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# Anti-Z-DNA antibody binding can stabilize Z-DNA in relaxed and linear plasmids under physiological conditions

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**It is shown that anti-Z-DNA antibody binding can stabilize sequences of $d(CG/GC)_n$ and $d(CA/GT)_n$ in the Z-DNA conformation in a plasmid in the complete absence of supercoiling. This effect is quantitated by using antibody preparations of different affinities and varying concentrations. The $d(CG/GC)_n$ sequence can be stabilized under physiological conditions. This is the first demonstration that a region of Z-DNA can be stabilized by protein binding in a completely relaxed plasmid under physiological conditions. The antibody-Z-DNA complex in the relaxed plasmid is shown to be an equilibrium state and not a long-lived kinetic intermediate since specific binding of the antibody to linearized plasmids containing Z-forming sequences is observed.**

*Key words:* Z-DNA/anti-Z-DNA antibodies/protein-nucleic acid interaction/DNA conformation

## Introduction

Under physiological conditions, right-handed B-DNA is the predominant conformation of double-stranded DNA although it is in equilibrium with the left-handed Z-DNA form (Wang *et al.*, 1979). The latter makes only a small contribution to the equilibrium state of relaxed, unmodified double-stranded DNA. The factors that stabilize Z-DNA (reviewed in Rich *et al.*, 1984) include cations and anions, dehydrating solvents, numerous covalent modifications of DNA as well as negative supercoiling (Singleton *et al.*, 1982; Peck *et al.*, 1982; Nordheim *et al.*, 1982; Peck and Wang, 1983; Haniford and Pulleyblank, 1983a, 1983b). The B to Z transition driven by negative supercoiling has received the most attention since it is likely that negative supercoiling plays a role in stabilizing Z-DNA *in vivo*. Negative supercoiling is the most potent factor since small regions of Z-DNA buried in a predominantly B-DNA molecule can be effectively stabilized by negative supercoiling. These internal Z-DNA segments are bordered by B-Z junctions, the formtion of which requires +5 kcal/(mol of junction) (Peck and Wang, 1983). Z-DNA *in vivo* is likely to be found in such a form, thus it is particularly valuable to study mechanisms which can effectively stabilize these regions.

We might expect that the free energy of association of a specific Z-DNA binding protein would also contribute to the stabilization of Z-DNA. Perturbing effects of anti-Z-DNA antibodies on the salt-induced B-Z transition of poly(dG-dC).poly(dG-dC) have been observed (Malfoy and Leng, 1981; Jovin *et al.*, 1983). Revet *et al.* (1984) observed that if anti-Z-DNA antibodies were bound to supercoiled plasmids in very low ionic strength, and the plasmids were then cut with a restriction enzyme, some antibodies could be visualized on the linear plasmids in the electron microscope, suggesting that Z-DNA had been stabilized in the plasmid through its interaction with the anti-Z-DNA antibody. The stabilization of Z-DNA by Z-DNA-specific protein binding has not been extensively examined, although it is likely that Z-DNA binding proteins stabilize Z-DNA *in vivo*. We have been characterizing the effect of such protein binding on the B-Z equilibrium using anti-Z-DNA antibodies of differing relative affinities.

Anti-Z-DNA antibodies provide a good model system for examining specific Z-DNA-protein interactions because large quantitites of well characterized proteins are available (Lafer *et al.*, 1981, 1983). In another study (Lafer *et al.*, in preparation) we showed that anti-Z-DNA antibodies raised against poly(dG-dC).poly(dG-dC) in the Z-conformation could measurably shift the B-Z equilibrium towards Z-DNA in poly(dG-dC).poly(dG-dC) under very low as well as very high ionic strengths. We also showed that these antibodies could shift the B-Z equilibrium in a supercoiled plasmid so that the B to Z transition occurred at a lower superhelical density in the plasmid than that required to drive the transition in the absence of antibody binding. The magnitude of the perturbation in the equilibrium was dependent on the antibody concentration. For example, for a higher affinity antibody (G1eDE1), it was found that the antibody could be used as a non-perturbing probe for Z-DNA at $<5$ nM, but higher concentrations of antibody resulted in progressively larger perturbations in the equilibrium. Here we extend this study by demonstrating that antibody binding alone is sufficient to stabilize sequences of $d(CG/GC)_n$ and $d(CA/GT)_n$ in the Z-DNA conformation in a plasmid in the complete absence of negative supercoiling. This effect is quantitated using two antibody preparations of differing affinities and varying concentrations. It is shown that the $d(CG/GC)_n$ sequence can be stabilized in the Z form under physiological conditions, making this the first observation of a region of Z-DNA stabilized by protein binding in a plasmid in the absence of supercoiling and under physiological conditions. It is further shown that this antibody-Z-DNA complex is not simply a long-lived kinetic intermediate, but is an equilibrium state which can be reached from two directions: the antibody can be bound to a supercoiled plasmid in which Z-DNA is already present and the plasmid relaxed to completion without loss of Z-DNA or antibody binding. Alternatively, the antibody can be incubated with linearized plasmids containing sequences that can form Z-DNA, resulting in the specific association of the antibody with those Z-forming sequences.

## Results

### *Stabilization of Z-DNA by antibody binding to plasmids under physiological conditions*

If the binding of a protein to a double-stranded closed circular DNA molecule is associated with an unwinding of the DNA helix, the unwinding can be measured by relaxing DNA with a topoisomerase, removing the protein from the DNA, and then analysing the circular DNA molecule on a gel to determine the number of negative supercoils generated by the bound protein. Such an approach has been used to measure the amount of unwinding generated by the binding of *lac* repressor (Kim and Kim, 1983).

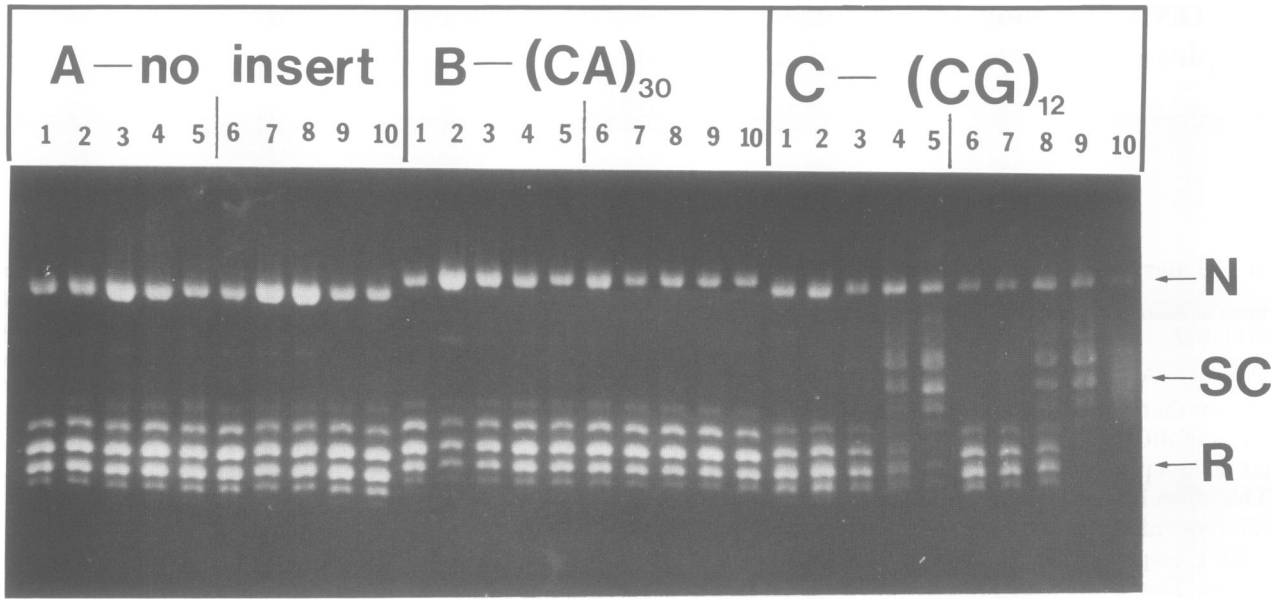


**Fig. 1.** Tritium-labeled plasmids at bacterial superhelical density were extensively relaxed with topoisomerase I in 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA in the presence of varying concentrations of anti-Z-DNA antibodies. **(A)** plasmid pDPL6 relaxed in the presence of G10cDE2 antibody **(lanes 1 – 5)** or G1eDE1 antibody **(lanes 6 – 10)**. The specific antibody concentration spans a 10 000-fold range: 0.3 nM **(lanes 1, 6)**, 3 nM **(lanes 2, 7)**, 30 nM **(lanes 3, 8)**, 300 nM **(lanes 4, 9)** and 3000 nM **(lanes 5, 10)**. **(B)** pDPL6 with an insert of $d(CA/GT)_{30}$ and **(C)** pDPL6 with an insert of $d(CG/GC)_{12}$. Antibody concentrations are the same as in **A**. The gel contains 1 μg/ml chloroquine so that the fully relaxed plasmid runs faster than the supercoiled material. Nicked (N), supercoiled (SC), relaxed (R); arrows indicate the center of each distribution.

Since the stabilization of one turn of Z-DNA results in a net unwinding of approximately two superhelical turns, we were able to use such an approach to demonstrate that the binding of antibody stabilized Z-DNA.

Three different plasmids were used in these studies. The host vector, pDPL6 (Haniford and Pulleyblank, 1983a, 1983b), is a 2.2-kb pBR322 derivative lacking the 14-bp Z-forming region of pBR322 (Nordheim *et al.*, 1982; Azorin *et al.*, 1983). The other plasmids contain inserts of alternating purine-pyrimidine sequence, one an inset of $d(CA/GT)_{30}$, the other an insert of $d(CG/GC)_{12}$. We have characterized the two antibody preparations used in these experiments (Lafer *et al.*, in preparation). The G10cDE2 preparation was found to be of lower affinity than the G1eDE1 preparation. This was determined by an analysis which compared the relative affinities of the two antibody preparations as well as by examining the extent of perturbation of the B-Z equilibrium induced by each preparation. Approximately an order of magnitude more of the G10cDE2 preparation was required to effect as large a shift in B-Z equilibrium as was produced by a given concentration of the G1eDE1 preparation.

As described in Materials and methods, plasmid DNA at bacterial superhelical density was incubated with either of the antibody preparations at an antibody concentration that spanned a 10 000-fold range from 0.3 nM to 3000 nM anti-Z-DNA specific antibody in 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA. Topoisomerase I was then added at a 10-fold excess and the reaction was allowed to proceed for 12 h at room temperature. This was followed by a second addition of topoisomerase I and a second 12-h incubation to insure complete relaxation. SDS and protease were then added to dissociate the antibody-DNA complex and the DNA was run on a gel containing 1 μg/ml chloroquine. The presence of chloroquine in the gel introduces positive supercoils in the DNA and resolves the fully relaxed species from those containing a number of supercoils. The results of this experiment are seen in Figure 1. In this gel the fully relaxed DNA (R) is running lower (more positively supercoiled) than the DNA that contained negative supercoils (SC). The plasmid lacking a Z-forming insert relaxes to completion at all antibody concentrations, as does the plasmid with the $d(CA/GT)_{30}$ insert. The plasmid with the $d(CG/GC)_{12}$ insert behaves differently: at high antibody concentrations we observe a bimodal distribution in the superhelical density of this plasmid. Part of this distribution corresponds to the completely relaxed form of the plasmid but a fraction of the plasmid remains supercoiled (SC).

The fraction of the plasmid that remains supercoiled increases with increasing antibody concentration and in Figure 1 lane 10C (highest concentration of the high affinity antibody) all of the DNA is supercoiled. The center of the topoisomer distribution of the supercoiled fraction is shifted by approximately five supercoils from the fully relaxed distribution. This corresponds to the entire 24-bp insert being maintained in the Z-form since 4.2 turns are unwound in the DNA due to formation of 24 bp of Z-DNA and 0.8 turns are unwound due to formation of two B-Z junctions (Peck and Wang, 1983). Further, it is evident that the high affinity antibody (lanes 6 – 10) is more effective at stabilizing the insert in the Z-form that the lower affinity preparation (lanes 1 – 5). This effect is quantitated in Figure 2. These plasmids had been labeled *in vivo* with tritium. The bands of DNA were cut from the gel and the percentage of plasmid in the relaxed and supercoiled distributions was accurately determined by measuring the amount of radioactivity in the gel fragment. To achieve the same amount of stabilization observed with a given concentration of high affinity G1eDE1 antibody required approximately an order of magnitude greater concentration of the G10cDE2 antibody.

*The sequence $d(CA/GT)_n$ can be stabilized in the Z-DNA conformation by antibody binding at lower ionic strength conditions*

The energetics of the B to Z transition as a function of ionic strength are complex. NaCl concentrations >1.5 M stabilize Z-

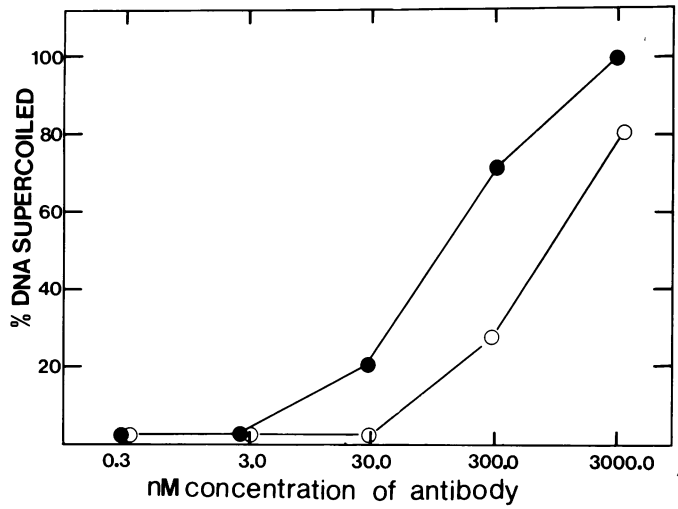


**Fig. 2.** Quantitation of the stabilization of the $d(CG/GC)_{12}$ sequence by anti-Z-DNA antibody binding. The amount of supercoiled plasmid in Figure 1C was quantitated by cutting out the sections of the gel containing the supercoiled or relaxed distribution of topoisomers and measuring the amount of radioactivity. The percentage of plasmid remaining supercoiled after extensive relaxation in the presence of increasing concentrations of G1eDE1 (solid circles) or G10cDE2 (open circles) is plotted. The molar concentration of plasmid was 35 nM.

DNA relative to B-DNA (Pohl and Jovin, 1972; Pohl, 1983). However, increasing ionic strength in the 0–300 mM range destabilizes Z-DNA (Azorin *et al.*, 1983; Peck and Wang, 1983). Further, since the B-Z junction may have some single-stranded character (Singleton *et al.*, 1983) and increasing ionic strength is known to stabilize double-stranded DNA, it might be expected that the B-Z junction would be more stable under lower ionic strength conditions. In addition, at lowered ionic strengths, electrostatic interactions between the antibody and the DNA will be enhanced. This would increase the relative affinity of the antibody and its ability to stabilize Z-DNA. For these reasons, even though no stabilization of the $d(CA/GT)_n$ insert in the Z-DNA conformation by antibody binding was seen in 140 mM NaCl, we might expect to observe stabilization in lower ionic strengths.

When the experiment described in Figure 1 was repeated with the $d(CA/GT)_{30}$ plasmid and the host vector in 50 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, it was found that the $d(CA/GT)_{30}$ sequence could be stabilized in the Z-DNA conformation (Figure 3). However, stabilization could be observed only at the highest concentrations (3 $\mu$M) of the high affinity antibody, G1eDE1. The low affinity antibody G10cDE2 failed to stabilize the $d(CA/GT)_{30}$ sequence in Z-DNA (data not shown). The host vector could be relaxed to completion in the presence of either antibody preparation. The stabilization effected by the G1eDE1 antibody was reproducible: 15–20% of the covalently closed circular molecules retained 6–7 supercoils after extensive treatment with topoisomerase I. Since complete transition of the $d(CA/GT)_{30}$ insert to Z-DNA results in a net unwinding of 11.4 turns, this represents only a partial stabilization of the insert in the Z-DNA conformation.

*The anti-Z-DNA antibody-stabilized Z-DNA region in a relaxed plasmid is not a long-lived kinetic intermediate but an equilibrium state; binding to linear DNA*

One interpretation of the experiments described is not that the binding of the anti-Z-DNA antibody stabilizes Z-DNA *per se*, but that the antibody-Z-DNA complex is a long-lived kinetic intermediate. That is, since the experiment began with a supercoiled plasmid which contained Z-DNA and was then relaxed in the presence of the antibody, it could be argued that the stabilization observed is due to the antibody having a very slow off rate. Given time the antibody might become unbound and release the Z-DNA from the plasmid. The on rate of the antibody onto spontaneously forming Z-DNA might then be insufficient to form Z-DNA as quickly as it is being lost. Effectively this would mean that the antibody would not have sufficient binding energy to stabilize Z-DNA. This is unlikely to be a valid interpretation for the following reasons. The antibody concentration required to achieve 50% stabilization of relaxed $d(CG/GC)_{12}$ plasmid was one order of magnitude greater than that required to achieve 50% binding of the plasmid in a nitrocellulose filter binding assay when it is supercoiled (Lafer *et al.*, in preparation). Therefore when the antibody concentration is insufficient to drive the formation of Z-DNA, the antibody is released and the plasmid relaxes to completion implying that a very slow off rate cannot be the explanation for stabilization. At the very high antibody concentrations used in these experiments the antibody binds maximally to Z-DNA in both the host vector and the $d(CA/GT)_{30}$ plasmid, yet these plasmids relax to completion, which argues that if the sequence to which the antibody is bound is too energetically disfavored to be maintained in the Z-DNA conformation, that sequence will revert to B-DNA as torsional strain is released. The treatment with topoisomerase used in these experiments was extensive: a 20-fold excess of topoisomerase was used over a 24-h incubation. If this treatment is continued with the addition of fresh topoisomerase over a further 24 h, we find that the supercoiled plasmid does not relax further, demonstrating that, at least over this time course, the antibody-Z-DNA complex is stable (data not shown).

To demonstrate rigorously that the Z-DNA stabilized by the anti-Z-DNA antibody represented an equilibrium state for sufficiently high concentrations of antibody it was necessary to show that this state could be approached from a direction which begins with a plasmid that is not supercoiled. The high concentration of G1eDE1 antibody (3 $\mu$M) was incubated with radiolabeled linearized $(CG)_{12}$ plasmid and the host vector in 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, and binding of the antibody was assayed by nitrocellulose filter binding. As the incubation progressed over a 72-h period at 37°C, specific association of the antibody with the $d(CG/GC)_{12}$ plasmid was observed (Figure 4, dashed lines). This binding was slow since, even after a 72-h incubation, only 20% binding to the $d(CG/GC)_{12}$ plasmid was observed. This was expected since the antibody has no catalytic activity; it can only bind to Z-DNA which forms spontaneously. In a linear plasmid Z-DNA is sufficiently disfavored so that only an extremely small number of plasmids contain Z-DNA at any moment.

Since Z-DNA forms more readily in low ionic strengths it would be expected that the binding of antibody to linear plasmids would occur more rapidly at lower ionic strengths. If we repeat the experiment described in 0 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, binding is 20- to 30-fold faster (Figure 4, solid lines) than in 140 mM NaCl, although it is still slow since only 20% binding is observed after ~3 h of incubation. However these experiments demonstrate that the rate of spontaneous formation of Z-DNA in the $(CG)_{12}$ sequence, as well as the on rate of the G1eDE1 antibody onto these transient Z-DNA regions is clearly greater than the rate at which these antibody-Z-DNA complexes dissociate. The antibody-Z-DNA complex which forms is therefore an equilibrium state but this equilibrium is approached slowly from this direction.

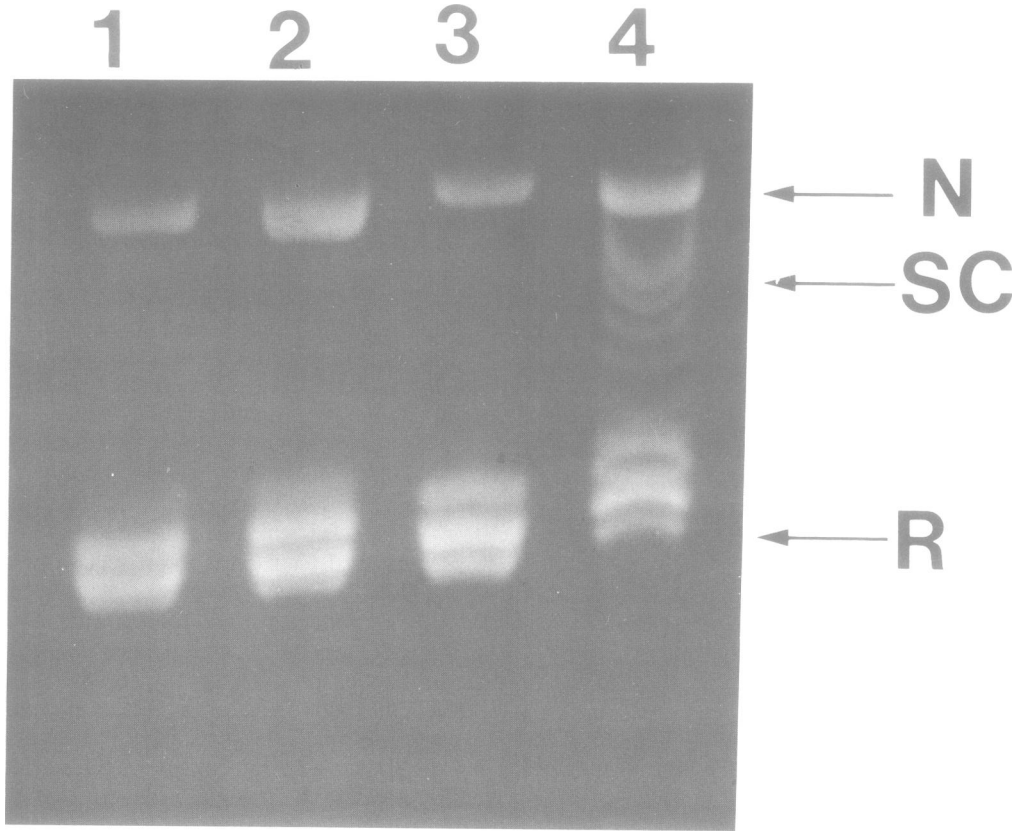


**Fig. 3.** Stabilization of the $d(CA/GT)_{30}$ sequence in the Z DNA conformation in a low ionic strength buffer. pDPL6 **(lanes 1, 2)** and pDPL6 containing a $d(CA/GT)_{30}$ insert **(lanes 3, 4)** at bacterial superhelical density were relaxed in the presence **(lanes 2, 4)** or absence **(lanes 1, 3)** of 3 μM G1eDE1 antibody in 50 mM NaCl, 10 mM Tris pH 7.5 1 mM EDTA. The gel contains 1 μg ml chloroquine so that the fully relaxed plasmid runs faster than the supercoiled species. Nicked (N), supercoiled (SC), relaxed (R); arrows indicate the center of each distribution.

We next asked if we could also observe binding and stabilization of Z-DNA by the antibody in a covalently closed, relaxed plasmid molecule. This addresses a different question from that asked by the experiment done with the linearized plasmids. The formation of Z-DNA in a relaxed covalently closed circular molecule generates positive supercoils; in the relaxed $(CG)_{12}$ plasmid, for insance, five positive supercoils would be generated if the entire insert undergoes the transition to Z-DNA. Thus there is an additional energetic barrier associated with the formation of Z-DNA in a relaxed but topologically constrained molecule, and this barrier may or may not abolish the stabilization of Z-DNA by the antibody. It depends upon whether or not the energy generated by the binding of the antibody molecules is sufficient to overcome the unfavorable free energy associated with both the formation of Z-DNA and these positive supercoils. Experiments were carried out to test this. High concentrations of the high affinity antibody preparation (G1eDE1) were incubated with the $(CG)_{12}$ plasmid at 37°C in 0 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, under conditions which were determined from the experiment with linear plasmids to be the most favorable for the formation of the Z-DNA-antibody complex. No filter binding of antibody to the relaxed plasmid was observed even after a 48-h incubation (data not shown) while the binding of the antibody to the linear plasmid under these conditions was measurable after a 1-h incubation (Figure 4).

Since the stabilization of Z-DNA by antibody binding in a relaxed plasmid leads to the formation of positive supercoils, stabilization might also be detected by treating a reaction mixture of relaxed plasmids containing $(CG)_{12}$ and anti-Z-DNA antibody with topoisomerase I. If stabilization had occurred negatively supercoiled plasmid molecules could be detected if the DNA topoisomers were resolved on an agarose gel after dissociation of the antibody by SDS-protease treatment. A reaction mixture of relaxed plasmid containing $(CG)_{12}$ and high concentrations of the high affinity antibody preparation (G1eDE1) was incubated in 10 mM Tris pH 7.5, 1 mM EDTA for 48 h at 37°C. This reaction was carried out in low salt conditions. The NaCl concentration was then brought to 50 mM and a 10-fold excess of topoisomerase I was added and the reaction was allowed to proceed for 12 h at 37°C. Agarose gel analysis showed that no negatively supercoiled plasmid was generated (data not shown). These experiments demonstrate that under these experimental conditions the G1eDE1 antibody does not bind and stabilize Z-DNA in the relaxed, covalently closed $(CG)_{12}$ containing plasmid while it does bind to the linearized $(CG)_{12}$-containing plasmid. We interpret this as indicating that the topological constraint imposed by the covalently closed, relaxed molecule, in terms of the positive supercoiling generated by Z-DNA formation, presents too great an energetic barrier. This argues that the binding of the anti-Z-DNA antibody to the linearized $(CG)_{12}$ plasmid involves the formation of a structure in the $(CG)_{12}$ insert that has a twist that differs from that of B-DNA. Since this binding is specific for the $(CG)_{12}$ insert this leads to the conclusion that this structure is Z-DNA.

## Discussion

In a study on the effects of antibody binding on the B-Z equilibrium in the synthetic polymer poly(dG-dC).poly(dG-dC),

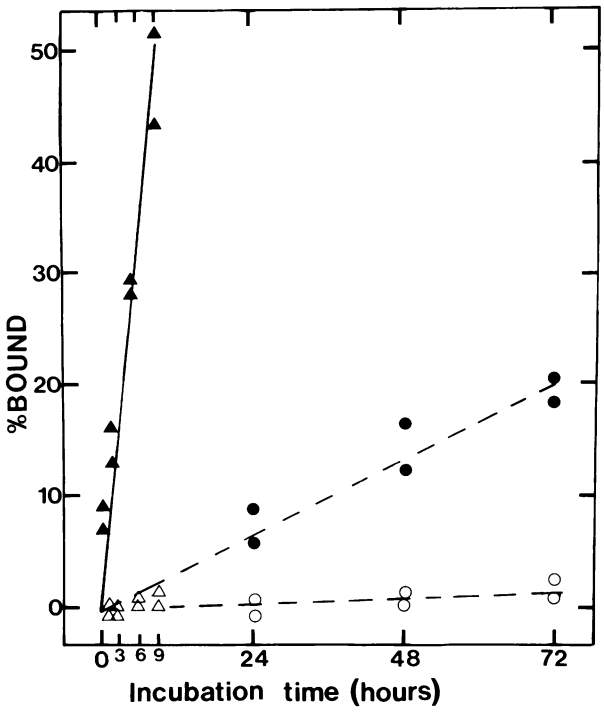


**Fig. 4.** Antibody binding to linear plasmids. Linearized $^{14}$C-labeled pDPL6 (open symbols) and $^{3}$H-labeled pDPL6 containing a d(CG/GC)$_{12}$ insert (solid symbols) were mixed together and incubated with 3 μM G1eDE1 antibody in 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, (circles) or 0 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA (triangles) at 37°C. Antibody binding to the linear plasmids was measured at periodic intervals by using nitrocellulose filters to separate protein-bound from protein-free DNA and counting the radioactivity in a Beckman LS7500 scintillation counter that independently monitored the $^{3}$H and $^{14}$C channels with automatic quench control operating. Duplicate time points are plotted.

we concluded that under high salt conditions the antibody stabilized Z-DNA cooperatively (Lafer *et al.*, in preparation). This seems to be the case also for the d(CG/GC)$_n$ sequence in plasmids. The distribution of superhelicities in the d(CG/GC)$_{12}$ plasmid in the experiment described in Figure 1 is bimodal. In lane 8c, for instance, ~35% of the plasmid is supercoiled with the center of the distribution occurring at five negative supercoils; the remainder of the plasmid is completely relaxed. The inert sequence is either completely in the Z-DNA conformation or completely in the B form. Had the antibody-stabilized transition not been cooperative we would have expected to see 100% of the plasmid remaining supercoiled with the center of the distribution at 1 − 3 negative supercoils, corresponding to part of the insert being in Z-DNA.

The antibody-stabilized transition in the d(CA/GT)$_{30}$ sequence in low ionic strength conditions does not appear to be cooperative since the amount of unwinding observed is consistent with partial stabilization of the insert sequence. However, these low salt conditions may lower the unfavorable free energy associated with junction formation and since the B-Z transition in d(CA/GT)$_n$ sequences is known to be less cooperative than the transition in d(CG/GC)$_n$ (Haniford and Pulleyblank, 1983a, 1983b) this result is not surprising.

We have found that Z-form poly(dG-dC).poly(dG-dC) can accommodate one anti-Z-DNA antibody molecule per 10 bp (by measuring the amount of DNA and antibody in immune precipitates formed in high antibody excess; data not shown). This suggests that more than one antibody molecule may bind to each insert so that the sequence is stabilized through the combined constributions of the free energies of more than one antibody-DNA interaction. The facility with which these sequences can be stabilized in Z-DNA is consistent with the known energetics of the formation of Z-DNA. The sequence d(CG/GC)$_n$ is known to form Z-DNA more readily than d(CA/GT)$_n$, which is known to form Z-DNA more readily than any sequence which occurs in our host vector. These antibodies were raised against the d(CG/GC)$_n$ form of Z-DNA and display some sequence preference for d(CG/GC)$_n$ in Z over d(CA/GT)$_n$ in Z, a 3- to 4-fold difference in relative affinity (data not shown). However, this affinity difference alone cannot explain the large differences observed in the degree of stabilization effected by the antibody. In this respect it is important to note that these are polyclonal antibody preparations. Monoclonal antibody preparations have been made and characterized (Moller *et al.*, 1982; Nordheim *et al.*, 1985; Pohl, 1983). They are broadly of two types, one group that combines only with d(CG/GC)$_n$ in the Z-DNA conformation and another group that combines with other sequences such as d(m$^5$C-G/G-$^5$mC)$_n$ and d(CA/GT)$_n$ in the Z-conformation. This behavior has been interpreted as indicating that the first group of monoclonal antibodies is binding to the bases, and is therefore sensitive to sequence, while the latter group is largely binding to the Z-DNA backbone and is less sensitive to sequence. It is likey that the polyclonal preparation contains both types of antibodies. Both of these can act to stabilize the d(CG/GC)$_{12}$ insert in the Z-conformation, but only those that bind to the backbone can stabilize the d(CA/GT)$_{30}$ insert as Z-DNA. This might lead to a loss of stabilization of the d(CA/GT)$_{30}$ insert relative to the d(CG/GC)$_{12}$ insert and contribute, in part, to the observed differences.

The negative supercoiling driven B to Z transition is rapid (Peck, 1984), but we found that the transition driven by an anti-Z-DNA antibody, as measured by binding to linearized plasmids, was very slow. This was not unexpected; negative supercoiling provides not only a source of free energy which stabilizes Z-DNA but also puts a focused strain on the molecule which drives the initial formation of Z-DNA. In a linear molecule there is no such strain and Z-DNA forms rarely. The antibody cannot catalyze the B to Z transition, therefore accumulation of antibody-Z-DNA complexes is slow since it occurs only through antibody binding of these transient Z-DNA regions. The binding is more rapid in low ionic strength buffer conditions than under physiological conditions. In using the term 'physiological conditions' we mean a buffering medium that is approximately isotonic with human serum, even though the ionic environment which DNA experiences in the nucleus may be significantly different. Ionic concentrations near physiological are particularly unfavorable for the formation of Z-DNA relative to ionic strengths which are much higher or lower. If the nuclear environment differs significantly from the extracellular environment it might possibly be more, not less, amenable to the formation of Z-DNA. For instance, the presence of spermine, which is known to stabilize Z-DNA (Behe and Felsenfeld, 1981) in the nucleus could make a significant contribution.

These results suggest that there might be a role for a Z-DNA 'flippase', that is, an enzyme catalyzing the B-Z transition, in a cell. In either relaxed DNA or in DNA that is not sufficiently supercoiled to drive the B to Z transition but can form Z-DNA if stabilized by interaction with a Z-DNA binding protein, a putative 'flippase' could play a role by rapidly generating transient Z-DNA regions which might then be bound by a Z-DNA binding protein.

The results with linear plasmids are provocative in that they

suggest that observations from experiments in which proteins are bound to linear DNA molecules do not necessarily imply that such binding does not involve Z-DNA (or even some other non-B-DNA conformation). The binding of the restriction endonuclease *Eco*RI to its DNA substrate, for instance, has been shown to result in considerable distortion of the DNA (Frederick *et al.*, 1984). Effectively, *Eco*RI binds to a DNA structure which does not occur in the absence of its binding. Whether a protein can stabilize a given sequence in the Z-DNA conformation depends on how much energy is gained from its interaction with the sequence in the Z-DNA conformation and how much energy is required to maintain that sequence as Z-DNA. The energy of binding can be greater if the number of base pairs involved in the protein DNA interaction is larger. In particular, the energy of binding will be larger for sequence-specific Z-DNA binding proteins in which energetic contributions to the free energy of binding come both from phosphate backbone interactions as well as specific protein-base contacts.

## Materials and methods

*Nucleic acids*

pDPL6, a pBR322-derived plasmid, pDHf14, which was derived by cloning $(dC\text{-}dA)_{30}\cdot(dT\text{-}dG)_{30}$ into pDPL6, and pDHg16, which was derived by cloning $(dC\text{-}dG)_{12}\cdot(dC\text{-}dG)_{12}$ into pDPL6 (Haniford and Pulleyblank 1983a, 1983b, were kindly provided by Dr. David Pulleyblank and David Haniford (University of Toronto). Radioactive plasmids were prepared by growth of appropriately transformed DH1 TL3 ($Thy^-$) *Escherichia coli* in 500 ml cultures of minimal media supplemented with 2 μg/ml cold thymidine and 1 μCi/ml [$^3$H]thymidine. Typical yields of plasmid were 0.25 mg per 500 ml culture labeled to $2 \times 10^4$ c.p.m./μg.

*Antibodies*

G10 and G1 designate goats that were immunized with Br-poly(dG-dC)·poly(dG-dC) as described previously (Lafer *et al.*, 1981, 1983). G10cDE2 and G1eDE1 denote goat IgG preparations purified by DEAE-cellulose column chromatography. The specific anti-Z-DNA antibody content was ~3% of the total protein in both preparations, as determined by quantitative immunoprecipitation with poly(dG-dC)·poly(dG-dC) in 4.0 M NaCl. In all the experiments described the concentration of antibody used is expressed as Z-DNA-specific IgG rather than total IgG.

*Detection and measurement of Z-DNA in covalently closed circular plasmids*

1 μg of radiolabeled plasmid at bacterial superhelical density was suspended in 20 μl of buffer with anti-Z-DNA antibody at concentrations ranging from 0.3 nM to 3000 nM. The buffer used was either 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA or 50 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA. The plasmid was incubated for 30 min at room temperature followed by addition of 10 units of topoisomerase I (B.R.L.) followed by a 12-h room temperature incubation. A second addition of 10 units of topoisomerase was made, followed by a second 12-h incubation. In some cases fresh topoisomerase I was added and the incubation continued for another 24 h. 10 μl of 10% SDS containing 5 mg/ml protease (from *Streptomyces griseus*, Sigma) was added to each reaction mixture and then incubated for 1 h at 37°C to dissociate the antibody-DNA complex. 10 μl of each reaction mixture were then run on a 1% agarose gel containing 1 μg/ml chloroquine in TBE buffer (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA). To quantitate the amount of DNA that was supercoiled, sections of the gel containing the bands were cut out and placed in scintillation vials with 2 ml of 1 N HCl and heated to dissolve the agarose. 10 ml of aquasol were added and the radioactivity counted in a Beckman LS7500 scintillation counter with automatic quench control operating.

*Binding of antibody to linearized plasmids*

5 μg each of $^{14}$C-labeled pDPL6 and $^3$H-labeled pDPL6 containing a $d(CG/GC)_{12}$ insert were linearized with *PstI*, mixed together, ethanol precipitated, and resuspended in 50 μl of 3 μM G1eDE1 antibody in either 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, or 0 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, and incubated at 37°C. At periodic intervals, 5 μl of this reaction mixture were removed and diluted into 500 μl of buffer containig 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, and incubated for 1 h at room temperature in order to dissociate non-specific assocation but not tight, specific binding. This was followed by filtration on Millipore type HA 0.45 μm nitrocellulose filters. Prior to filtration the filters were pre-treated with 0.3 M NaOH for 10 min, washed extensively in five changes of distilled water, placed in a vacuum manifold, and washed with 1 ml of buffer containing 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA. After the reaction mixtures were passed through the filters, the filters were washed twice more with 1 ml of buffer containing 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, dried, and counted in a Beckman LS7500 scintillation counter using a counting program that separately monitored $^3$H and $^{14}$C with automatic quench control operating.

*Binding of antibody to relaxed plasmids*

5 μg each of $^{14}$C-labeled pDPL6 and $^3$H-labeled PDPL6 containing a $d(CG/GC)_{12}$ insert were relaxed to completion with topoisomerase I, mixed together, ethanol precipitated, and resuspended in 50 μl of 3 μM G1eDE1 antibody in 0 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, and incubated at 37°C. At periodic intervals 5 μl of this reaction mixture were removed and diluted into 500 μl of buffer containing 140 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, and incubated for 1 h at room temperature in order to dissociate non-specific association but not tight, specific binding. This was followed by filtration on Millipore type HA 0.45 μm nitrocellulose filters as described above.

*Topoisomerase treatment of relaxed plasmid-antibody reactions*

1 μg of completely relaxed plasmid [either pDPL6 or pDPL6 containing a $d(CG/GC)_{12}$ insert] was suspended in 20 μl of 3 μM of G1eDE1 antibody in 0 mM NaCl, 10 mM Tris pH 7.5, 1 mM EDTA and incubated at 37°C for 48 h. The NaCl concentration was brought to 50 mM by the addition of 20 μl of 100 mM NaCl solution and then 20 units of topoisomerase I were added. The topoisomerase reaction was allowed to proceed for 12 h at 37°C and the resultant distribution of topoisomers was analysed by agarose gel electrophoresis as described above.

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