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# Topoisomer gel retardation: detection of anti-Z-DNA antibodies bound to Z-DNA within supercoiled DNA minicircles

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## ABSTRACT

Small DNA fragments of approximately 350 bp in length, either with or without $d(CG)_n$ tracts, are ligated into underwound DNA minicircles to generate topoisomeric rings with different topological linking numbers, Lk. These minicircles, differing by an Lk of one, can be separated by acrylamide gel electrophoresis. Furthermore, electrophoresis can be used to reveal DNA double helix conformational changes that are induced by supercoiling, such as left-handed Z-DNA. When anti-Z-DNA antibodies are added to such minicircles, their binding leads to a selective retardation of the electrophoretic migration of the Z-DNA containing circles. This effect is not seen with relaxed minicircles and those with insufficient torsional stress to induce a conformational transition. Thus the technique of 'topoisomer gel retardation' presents a very sensitive assay for the identification of proteins that selectively bind to DNA conformations stabilized by negative DNA supercoiling.

## INTRODUCTION

The conformation of the DNA double helix can be polymorphic and can thereby exert significant influence upon the binding of proteins to DNA. Not only is the specificity in protein-DNA interaction determined by the primary nucleotide sequence of the DNA binding site but also by DNA structural deformations that may be required to occur within the DNA-protein complex. The latter situation has been analyzed at high resolution in co-crystals of EcoRI restriction endonuclease (1) or phage 434 repressor (2) with their respective DNA binding sites. Similarly, complex formation between the CAP catabolite activator protein and its DNA binding site involves a structural double helix deformation, namely bending of DNA (see e.g. (3)). A special case in which the binding of proteins to DNA is determined almost exclusively by DNA conformation is represented by the

interaction of anti-Z-DNA antibodies (4-7) with left-handed Z-DNA (6-12).

Secondary structure of the DNA double helix is influenced greatly by DNA tertiary folding: DNA supercoiling provides free energy for a variety of DNA structural transitions (for review: 13). These include Z-DNA formation (10, 11), cruciform extrusion (14-16), local DNA melting (17), and other DNA polymorphism (18, 19).

In order to facilitate the identification of proteins that specifically interact with sites of supercoil-induced alterations in DNA conformation, we make use of the gel retardation assay (20, 21) to separate protein-free DNA from DNA-protein complexes by acrylamide gel electrophoresis. We first show that small DNA circles differing by units of one in their topological linking number Lk, can be separated from each other on acrylamide gels. Second, we demonstrate that protein-free forms of these topological isomers (topoisomers) can also be separated from their counterparts that form specific complexes with proteins. Using this new assay of `topoisomer gel retardation', we can identify supercoil-induced changes in DNA structure as well as the specific recognition of such structures by proteins. As a model system we investigate the affinities of monoclonal anti-Z-DNA antibodies for their left-handed Z-DNA antigen.

## MATERIALS AND METHODS

**Plasmids.** Plasmid pBR322 (22) and its $d(CG)_n$-containing derivatives pLP14 and pLP32 have been described (23). pAN700 is a derivative of pUC7 (24), which has inserted at its polylinker-HincII site a pUC18-PvuII fragment (25) extending from pUC18 coordinates 302 to 628.

Plasmids pAN014 and pAN022 are pUC18 derivatives containing at their single BamH1 sites d(CG) inserts of 14 and 22 bp, respectively. Plasmids pFP316 and pUC32 are pUC8 derivatives containing d(CG) inserts of 16 and 32 bp at their single BamH1 sites. Plasmids pAN701, pAN702, pAN703 and pAN704 are all derived from pUC7 by inserting into its HincII site the $d(CG)_n$-containing PvuII fragments of plasmids pAN014, pFP316, pAN022

and pUC32, respectively. Plasmids pFP016 and pUC32 were obtained from Dr. F. Pohl and Dr. M. Darby, respectively. The plasmid constructions were performed according to routine cloning techniques (26).

Antibodies. Monoclonal anti-Z-DNA antibody Z22 (27, 28) was prepared from mouse ascites fluid according to standard procedures (29) using protein A affinity chromatography. For the generation of Z22 Fab fragments papain cleavage was employed (30), followed by protein A chromatography. Purity of Fab protein was checked by SDS gel electrophoresis (31) and the material was stored frozen in PBS (10mM sodium phosphate, pH=7.2, 150mM NaCl).

Generation of DNA minicircles.

TaqI restriction fragments of pBR322, pLP14 and pLP32, as well as EcoRI restriction fragments of the pAN700 to pAN704 series of plasmids, were dephosphorylated with calf intestine alkaline phosphatase (Boehringer, Mannheim), phenol extracted twice and purified by gel electrophoresis and electroelution out of the gel. Purified restriction fragments were radiolabelled with [$^{32}$P-$\gamma$] ATP using polynucleotide kinase. Labelled fragments were ligated at low DNA concentration (not exceeding 1$\mu$g/ml) into unit length circles. Ligation reactions, which contained ethidium bromide (EtBr) at the concentrations indicated in figure 2A, were usually done at 4°C (see below). Individual topoisomers were identified and their topological linking difference ($\Delta$Lk) determined according to their appearance in ligation reactions containing increasing concentrations of EtBR. Topoisomers were identified by the band counting method (32).

Antibody binding reactions. Binding reactions were performed in 10$\mu$l reaction volumes usually containing 0.2pg radiolabelled minicircle DNA and antibody protein at the indicated concentrations. The reaction buffer contained 5mM sodium phosphate (pH=7.2) and 75mM NaCl. In the competition experiments of figure 5 competitor DNAs were added at the amounts indicated in the figure legend. Reaction mixtures were incubated for 20 min. on ice.

**Gel electrophoresis.** Electrophoretic separation of topoisomeric minicircles from each other, as well as from their protein-bound counterparts, was achieved in 4% acrylamide (29:1) gels, buffered with 0.5xTBE (TBE: 90mM Tris borate, pH=8.3, 2.5mM EDTA), that were run in the cold room (4°C) for 6 hours at 30mA. The gels were exhaustively electrophoresed prior to loading. The gels were dried in vacuo and autoradiographed for about 12 hours at -70°C using two intensifying screens.

## RESULTS

### Construction of topoisomeric DNA minicircles.

To study supercoil-induced DNA conformational changes and the resulting interaction of proteins with alternative DNA structures, we made use of the observation that the topoisomeric forms of small DNA circles (between 200 to 600 bp in length) can be separated by acrylamide gel electrophoresis (33, 34). Since the B-Z transition is a DNA conformational change particularly responsive to torsional stress (6, 23, 35, 36), we constructed plasmid molecules that would easily permit the generation of small restriction fragments with the potential to form Z-DNA that could be ligated into minicircles. One such series of plasmids based on the pBR322/pLP14/pLP32 constructs (23) (figure 1A). The isolated 313 bp Taq fragment from pBR322 or the correspondingly longer $d(CG)_n$-containing fragments from pLP14 and pLP32 was circularised by ligation. A second set of constructs was generated using pUC plasmid sequences (25). Initially a 322 bp PvuII-fragment from plasmid pUC19 or the correspondingly longer $d(CG)_n$-containing pUC fragments shown in figure 1B was inserted into the symmetric polylinker of the pUC7 vector. EcoRI digestion yields fragments from the pAN700 to pAN704 series of plasmids that can be ligated into minicircles containing $d(CG)_n$ inserts, with n ranging from 0 to 16.

### Separation of minicircle topoisomers by acrylamide gel electrophoresis.

In order to establish conditions for the separation by acrylamide gel electrophoresis of topoisomeric DNA minicircles that differ from each other by steps of one in linking number ($\Delta Lk=1$), we first circularized the pBR322 313bp Taq restriction

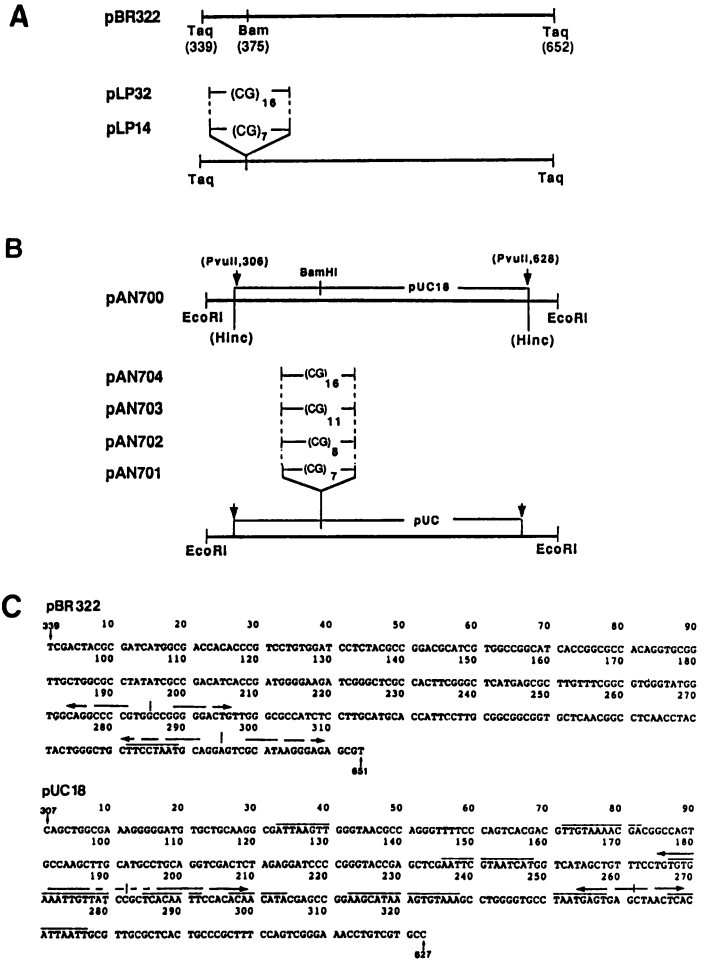


**Figure 1: Plasmid constructs for the generation of minicircles.**

(A) Coordinates of the 313bp TaqI restriction fragment of pBR322 used for circularisation into DNA minicircles. pLP32 and pLP14 are derivatives of plasmid pBR322 which contain $d(CG)_n$ insertions at the single BamHI site (23).

(B) The pAN700 to pAN704 series of plasmids permitted the generation of an EcoRI restriction fragment for ligation into DNA minicircles. The EcoRI fragment of pAN700 contains pUC18 sequences from position 306 to 628 in addition to the pUC7 polylinker sequence. The $d(CG)_n$ inserts present in plasmids pAN701 to pAN704 are indicated.

(C) Primary nucleotide sequences of the pBR322 (22) and pUC18 (25) fragments used for the construction of DNA minicircles. Symmetrical nucleotide segments are indicated by horizontal arrows and regions of high AT-content are overlined.

fragment that spans nucleotide positions 339 to 652 (figures 1A, 1C). The $^{32}$P-$\gamma$ endlabelled Taq fragment was ligated into circles in the presence of increasing amounts of the DNA intercalating agent ethidium bromide (EtBr). After completion of the ligation reaction and removal of ethidium bromide, a reasonable separation of individual topoisomeric forms of the generated minicircles can be achieved by electrophoresis on an acrylamide gel (figure 2A). Minicircles of increasing torsional stress migrate with increasing velocity, due to compensating changes in writhe (Wr) according to the relationship (37)

$$\Delta Lk=\Delta Tw+\Delta Wr,$$

where $\Delta$Tw represents a change in helial twist (Tw).

The gel electrophoretic separation of the topoisomers permits the identification of their differences in linking number ($\Delta$Lk) by simple band counting (32). There is a continuous increase in the migration of minicircles up to a linking deficit of $\Delta$Lk=-4 (figure 2A, lane 13). With samples of $\Delta$Lk=-5 a discontinuity in migration behaviour was observed (figure 2A, lane 14), indicating a local conformational change (denoted X) in the double helix of the minicircle (see below).

All of the covalently closed, negatively supercoiled circles of figure 2A, lanes 2 to 15, are converted to the relaxed form upon treatment with the nicking-closing enzyme topoisomerase I (not shown). In addition we find a considerable proportion present as nicked circles (nic) (figure 2A) that we attribute to incomplete ligation.

**DNA double helix conformational changes within supercoiled minicircles.**

The LP14-Taq minicircle (figure 1A) contains a d(CG)$_7$ segment within the BR322-Taq minicircle. It displays a discontinous gel electrophoretic migration behaviour upon an increase in linking deficit from $\Delta$Lk=-1 to $\Delta$Lk=-2. The topoisomer with $\Delta$Lk=-2 migrates at the position of the relaxed circles (figure 2B, lanes 7-10), thereby exhibiting a change in writhe expected to occur upon flipping the d(CG)$_7$ segment into the left-handed Z-DNA conformation (23). This topoisomer is designated -2$_z$ in

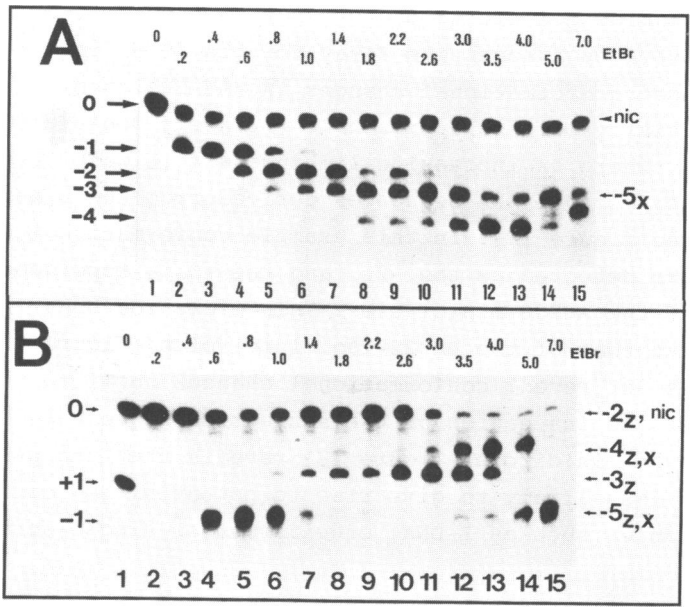


**Figure 2: Acrylamide gel electrophoresis of DNA minicircles generated by circularisation in the presence of ethidium bromide.**

(A) BR322-Taq minicircles are generated by ligation into circles in the presence of 0-7.0μM EtBr, as indicated above the gel lanes. The resulting minicircles are underwound proportionally to the EtBr concentration present during circularization. The linking differences (ΔLk) of the individual topoisomers are indicated at the left and right margins. Nic refers to nicked ligation products that migrate as relaxed circles.
(B) LP14-Taq minicircles of increasing linking deficit. Abbreviations and symbols are used as in (A). A change in writhe, due to a B-Z transition within the minicircle, is seen upon comparison of topoisomeric distributions in lanes 6 and 7. A second conformational change of unidentified nature (called X) is seen in lanes 12-14. Topoisomers of ΔLk=-2 to ΔLk=-5, which contain stabilised Z-DNA transitions or the unidentified X transition, are indicated by Z or X, respectively.

figure 2B. As the torsional stress is increased the $-3_z$ topoisomer shows more rapid migration which indicates an increase in writhe. At yet higher torsional stress the $-4_z$ topoisomer displays a second step of reduced migration (figure 2B, lanes 12 to 14). This second discontinuity in migration indicates a second DNA structural change of an unidentified nature and this topoisomer is therefore labeled $-4_{zx}$. These results demonstrate

that acrylamide gel electrophoresis of topoisomeric DNA minicircles provides a sensitive assay for the detection of supercoil-induced conformational changes in the DNA double helix. The sensitivity of this approach is further demonstrated in figure 3A, where an increasing linking deficit was introduced into minicircles of DNA sequences derived from the plasmid pAN700 (see figure 1B). In this example conformational DNA changes are detected as the $-2_x$ and the $-3_{x,y}$ topoisomer species of the AN700-minicircles. This migration behaviour contrasts with that of the BR322-Taq minicircles (figure 1A), which does not reveal conformational changes until to the -4 topoisomer. An inspection of the nucleotide sequences of these two types of minicircle (figure 1C) reveals that the pBR322 sequence is less likely to give rise to potential DNA conformational changes such as Z-DNA, cruciforms, or single-stranded regions.

When a $d(CG)_n$ segment is inserted into the AN700-RI minicircles (the AN701 to AN704 series of minicircles; see figure 1B), this sequence permits the B-Z transition to occur at negative superhelical densities lower than those required for inducing the unidentified X- and Y-transitions (figure 3B, lanes 6-8).

**Binding of anti-Z-DNA antibodies to supercoiled minicircles causes gel retardation of specific topoisomers.**

Anti-Z-DNA antibodies interact specifically with Z-DNA stabilized by supercoiling (6, 7, 12). We used Fab protein fragments of the monoclonal anti-Z-DNA antibody Z22 (27, 28) to determine how the formation of complexes between these antibodies and Z-DNA-containing supercoiled minicircles affects the migration of the minicircles in acrylamide gels. The addition of an increasing amount of antibody protein to the LP14-minicircles leads to a shift towards slower migration of the covalently closed minicircles (topoisomers -1, $-2_z$, and $-3_z$) but not of the nicked species (figure 4A). The $-2_z$ and $-3_z$ topoisomers are shifted at a protein concentration of 0.182$\mu$g/ml (figure 4A, lane 5) whereas the -1 topoisomer is slowed only at a higher antibody concentration of 0.365$\mu$g/ml (figure 4A, lane 6). The observation that the antibody binds to the -1 topoisomer from the LP14-minicircle in spite of the fact that its

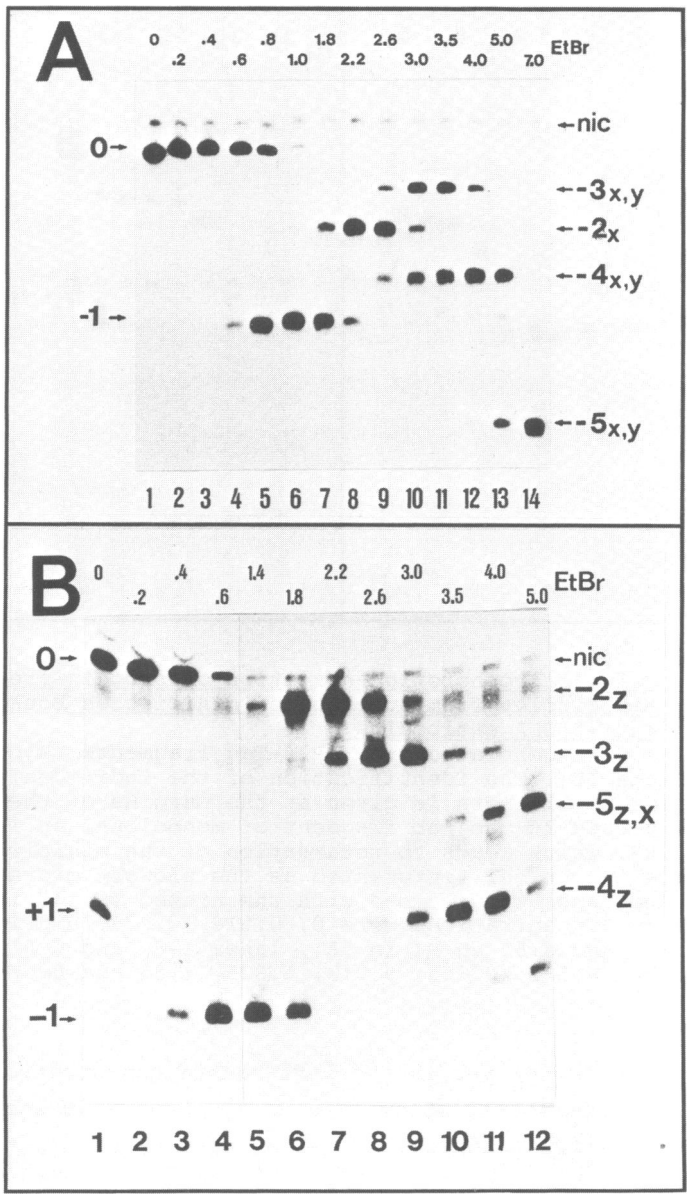


**Figure 3: Acrylamide gel electrophoresis of topoisomeric minicircles derived from pUC sequences.**
(A) and (B) show electrophoretic separation of minicircles generated by ligation of AN700-RI fragments and AN701-RI fragments, respectively. Abbreviations and symbols are used as in figure 2.

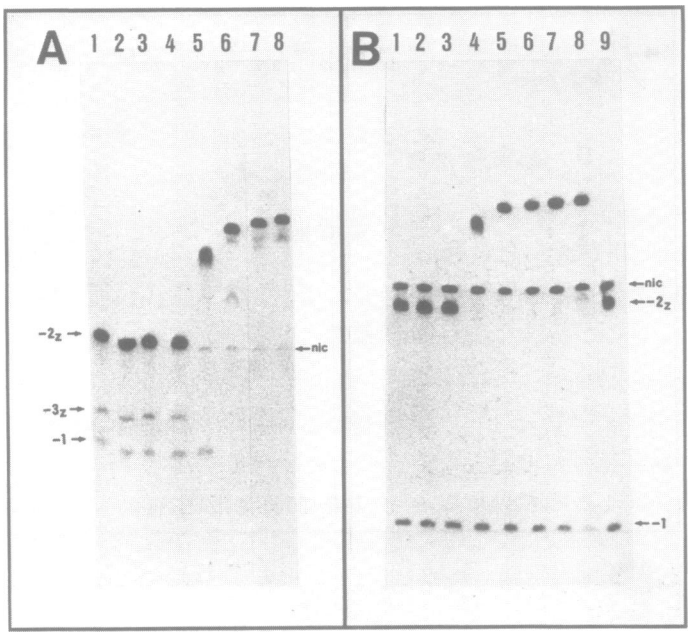


**Figure 4: Gel electrophoretic separation of protein-free DNA minicircles from supercoiled minicircles bound by anti-Z-DNA antibodies.**

Minicircles were generated from LP14-Taq fragments (A) or LP32-Taq fragments (B). The identification of the individual protein-free topoisomers is given at the margins of the gels. The protein used is the Fab fragment of monoclonal anti-Z-DNA antibody Z22, which leads to retardation of the minicircle topoisomers in the gel represented as the slowest migrating band. No complexes were formed with the nicked (nic) circles. The antibody concentrations were 0, 0.018, 0.036, 0.091, 0.182, 0.365, 1.825 and 3.65 $\mu$g/ml in (A), lanes 1-8, and 0.018, 0.036, 0.091, 0.182, 0.365, 0.913, 1.825, 3.65 and 0$\mu$g/ml in (B), lanes 1-9.

$d(CG)_7$ insert is not yet in the left-handed conformation, is analyzed in more detail below (figure 6). Figure 4B shows that the LP32-minicircles containing Z-DNA, namely the $-2_z$ topoisomers, are also bound by the antibodies above a protein concentration of 0.182$\mu$g/ml (figure 4B, lane 4) and are shifted to a slower migrating form. In these minicircles the -1 topoisomer species is bound by the antibody only at the highest concentration of 3.65$\mu$g/ml (figure 4B, lane 8), while the nicked material is again not recognized by the protein.

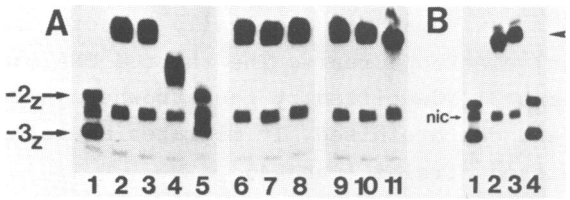


Figure 5: Characterization of the complexes between DNA minicircles and anti-Z-DNA antibodies.
(A) Competition experiments. Radiolabelled AN702-EcoRI minicircles (lane 1) are bound by Z22 antibody Fab fragments in the absence (lane 2) or presence of various competitor DNAs (lanes 3-11). The type and amount of competitor DNAs were: supercoiled, Z-DNA containing plasmid pUC32 at 0.1$\mu$g (lane 3), 1$\mu$g (lane 4) and 4$\mu$g (lane 5); HaeIII digested plasmid pUC32, at 1$\mu$g (lane 6), 4$\mu$g (lane 7) and 7$\mu$g (lane 8); supercoiled vector pUC18 without Z-DNA insert at 0.1 $\mu$g (lane 9), 1$\mu$g (lane 10) and 4$\mu$g (lane 11).
(B) Evidence for topological integrity of retarded topoisomers. Minicircles used in (A), lane 1, were bound by 3.6ng (lane 2) and 36ng (lane 3) of Z22 Fab fragments. The retarded material (indicated by the arrowhead) was electroeluted, deproteinised by phenol extraction and electrophoresed (lane 4). Topoisomer identifications are as indicated in figure 2.

Figure 4 demonstrates that gel retardation can be used to identify the differential interaction of anti-Z-DNA antibodies with supercoiled DNA circles of different linking numbers. Since by definition topoisomeric circles are identical in their nucleotide sequence, the observed differential protein affinities are due to the presence of structural DNA polymorphism, in this case Z-DNA, that is stabilized by DNA topological constraints.

The conclusion that the antibody is recognising left-handed Z-DNA structures is further substantiated by competition experiments, as shown in figure 5A. Using radioactive minicircles of the AN702-EcoRI fragment containing $d(CG)_8$, Fab-DNA complex formation can be competed by unlabelled, supercoiled pUC32 plasmids that contain a left-handed Z-DNA segment (figure 5A, lanes 3-5) but not by linear fragments of this plasmid obtained by HaeIII restriction digestion (lanes 6-8). The supercoiled pUC8 vector plasmid, which does not contain a $d(CG)_n$ insert, also does not compete efficiently (figure 5A, lanes 9-11).

Figure 5B demonstrates that the slow migrating forms of the DNA-protein complex remain topologically intact during antibody binding and gel electrophoresis. The shifted DNA material (figure 5B, lane 3; identified by the arrowhead) was extracted from the gel and deproteinised. It migrates as forms $-2_z$ and $-3_z$ with no nicked circles (lane 4).

Affinity of Z22 Fab fragments to Z-DNA in supercoiled minicircles.

The high sensitivity of the topoisomer gel retardation assay for detecting complexes of DNA minicircles with protein allowed us to measure the protein binding affinities to different DNA topoisomers. Figure 6 displays a fine-titration of Z22 Fab antibodies with topoisomers of AN701-minicircles (figure 6A) and LP14-minicircles (figure 6B), both of which contain $d(CG)_7$ inserts. In either case the $-2_z$ and $-3_z$ topoisomers are shifted by antibody at a protein concentration of 0.52$\mu$g/ml and 1.8$\mu$g/ml, respectively (figure 6A, lane 6; figure 6B, lane 7). Further increases in protein lead to a progressively slower migration behaviour until a final form is reached at 18$\mu$g/ml protein solution. Increasing the antibody concentration beyond this value results in nonspecific retention of the entire DNA material in the loading pocket at the origin of the gel (not shown).

In the AN701-minicircles the -1 topoisomer is not bound specifically by antibody (figure 6A). In contrast, the -1 topoisomers of LP14-minicircles are bound by the Z22 Fab fragments at a concentration of 73$\mu$g/ml (figure 6B, lane 10). This latter concentration is about 5x higher than that required for recognition of the $-2_z$ and $-3_z$ topoisomers. This suggests that the equilibrium between the B- and Z-DNA forms of the same $d(CG)_7$ insert may be influenced by Z22 antibodies in the case of the -1 topoisomer of LP14-minicircles, but not to the same extent in the sequence context of the -1 topoisomer of AN701-minicircles. In no case was binding to the nicked minicircles observed in the range of protein concentrations permitting specific interaction with DNA.

The titration of antibody shown in figure 6 allows for the estimation of affinity constants associated with Fab fragments

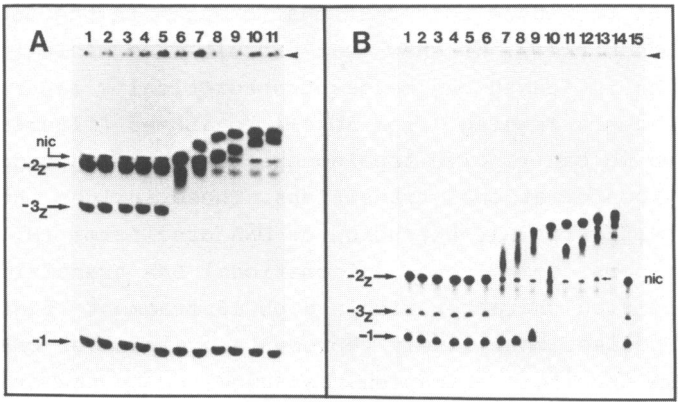


**Figure 6: Fine-titration of Z22 Fab antibody in the binding reaction to topoisomeric minicircles.**
The minicircles were generated from the endlabelled DNA fragments AN701-EcoRI (A) and LP14-Taq (B), both containing $d(CG)_7$ insertions. The ng amounts of protein added were in (A): 0 (lane 1), 0 (lane 2), 0.65 (lane 3), 1.3 (lane 4), 2.6 (lane 5), 5.2 (lane 6), 10.5 (lane 7) 21 (lane 8), 45 (lane 9), 90 (lane 10), 180 (lane 11). The ng amounts of protein added in (B) were: 0 (lane 1), 1.83 (lane 2), 3.65 (lane 3), 7.3 (lane 4), 11 (lane 5), 14.6 (lane 6), 18.25 (lane 7), 27.5 (lane 8), 36.5 (lane 9), 73 (lane 10), 145 (lane 11), 182 (lane 12), 365 (lane 13), 730 (lane 14), 0 (lane 15).
Identification of topoisomers is given at the margins. The arrowheads point towards the origins of the gels.

of the monoclonal Z22 anti-Z-DNA antibodies. For the $-2_\infty$ and $-3_\infty$ topoisomers we calculate an affinity constant of $5 \times 10^{-8}$ M, while an affinity constant of $8 \times 10^{-7}$ M is found for the -1 topoisomer of the LP14-minicircle. These calculations assume a 100% active preparation of antibody.

## DISCUSSION

In the present work we describe a very sensitive approach for the analysis of DNA-protein interactions that are under the influence of DNA supercoiling. We apply this technique,called topoisomer gel retardation, to an analysis of the interaction between monoclonal anti-Z-DNA antibodies (Z22 Fab fragments) with individual topoisomeric forms of small DNA circles. Topoisomer gel retardation combines the advantages of the conventional gel retardation assay (20, 21) with the power of DNA

supercoiling to induce conformational changes in the double helix of DNA circles. We show that protein-free minicircles of about 350 bp in length can be electrophoretically separated into topoisomers ranging from $\Delta Lk=+1$ to $\Delta Lk=-5$ (figures 2, 3). In addition we can resolve topoisomers that have undergone internal DNA conformational transitions. These include the B-Z transition (figure 2B), extrusion of DNA cruciforms (M. Bianchi, EMBL; pers. comm.) and conformational DNA transitions of yet unidentified character within a pUC18 fragment (figures 1B, 1C, 3A). The last most likely represents examples of DNA melting and DNA cruciform extrusion, as suggested by the primary nucleotide sequence (figure 1C). A gel analysis system very similar to that reported herein was recently described (38).

In addition to resolving individual topoisomeric minicircles our acrylamide gel system also permits the separation of protein-free topoisomeric minicircles from the corresponding protein-bound species (figures 4-6). Consequently the topoisomer gel retardation assay facilitates the study of DNA-protein interactions that are under the influence of DNA supercoiling or that are directed by supercoil-induced DNA structural changes. Some examples include integrative recombination (39), initiation of transcription (40), cruciform extrusion (14-16) or Z-DNA formation (10, 11).

We have also used the topoisomer gel retardation assay to analyse in detail the influence of DNA supercoiling on DNA loop formation in the lac operator/repressor system. Negative DNA supercoiling was found to stabilise looped complexes between lac repressor and two operators, as well as influence the spacing requirement of two operators for DNA loop formation (Krämer, Amouyal, Nordheim and Müller-Hill, 1988, EMBO J., in press).

In the present analysis the topoisomer gel retardation technique has revealed a high affinity of monoclonal anti-Z-DNA antibodies to their left-handed antigen present in supercoiled minicircles. The calculated affinity constants for Fab fragments of the Z22 antibody was $\approx 5 \times 10^{-8}$M. Although no specific binding was observed to nicked or completely relaxed forms, in

some circumstances the antibody was shown to affect the B-Z equilibrium in $d(CG)_7$ segments present in low superhelical density minicircles (figure 6B), yielding an affinity constant of $8x10^{-7}M$.

The separation of topoisomeric DNA minicircles, as achieved by our use of acrylamide gels, allows therefore for the estimation of differential binding affinities of proteins to circular DNA binding substrates of different superhelical density. In earlier studies (41, 42) Pohl and collegues already used gel mobility shifts to demonstrate the binding of anti-Z-DNA antibodes to supercoiled plasmids and form V DNA. In these experiments complete plasmids were used and the agarose gels employed did not permit separation of individual topoisomers of the circular DNAs.

The Fab fragments of D11, a different anti-Z-DNA monoclonal antibody, were calculated previously to have a Z-DNA binding affinity approximately ten times higher than the value we estimate here for our Z22 antibody (43).

Topoisomer gel retardation permits the clear separation of protein-free topoisomeric minicircles from their protein-bound forms. Such separation can aid the detailed characterisation of DNA-protein contacts, e.g. by using the different protocols of chemical modification-interference or chemical footprinting studies (12). We expect that the assay described here will also prove useful in the search for natural Z-DNA binding proteins (44-47) since it offers a rapid and sensitive means to screen for such binding activities. In particular, the stabilisation of Z-DNA at physiologically low ionic strengths by DNA supercoiling obviates the need for artificial Z-DNA assay-substrates stabilized by chemical DNA modification (48).

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