of the inserts from right to left under physiological conditions. For a negatively supercoiled DNA with a fixed linking number, this flipping reduces its superhelicity and, therefore, is accompanied by a shift of its electrophoretic mobility in agarose gel. Quantitation of the mobility shifts indicates that essentially the whole stretch of contiguous alternating d(pCpG) flips to the left-handed helical form when the negative superhelical density (specific linking difference) is ≥ 0.03.**

Interest in DNA structural polymorphism and its plausible biological significance has reached a new zenith with the establishment of the left-handed helical structures of several crystals of oligodeoxyribonucleotides of alternating d(pCpG) sequence (1, 2). In solution, it has been known since the work of Pohl and Jovin (3) that the conformation of $d(pCpG)_n \cdot d(pCpG)_n$ is drastically affected by salt concentration. The inversion of the circular dichroism spectrum of this polymer by the addition of molar amounts of salt has led the authors to suggest the possibility of a salt-induced transition of the polymer from the right-handed to the left-handed helical form. Recent Raman scattering studies (4) comparing the spectra of the low-salt and high-salt solution forms with the spectrum of a crystal of an oligomer of alternating d(pCpG) in its left-handed Z structure has confirmed the earlier suggestion. NMR data also have lent support to this notion (5). Spectroscopic measurements with DNA restriction fragments containing alternating d(pCpG) sequences have demonstrated further that the salt-induced transition can also occur when the alternating d(pCpG) sequence is bounded by right-handed helical regions (6). Intact plasmid DNAs containing this sequence show a large reduction in electrophoretic mobility, relative to that of the same DNAs in the nicked form, when the low-salt electrophoresis buffer is replaced by one containing several molar salts (6). This large reduction in mobility is consistent with the expected reduction in negative superhelicity when the $d(pCpG)_n$ sequence flips from the low-salt right-handed form to the high-salt left-handed form.

In this communication, sedimentation and gel electrophoresis analyses of plasmid DNAs containing alternating d(pCpG) inserts of different lengths are reported. In agreement with previous workers, it is shown that the sequence juxtaposed to right-handed DNA on both sides undergoes a salt-induced flipping of its helical sense. In addition, the sharp dependence of this transition on the superhelicity of the DNA is demonstrated. For alternating d(pCpG) inserts ranging from 24 to 42 base pairs, the left-handed helical conformation is the stable one under physiological conditions at a negative superhelicity significantly lower than that of a typical DNA from natural sources.

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MATERIALS AND METHODS

Plasmid Construction. The octamer d(CpGpCpGpCpGpCpG) [$d(CpG)_4$] first was phosphorylated by using T4 polynucleotide kinase and then was ligated with T4 ligase into multimers. The reaction mixture was fractionated by electrophoresis on a polyacrylamide gel, and individual bands corresponding to multiples of the self-complementary octamer were isolated. These multimers were then ligated into the filled-in *Bam*HI site of pBR322. Transformation of *Escherichia coli* cells yielded clones which were screened for inserts by electrophoresis of the *Hae* III restriction enzyme digest of plasmid DNA. Plasmids pLP24 and pLP32 were constructed in this way. Plasmids pLP14 and pLP42 were obtained by a "bootstrap" procedure. The *Hae* III fragment of plasmid pLP32 containing the alternating d(pCpG) insert was first isolated. This DNA was partially digested with restriction endonuclease *Hha* I to cleave within the alternating d(pCpG) sequence. Commercially available $d(pCpG)_n \cdot d(pCpG)_n$ also was digested partially with *Hha* I. These DNAs were then ligated together and, after inactivation of ligase by heating at 65°C, digested with *Bam*HI. Ligation into *Bam*HI-digested pBR322 was followed by transformation and subsequent restriction digest screening.

Nucleotide sequence determination of the appropriate insert containing restriction fragments was done by the procedure of Maxam and Gilbert (7).

Topoisomerase I Treatment and the Preparation of DNA Samples of Different Linking Numbers. Calf thymus topoisomerase I was the preparation of K. Javaherian. Relaxation of a plasmid DNA was carried out by treatment with the topoisomerase at 25°C in 10 mM Tris·HCl, pH 8/0.1 mM EDTA/0.2 M NaCl. Various amounts of ethidium bromide were present during the relaxation to give DNA samples of different linking numbers. After overnight incubation, samples were extracted twice with phenol and extensively dialyzed. Ethidium bromide was effectively removed during the phenol extraction step. Agarose gel electrophoresis in the presence of various amounts of ethidium bromide (8) was used to determine the center of the thermal distribution of topoisomers (9). The linking difference $\alpha - \alpha^0$ of each DNA sample, where α is the average linking number of the sample and α^0 is that of the same DNA when relaxed (10), was determined from the separation between the center of the thermal distribution of topoisomers of the sample and that of the sample relaxed in the absence of ethidium bromide by using the band counting method of Keller (8). A set of pBR322 DNA standards with overlapping distributions of to-

poisomers were used in the band counting. It should be pointed out that the linking difference so determined refers to that under the topoisomerase treatment conditions.

Sedimentation. All sedimentation rates were measured at 25°C and 25,481 rpm (Spinco AnG rotor) in double-sector cells. Absorption scans at 265 nm were taken every 16 min.

RESULTS

A Salt-Induced Conformational Change of Plasmid pLP32 DNA That Contains a $d(pCpG)_{16}$·$d(pCpG)_{16}$ Insert. Restriction mapping of purified pLP32 DNA and sequence determination of the region containing the insert showed that a 36-base-pair sequence $(pCpG)_{16}$pGpApTpC had been cloned into the original plasmid: $(pCpG)_{16}$ presumably came from the insertion of four copies of the phosphorylated $d(pCpG)_4$ octamer, and pGpApTpC, from the repaired *Bam*HI site.

To test whether high concentrations of salt can induce a transition of the alternating d(pCpG) insert from the right-handed helical form to the left-handed one, as one might expect from previous studies with $d(pCpG)_n$·$d(pCpG)_n$ (3) and from the recent work with restriction fragments containing alternating d(pCpG) sequences (6), the sedimentation coefficient of a covalently closed pLP32 DNA sample was measured as a function of salt concentration. If a salt-induced flip of the helical hand of the $d(pCpG)_{16}$ insert occurs, a corresponding change in the superhelicity of the DNA and, hence, its sedimentation rate should result.

Fig. 1 shows the dependence of the sedimentation rate on superhelicity for the control pBR322 DNA. The relative sedimentation coefficient is expressed as the ratio of the sedimentation coefficient of the covalently closed form to that of the linear form, and the superhelicity is expressed as the linking difference $\alpha - \alpha^0$, where α is the linking number and α^0 is the same quantity when the DNA is relaxed. It should be pointed out that, although α is a topological invariant, the linking number α^0 of a DNA when fully relaxed is clearly dependent on temperature, salt concentration, etc. For convenience, values of α^0 will be taken as those under the conditions of topoisomerase treatment (in 0.2 M NaCl/10 mM Tris·HCl, pH 8/0.1 mM EDTA at 25°C). Sedimentation runs carried out in 2.55 and 3.5 M salt gave virtually superimposable data, and a single curve is drawn in Fig. 1.

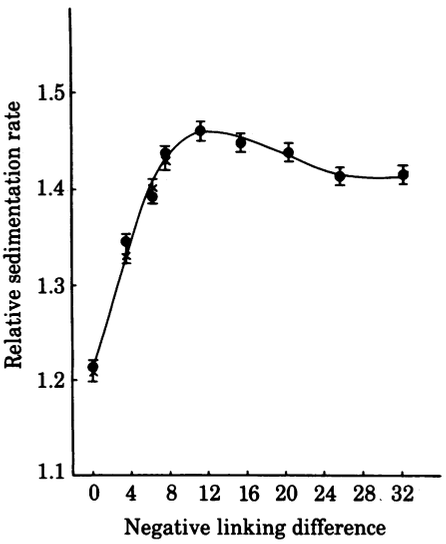

FIG. 1. The relative sedimentation rate of pBR322 as a function of linking difference. The sedimentation rate is relative to that of linear pBR322, and the linking difference refers to values at 25°C in 0.2 M Na^+. ●, Sedimentation in 2.55 M NaCl; +, sedimentation in 3.5 M NaCl. Data points are an average of at least 2 and as many as 11 separate determinations.

The $d(pCpG)_{16}$-containing pLP32 DNA and its pBR322 control were relaxed in the presence of calculated amounts of ethidium bromide with DNA topoisomerase I from calf thymus. After enzyme inactivation and ethidium bromide removal, the Gaussian center of the Boltzmann distribution of topoisomers for both plasmids was measured by agarose gel electrophoresis (9) to have a linking difference of -6.3 ± 0.3 (as before, α^0 refers to 25°C and 0.2 M NaCl). From Fig. 1, it is clear that this degree of superhelicity lies in a region where the sedimentation coefficient is sensitive to supercoiling.

In Fig. 2, the ratio of the sedimentation coefficients of pBR322 and pLP32 is depicted as a function of the salt concentration of the sedimentation medium. Over this salt range, the relative sedimentation coefficient of pBR322 DNA itself was little affected; the 14% increase in the ratio of the sedimentation coefficient of pBR322 to that of pLP32 when the salt concentration was increased from 2 to 3.5 M reflects a 14% decrease of the sedimentation coefficient of pLP32 DNA over this range of salt concentration.

The salt-induced transition is most likely due to the flipping of the alternating d(pCpG) insert from a right-handed helical form to a left-handed helical form. In 90 mM Tris borate, pH 8.3/2.5 mM Na_3EDTA, band-shift measurements (11, 12) with slightly supercoiled DNA samples containing the $d(pCpG)_n$ inserts are consistent with a right-handed 10.5-fold helical structure for the alternating d(pCpG) inserts (data not shown). When a 10.5-fold right-handed helical segment of 32 base pairs is changed to a 12-fold left-handed Z structure (1), the total decrease in helical twist is 5.7 turns. Thus, by assuming that the boundaries between right- and left-handed regions are fairly short, α^0 for pLP32 is expected to decrease by about 5.7 turns if the $d(pCpG)_n$ insert is in the left-handed helical form. The 14% decrease in the relative sedimentation coefficient of the pLP32 sample used in the analysis corresponds to a drop of its sedimentation coefficient from 1.40 to 1.23 times that of the linear form. Because the size of pLP32 is essentially the same as that of pBR322, this magnitude of change in the sedimentation rate corresponds to a change of linking difference of about 5.6 (See Fig. 1). This is in good agreement with the expected change discussed above.

An alternative interpretation of the salt-induced transition is the formation of a cruciform structure from the palindromic sequence containing the alternating d(pCpG) region. The total length of the palindromic sequence in pLP32 is 42 base pairs [$d(pCpG)_{32}$ insert with 5 base pairs on either side]. The expected change in linking difference for cruciform formation is

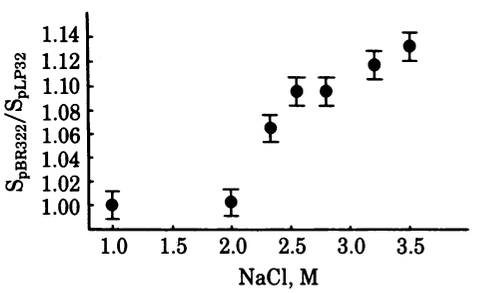

FIG. 2. The dependence of the relative sedimentation rate of pLP32 on NaCl concentration. The ratio of the sedimentation coefficients of pBR322 and pLP32 was shown as a function of NaCl concentration in the sedimentation medium. The linking difference of both samples at 25°C and in 0.2 M Na^+ was measured to be -6.3 ± 0.3. Samples were incubated at the stated salt concentration for at least 1 day prior to centrifugation.

about 42/10.5 or 4. The measured change of linking difference of 5.6 is substantially larger. Additional reasons against the cruciform interpretation will be discussed later.

The Rate and Reversibility of the Salt-Induced Conformational Change in the Cloned Alternating d(pCpG) Sequence. The data in Fig. 2 were obtained with samples that had been incubated at the stated salt concentration for at least 24 hr prior to centrifugation. This long incubation was necessary because of the slow rate of transition. At 2.55 M salt, for example, the half-time was measured to be of the order of half a day at 25°C for the particular pLP32 DNA sample. Preliminary results indicate that the rate of the salt-induced transition is accelerated by higher temperature, higher salt concentration, and increasing negative superhelicity.

The reversibility of the salt-induced transition was tested with the sample of pLP32 DNA, and the control pBR322 DNA equilibrated at 2.55 M salt. The ratio of the sedimentation coefficients of this pair of DNAs is about 1.10 (Fig. 2) at this salt concentration. The ratio becomes 1.00 upon diluting both DNAs to a final concentration of 2.0 M. This indicates that the salt-induced transition can be readily reversed.

$Co(NH_3)_6^{3+}$ Induction of the Flipping of the Helical Structure of Alternating d(pCpG). As shown by Behe and Felsenfeld (13), the left-handed helical form of $d(pCpG)_n \cdot d(pCpG)_n$ was stabilized by the presence of the trivalent ion $Co(NH_3)_6^{3+}$. For DNA containing internal oligomeric alternating d(pCpG) inserts, the addition of $Co(NH_3)_6^{3+}$ also induced a flip of the helical sense of the sequence (Fig. 3).

Fig. 3 is a composite of sections of three agarose gels in which the DNA samples had been electrophoresed in different buffer systems. The electrophoresis buffer for the gel in Fig. 3 *Top* was 90 mM Tris borate, pH 8.3/2.5 mM Na_3EDTA. The other gels contained 450 nM chloroquine (Fig. 3 *Middle*) and 100 μM $Co(NH_3)_6^{3+}$ (Fig. 3 *Bottom*) in addition to the Tris borate/EDTA buffer. Lanes b, d, and f contained a pBR322 DNA sample with a linking difference of -11.3 ± 0.3 (with respect to the same DNA relaxed in 0.2 M NaCl at 25°C). This DNA served as a reference for samples run in adjacent lanes: pLP14 {d[$(pCpG)_7$pG$(pCpG)_2$]} in lane a, pLP24 {d[$(pCpG)_{12}$pG-$(pCpG)_3$pG$(pCpG)_3$]} in lane c, pLP32 {d[$(pCpG)_{16}$]} in lane e, and pLP42 {d[$(pCpG)_{21}$pG$(pCpG)_2$]} in lane g. The sequence indicated in the braces after each of the cloned DNAs is the sequence inserted at the DNA polymerase-repaired *Bam*HI site of pBR322. All DNAs of the pLP series shown in the figure were prepared in such a way that their superhelical densities or specific linking differences $[(\alpha - \alpha^0)/\alpha^0]$ were about the same.

The effect of $Co(NH_3)_6^{3+}$ addition can be seen by comparing, for example, the pLP32 sample (Fig. 3, lane e) and the reference pBR322 DNA (Fig. 3, lane f). The gel in Fig. 3 *Middle* was run in the presence of chloroquine so that the magnitudes of the specific linking differences of the samples were reduced to permit the resolution of the individual covalently closed topoisomers (14). Clearly the pLP32 sample in the chloroquine gel is more negatively supercoiled than the pBR322 reference. However, in Fig. 3 *Bottom*, the same pair of samples run in the gel containing $Co(NH_3)_6^{3+}$ shows an inversion of the relative degrees of negative supercoiling. Measurements from microdensitometer tracings show that, as a result of the addition of $Co(NH_3)_6^{3+}$, the ladder of pLP32 topoisomers is displaced upward by 5.5 ± 0.3 bands, relative to the ladder of the control pBR322 topoisomers.

By making the assumption that the electrophoretic mobility of a supercoiled DNA is dependent on its average writhe, *Wr*, but not on its twist number, *Tw*, it can be readily shown that the upward shift of the pLP32 topoisomers relative to the control pBR322 topoisomers represents an extra reduction in *Tw* by 5.5 turns for pLP32 when $Co(NH_3)_6^{3+}$ was added. [This follows from the relation $\alpha = Tw + Wr$ (15, 16) and the fact that the linking numbers of the samples were unchanged.] As was the case with the salt-induced conformational change described earlier, this change in the helix twist is most likely a result of the flipping of the alternating d(pCpG) segment to a left-handed form by the addition of $Co(NH_3)_6^{3+}$.

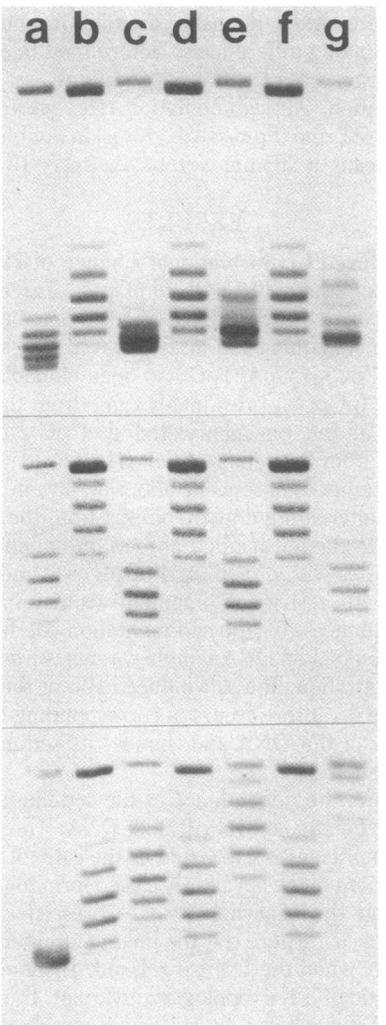

FIG. 3. Agarose gel electrophoretic patterns of plasmids containing alternating d(pCpG) inserts. The same DNA sample was electrophoresed through a given lane on three gels in electrophoresis buffer only (*Top*) or in that buffer containing 450 nM chloroquine (*Middle*) or 100 μM Co(III) hexamine (*Bottom*). Except for these additions, the electrophoresis conditions of the three gels were identical. pBR322 with a linking difference of -11.3 (lanes b, d, and f) is shown between plasmids containing $d(pCpG)_n$ inserts: pLP14 (lane a), pLP24 (lane c), pLP32 (lane e), and pLP42 (lane g).

Similar measurements show that the extra reduction in *Tw* by $Co(NH_3)_6^{3+}$ was 7.3 ± 0.3 for pLP42 and 4.1 ± 0.3 for pLP24. The topoisomer bands of pLP14 were insufficiently resolved in the $Co(NH_3)_6^{3+}$-containing gel to permit a reliable measurement of the change in *Tw*.

Flipping of the Helical Structure of Alternating d(pCpG) Under Physiological Conditions in a Negatively Supercoiled DNA. Because the flipping of a right-handed helical segment to a left-handed one reduces the superhelicity of a negatively supercoiled DNA, it follows immediately from the known thermodynamics of DNA supercoiling (10, 17, 18) that such a process should be favored in a negatively supercoiled DNA.

Initial experimental evidence confirming the above theoretical expectation came from an experiment in which samples of pLP32 and pBR322 were relaxed by calf thymus DNA topoiso-

merase I in a medium that was essentially identical to the $Co(NH_3)_6^{3+}$-containing electrophoresis buffer used for the gel in Fig. 3 *Bottom*. The enzyme was active in the medium, and the DNA samples became fully relaxed. When the relaxed samples were examined in a chloroquine gel without the trivalent ion, however, the topoisomer ladders of the two DNAs turned out to be not very different. If the presence of $Co(NH_3)_6^{3+}$ flips the alternating d(pCpG) segment in pLP32 to the left-handed form when the DNA is free from topological constraint due to the presence of the topoisomerase, and the same segment subsequently flips back to the right-handed form in the absence of the topoisomerase upon removal of the trivalent ion, the DNA should become more negatively supercoiled relative to the pBR322 control. In the presence of chloroquine during gel electrophoresis, then, the pLP32 sample would be expected to be less positively supercoiled than the pBR322 control. Because this was not observed, it follows that even in the presence of $Co(NH_3)_6^{3+}$, the alternating d(pCpG) segment in pLP32 is not in the left-handed helical form when the DNA is relaxed. Therefore, the flipping of this segment to the left-handed helical structure seen for pLP32 (Fig. 3 *Bottom*, lane e) is a consequence of *both* the presence of the trivalent ions and the fact that the DNA is negatively supercoiled.

The superhelicity effect on the helical geometry of the cloned alternating d(pCpG) segment also could be seen directly in the gel (Fig. 3 *Top*) that contained no trivalent ion. Although the pBR322 control (lane f) showed the normal Gaussian distribution of topoisomers, the pLP32 sample (lane e) showed an abnormal distribution. The abnormality can be attributed to the strong coupling between the flipping of the $d(pCpG)_n$ segment and the linking number of a particular topoisomer.

Imagine a normal Gaussian distribution of topoisomers of a negatively supercoiled DNA containing an alternating d(pCpG) insert so that the insert is in the right-handed helical form. For those topoisomers in the distribution that have lower linking numbers (and, hence, are more negatively supercoiled), the free energy of supercoiling may be sufficient to effect a flipping of a part of the alternating d(pCpG) sequence into the left-handed helical form; this flipping lowers their negative superhelicity and their free energies and also reduces their mobilities in the gel. This results in the piling up of topoisomers at the position of the intense dark band (Fig. 3 *Top*, lane e). In other words, the dark band represents the species with a critical linking difference at which right- and left-handed helical forms of alternating d(pCpG) are in equilibrium. A topoisomer with a large negative linking difference will convert a sufficient number of base pairs of the alternating d(pCpG) sequence to the left-handed form so that the magnitude of its linking difference is reduced to this critical value. By comparing the position of the dark band to the positions of the topoisomers of the pBR322 control of known linking differences, the critical specific linking difference was estimated to be around -0.03.

If the DNA is sufficiently negatively supercoiled so that essentially the whole alternating d(pCpG) segment is flipped to the left-handed form, a Gaussian distribution of the topoisomers should again be observed. Fig. 4 shows that this is the case; samples run in the gels in Fig. 4 corresponded to the ones run in Fig. 3 but were made to have higher negative superhelicities than the samples in Fig. 3. The pBR322 control in Fig. 4 (lanes b, d, and f) was more negatively supercoiled, by 4.2 turns, than the pBR322 control in Fig. 3. Note that pLP32 DNA (lane e), which was more negatively supercoiled than the pBR322 control (lane f) when the superhelicities of the DNAs were reduced by the addition of chloroquine (Fig. 4 *Middle*), became the less negatively supercoiled relative to the pBR322 control when the negative superhelicities of the pair were increased by the omission of chloroquine (Fig. 4 *Top*). The extra reduction in *Tw* for pLP32 in Fig. 4 *Top* relative to the same DNA in Fig. 4 *Middle* was 5.5 bands, which was identical to that observed with the addition of $Co(NH_3)_6^{3+}$ (Fig. 4, compare *Top* and *Bottom*; an identical result also was obtained with the less negatively supercoiled DNA samples used in Fig. 3, as described earlier).

Flipping of the Helical Sense of Noncontiguous Alternating $d(pCpG)_n$ Sequences. Among the clones containing $d(pCpG)_n$ inserts whose sequences have been determined, pLP32 is the only one that contains one contiguous stretch of alternating d(pCpG). The insert in pLP24, for example, is $d[(pCpG)_{12}pG(pCpG)_3pG(pCpG)_3]$. It is of interest to examine whether small stretches of alternating d(pCpG) separated by a single base pair can flip together to the left-handed helical form. As described earlier from data shown in Fig. 3, the extra change in *Tw* upon addition of $Co(NH_3)_6^{3+}$ was 5.5 ± 0.3 for pLP32 and 4.1 ± 0.3 for pLP24. Because the total length of $d[(pCpG)_{12}pG(pCpG)_3pG(pCpG)_3]$ is 6 base pairs longer than $d(pCpG)_{16}$, the smaller change observed for pLP24 suggests that only the longest stretch of alternating d(pCpG) was undergoing $Co(NH_3)_6^{3+}$-induced transition for the sample shown in Fig. 3. The expected reduction in *Tw* when $d(pCpG)_{12}$ changes from a 10.5-fold right-handed helix to a 12-fold left-handed helix is 4.3, in good agreement with the experimental

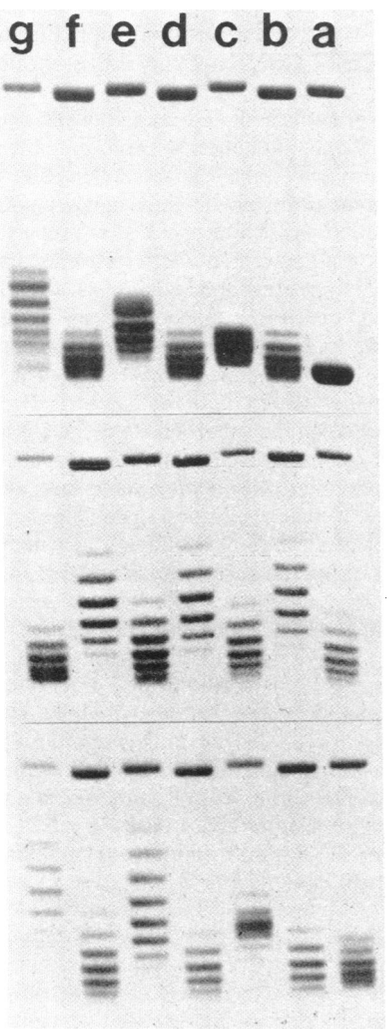

FIG. 4. Agarose gel electrophoretic patterns of plasmids containing alternating d(pCpG) inserts. The electrophoresis conditions of the three gels are identical to those shown in Fig. 3. The DNA samples are identical to those shown in Fig. 3 except that all of the plasmids shown here are more negatively supercoiled. The pBR322 DNAs in lanes b, d, and f have a linking difference of -15.5.

observation of 4.1 ± 0.3. Similarly, for the pLP14 sample (Fig. 4, lane a), in the presence of $Co(NH_3)_6^{3+}$, an extra change of about 2.5 in *Tw* suggests that the 14-base-pair stretch of $d(pCpG)_7$ may have flipped to the left-handed helical form.

The more negatively supercoiled pLP24 sample in Fig. 4 *Bottom*, lane c, however, showed an abnormal topoisomer distribution. Because essentially the whole $d(pCpG)_{12}$ segment for the sample used in Fig. 3 was presumably in the left-handed helical form, the abnormal pattern of the sample shown in Fig. 4 suggests that the increment in negative superhelicity may drive the adjacent noncontiguous d(pCpG) stretches into the left-handed helical structure as well.

DISCUSSION

The sedimentation and gel electrophoresis results demonstrate that the alternating d(pCpG) sequence undergoes a salt-, $Co(NH_3)_6^{3+}$-, or DNA-negative-superhelicity-induced transition when this sequence is bounded by nonrepetitive DNA. The magnitudes of the changes in superhelicities accompanying the transition for clones containing alternating d(pCpG) inserts of different lengths indicate that, under appropriate conditions, the transition corresponds to the flipping of the alternating d(pCpG) inserts from a right-handed 10.5-fold helix to a left-handed structure, presumably the 12-fold Z-helix.

In the calculation of the magnitude of the change in helical twists for the salt-, $Co(NH_3)_6^{3+}$-, or DNA-negative-superhelicity-induced transition with pLP32, which contains the $d(pCpG)_{16}$ insert, it is assumed that all of the alternating C·G base pairs flip from one helical sense to the other. Therefore, the agreement between the calculated value and the observed change indicates that essentially the whole segment flips under appropriate conditions. Klysik *et al.* (6) have reported that based on spectroscopic measurements with restriction fragments containing internal alternating d(pCpG) inserts, about five d(pCpG) repeating units at each B/Z junction are not in the Z structure. This discrepancy points to the necessity of considering this transition from a thermodynamic point of view. The final state is expected to be dependent on the salt and trivalent ion concentrations, as well as on the superhelicity of the DNA if it is in the covalently closed form. It appears from the abnormal distributions of topoisomers observed in some cases (Fig. 3 *Upper*, lanes c, e, and g) that only a part of the alternating d(pCpG) sequence flips when there is insufficient driving force.

The results presented here demonstrate that the left-handed helical structure can be the stable form for an internal alternating d(pCpG) sequence under physiological conditions, provided that the DNA is sufficiently negatively supercoiled. For inserts containing 12 or more d(pCpG) units, the critical negative specific linking difference above which the left-handed form dominates is ≈0.03. This value is significantly lower than a negative specific linking difference of ≈0.07 for a typical covalently closed DNA from natural sources or the negative specific linking difference of about 0.05 for intracellular *E. coli* DNA, estimated (18) from the *in vivo* trimethylpsoralen photobinding data of Sinden *et al.* (20). For the shortest $d(pCpG)_n$ insert used, $d(pCpG)_7$, in the Tris borate/EDTA buffer, there is no indication of a transition of the helical sense at a negative specific linking difference of 0.03. With the addition of 100 μM of $Co(NH_3)_6^{3+}$, however, a change of the helical structure appears to occur at a slightly higher negative superhelicity. It is most likely that at a sufficiently high negative superhelicity, even for short alternating d(pCpG) sequences, the transition of the helical sense may occur in the absence of any trivalent ion.

Formation of a cruciform structure by the intrastrand pairing of the self-complementary $d(pCpG)_n$ sequence could also be driven by negative superhelicity (19, 21–26). As previously discussed, the possibility that the observed conformational transition involves formation of a cruciform is unlikely because the magnitudes of the observed changes in *Tw* are significantly larger than would be expected for cruciform formation; the observed salt and $Co(NH_3)_6^{3+}$ dependence of the transition is also in complete accord with the B-to-Z structure change but is difficult to explain on the basis of cruciform formation. Further confirmation of the B-to-Z interpretation comes from studies with rabbit antibodies that can recognize the Z structure. Preferential binding of the antibodies to plasmid DNAs containing alternating d(pCpG) inserts is observed only under the conditions described in this report with the inserts in the left-handed helical form. Details of antibody binding studies will be published elsewhere.

The negative-supercoiling-induced transition of alternating d(pCpG) sequences from the right-handed to the left-handed helical form represents one of the most dramatic effects of DNA supercoiling on its structure. It remains to be explored whether transitions of this nature or subtler structural changes that are effected by superhelicity may play a role in the regulation of gene expression.

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