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The influence of tertiary structural restraints on conformational transitions in superhelical DNA

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ABSTRACT

This paper examines theoretically the effects that restraints on the tertiary structure of a superhelical DNA domain exert on the energetics of linking and the onset of conformational transitions. The most important tertiary constraint arises from the nucleosomal winding of genomic DNA *in vivo*. Conformational transitions are shown to occur at equilibrium at less extreme superhelicities in DNA whose tertiary structure is restrained than in unrestrained molecules where the residual linking difference α_{res} (that part of the superhelical deformation which is not absorbed by transitions) may be freely partitioned between twisting and bending. In the extreme case of a rigidly held tertiary structure, this analysis predicts that the B-Z transition will occur at roughly half the superhelix density needed to drive the same transition in solution, other factors remaining fixed. This suggests that superhelical transitions may occur at more moderate superhelical deformations *in vivo* than in solution. The influence on transition behavior of the tertiary structural restraints imposed by gel conditions also are discussed.

INTRODUCTION

A topological domain of DNA is a segment of duplex which is constrained so that its linking number is fixed. It may be comprised either of a closed circular DNA or of an internal loop within a longer molecule which is held at two anchor points in such a way that rotations about the duplex axis are precluded there. DNA within living systems commonly is constrained into topological domains whose superhelical deformations can be regulated independently. The genomic DNAs of both prokaryotes (1,2) and eukaryotes (3,4) are subdivided into multiple domains, while shorter DNAs frequently occur as single closed circular domains. In addition to this partitioning, both prokaryotic (5) and eukaryotic (6) genomic DNAs are complexed with basic proteins so that their tertiary structures are highly restrained. The association of eukaryotic nuclear DNA with histones to form nucleosomes within chromatin is reasonably well understood (7). Although the complex formed by prokaryotic genomic DNA with HU proteins is less well characterized at present, the resulting structure is qualitatively similar to the eukaryotic nucleosome (5). Superhelical deformations within DNA domains are known to be carefully regulated *in vivo* (8-10). Moreover, the modulation of superhelicity has been shown to affect the regulation of several important physiological functions (11). The most compelling evidence found to date for a putatively regulatory role for DNA superhelicity involves transcriptional initiation and promoter activity in prokaryotes (12,13). Transcriptional activation of specific eukaryotic genes also has been shown to be regulated by torsional stresses imposed on the domain by superhelicity (14-16).

Local conformational transitions can occur at susceptible sites within a DNA domain in response to imposed negative superhelicity. Such transitions may provide mechanisms whereby superhelicity modulates specific DNA functions. Z-DNA has been detected within transcriptional regulatory sequences within the SV 40 genome (17), and in transcriptionally active chromosomal regions (18). Nuclease digestion experiments also indicate that regions of altered DNA conformation occur at specific sites within transcriptionally active chromatin (19,20). *In vivo*, superhelical transitions would occur within domains where the DNA is wrapped into nucleosomes. This contrasts strongly with the *in vitro* conditions under which experiments investigating superhelical DNA secondary structure commonly are performed, where purified DNA is either free in solution or confined within a gel. In order to understand how these transitions might occur within living systems, it is important to consider how the tertiary restrictions imposed by nucleosomal winding would affect superhelical transconformational equilibria.

Although the temperature-induced twisting of relaxed domains is restrained by nucleosomal winding (21), when the domain is substantially supercoiled virtually all of the imposed linking difference is expressed through changes of twist (22). There is no evidence that substantial superhelicity alters either the nucleosome-dictated tertiary structure or the packing arrangement of the nucleosomes, nor that it preferentially deforms the inter-nucleosome linker regions. Present information indicates that, beyond a threshold of stress, the electrostatic association that holds the nucleosomal DNA-protein complex together cannot anchor the DNA against torsional deformations, although it still constrains the tertiary structure in an apparently unaltered conformation. The imposition of a substantial linking difference results in an approximately uniform torsional deformation of the nucleosome-associated DNA without appreciably affecting tertiary structure (22). In contrast, most experiments investigating supercoiled DNA are performed under conditions where imposed superhelicity may be partitioned between deformations of tertiary structure and twist.

The theoretical analysis presented here indicates that superhelical conformational transitions are expected to occur much more easily when the DNA's tertiary structure is restrained than when it is not, other factors remaining fixed. Briefly, when tertiary structure is determined by nucleosomal winding, the domain is not free to respond to imposed superhelicity with that mix of torsional and flexural deformations which would minimizes the free energy in the absence of restraints. Instead, it must accommodate the entire residual linking difference through torsional deformations. As shown below, this requires a higher free energy than is needed when both flexural and torsional deformations are possible. In consequence, conformational transitions will become energetically favored to occur at substantially less extreme negative superhelicities in restrained than in unrestrained domains.

SUPERHELICAL EQUILIBRIA AND THE ENERGETICS OF RESIDUAL LINKING

A DNA domain is constrained by the constancy of its linking number Lk to conformations in which its secondary and tertiary structures are topologically coupled through White's formula (23,24):

$$Lk = Tw + Wr. \quad (1)$$

The superhelicity imposed on a domain is expressed as its linking difference α. This is the amount by which the actual linking number Lk differs from its value Lk_0 in the relaxed state, in which both Tw and Wr have values characteristic of the nicked molecule under identical environmental conditions:

$$\alpha = Lk - Lk_0 = \Delta Tw + \Delta Wr. \tag{2}$$

Changes in Wr require flexure, while ΔTw may involve conformational transitions which alter the local helicity, or smooth torsional deformations of secondary structure, or both.

This definition of the linking difference is not the same as the one that is used elsewhere in the literature. Commonly α is measured under standard conditions, where the relaxed state is usually regarded as being unwrithed, $Wr_0 = 0$ (25). While that approach allows one to compare relative superhelicities of different molecules, it does not provide the information needed to analyze conformational equilibria of superhelical DNAs that are not in the standard environment. For this purpose the molecular linking difference must be measured relative to the relaxed state in the given environment, including any constraints on tertiary structure that might be present. It is important to note that the approach used here does not generally give the same values for the linking difference as those found when the molecule is referred to standard conditions.

Consider a DNA domain of specified sequence having linking difference α. There will be many conformational states accessible to this superhelical domain, in each of which α is accommodated by specific torsional and flexural deformations, and possibly also by local conformational transitions to alternative secondary structures. Precisely which flexural, torsional and transconformational ways of accommodating a given value of α are possible, and also their free energy costs, will depend on the length and base sequence of the DNA involved, as well as on the nature of the constraints imposed on the domain and environmental conditions (25-28). A free energy G_i is associated to each state of the system (here indexed by i), which is the sum of the free energies of all the component deformations and transitions of which that state is comprised. A dynamic superhelical equilibrium is determined by the competition among all the possible states of the system (28-30). This equilibrium is a mixture of all the states to which the molecule is susceptible, with the fractional occupancy f_i of state i determined by the contribution of its Boltzmann factor to the governing partition function:

$$f_i = \frac{\exp(-G_i/kT)}{\sum_i \exp(-G_i/kT)}. \tag{3}$$

If a parameter Q has value Q_i in state i, then its equilibrium value $Q(\alpha)$ at linking difference α will be its ensemble average over all the states in the equilibrium distribution:

$$Q(\alpha) = \sum_i f_i Q_i. \tag{4}$$

Any equilibrium property of interest can be computed this way, including the probability of transition, the free energy $G(\alpha)$, and the expected numbers of transformed base pairs and of sites of transition (28-30).

The states accessible to a superhelical DNA domain will depend upon the constraints imposed on its tertiary structure. For molecules in solution whose tertiary structures are not

externally constrained, the linking difference α may be partitioned freely between twisting and writhing. An intermediate level of constraint occurs in a gel, where the accessible tertiary structures are restricted by confinement within the network (31). When the tertiary structure of the domain is fixed by other factors, such as nucleosomal winding, any linking difference must be accommodated by torsional deformations. Because the accessible states vary with the identity of the DNA, the nature of its tertiary constraints and other environmental conditions, the superhelical equilibrium distribution will depend on these factors.

Statistical analysis of the distribution of topoisomers resulting from the ligation of nicked molecules in solution yields experimental estimates of the overall free energy associated to small linking differences. These results are consistent with an energy law, valid for small values of α, having a quadratic form:

$$G(\alpha) = K\alpha^2/2\,, \tag{5}$$

where $K = 2220RT/N$ and N is the number of base pairs in the domain (32). However, this simple quadratic relationship cannot be assumed to be accurate in ranges of superhelicity beyond that where it has been found experimentally to hold.

In the physiological range of superhelicities, $-.1 < \alpha/Lk_0 < -.03$, states involving local conformational transitions may be significantly occupied at equilibrium. Transition to an alternative secondary structure changes the local twist by an amount ΔTw_{trans}. In a negatively superhelical domain, transitions to conformations that are less twisted in the right-handed sense than the B-form will act to relieve the superhelical deformation of the balance of the domain. Although such transitions may be energetically disfavored in linear molecules, they will occur at equilibrium in susceptible negatively superhelical DNAs when the relief of deformation strain energy they afford exceeds their costs. There is strong experimental evidence for the occurrence of several types of transitions in negatively superhelical DNAs. These include strand separations (33-35), B-Z transitions (36-40) and cruciform extrusion (41-44).

In a domain undergoing transition, only the residual linking difference

$$\alpha_{res} = \alpha - \Delta Tw_{trans} = \Delta Tw_{res} + \Delta Wr, \tag{6}$$

that portion of α not absorbed by the change of twist consequent on transition, remains to be accommodated by other types of deformations. In the absence of transition $\alpha_{res} = \alpha$. When a DNA domain is free in solution, α_{res} may be partitioned between smooth torsional deformations ΔTw_{res} and bending deformations ΔWr so as to minimize the conformational free energy involved. When the tertiary structure is fixed by other factors, α_{res} must be expressed entirely as ΔTw_{res}.

To date the free energy associated to the residual linking difference α_{res} has only been determined in narrow ranges of superhelicities. Within each range a simple quadratic energy law represents the best current information regarding the free energy of residual linking:

$$G(\alpha_{res}) = \frac{K\alpha_{res}^2}{2}. \tag{7}$$

Although the actual relationship between free energy and α_{res} may be more complicated than this, the experimental determination of additional terms requires the measurement of higher

order effects. This quadratic expression will agree in magnitude, slope and curvature with the exact law within the experimental range of superhelicities sampled. However, care must be taken not to apply this quadratic expression to regimes of superhelicity beyond the range where it is known to hold. If, as the experimental evidence indicates, the actual law is more complicated, then the experimentally determined best-fitting quadratics of equation 7 will have different coefficients K in different regimes of superhelicity. Moreover, the value of K may also depend on sequence, so that values measured using one DNA might not apply to other molecules K also will depend upon environmental conditions, and on the nature of any constraints imposed on the domain.

For molecules free in solution near the relaxed state where $\alpha = \alpha_{res}$, a quadratic free energy of this type has been measured to high accuracy with $K = 2220RT/N$ (32). At substantial superhelicities a best-fitting quadratic free energy has been determined from the data of Peck and Wang (37) on B-Z transitions within circular supercoiled DNA molecules in a gel. This best-fitting quadratic free energy associated to α_{res} was found to have the value $K = 3015RT/N$ for $-20 < \alpha < -12$, the range where this transition was observed to occur (45). When the tertiary structure is rigidly fixed, any residual linking difference must be expressed entirely as twist, $\alpha_{res} = \Delta Tw_{res}$. In this case the free energy governing torsional deformations also is known to be quadratic (46-49), with the coefficient K related to the sequence-averaged torsional stiffness C of the DNA by $K = 4\pi^2 C/L$, where L is the length of the domain. The measured torsional stiffness of B-form DNA is $C = 2.4\times10^{-19}$ erg-cm, which gives the value $K = 6730RT/N$ for the free energy of residual linking in DNA whose tertiary structure is fixed.

LOCAL TRANSITIONS IN RESTRAINED AND UNRESTRAINED DNA

In this section the equilibrium transition behavior of superhelical DNA domains is analyzed in the presence and absence of tertiary structural restraints. For this purpose a simple example is used of a homopolymeric B-Z transition at a single susceptible site within a closed circular DNA. However, the techniques developed here may be applied to the quantitative analysis of the influence of tertiary restraints on any type of superhelical transition. The qualitative conclusions derived from the present analysis regarding the influence of tertiary restraints on B-Z transition behavior remain valid for other transconformation reactions, including denaturation and cruciform extrusion.

Because the transition of one dinucleotide repeat unit from B- to Z-form decreases the local twist by $A = 0.36$ turns, it alters the effective superhelical deformation on the rest of the domain a corresponding amount. In addition, each junction between B- and Z-forms has a local undertwist of θ turns. If there are n_Z dinucleotide repeat units transformed to the Z-form in r_Z runs, the residual superhelicity of the domain is

$$\alpha_{res} = \alpha + n_Z A + 2r_Z\theta. \tag{8}$$

When there is no transition, $\alpha_{res} = \alpha$.

The free energy associated to each conformational state is comprised of three contributions. In addition to the quadratic free energy associated to α_{res} by equation 7, each

transformed base pair requires free energy b. Also, a cooperativity free energy a is associated to the pair of junctions between the B- and the Z-forms found at the ends of each run of Z-form. The free energy of a state with n_Z Z-form dinucleotide repeat units in r_Z runs is

$$G(n_Z,r_Z,\alpha) = 2bn_Z + ar_Z + (K\alpha^2_{res}/2). \tag{9}$$

All states with the same number n_Z of Z-form repeat units and the same number r_Z of runs have identical free energies. There are $M(n_Z,r_Z)$ of these states, which in the case of a single site comprised of N_Z sequential repeat units is given by the combinatorial expression

$$M(n_Z,r_Z) = \binom{n_Z-1}{r_Z-1}\binom{N_Z-n_Z+1}{r_Z} = \frac{(n_Z-1)!\,(N_Z-n_Z+1)!}{(n_Z-r_Z)!\,(r_Z-1)!\,r_Z!\,(N_Z-n_Z-r_Z+1)!}. \tag{10}$$

Here the unique region susceptible to B-Z transition contains N_Z dinucleotide repeat units, hence $2N_Z$ base pairs.

A partition function is constructed for each fixed value of the linking difference α as the sum of the Boltzmann factors associated to each state of transition. The partition function governing this superhelical transition is:

$$Z = e^{-G(0,0,\alpha)/RT} + \sum_{n_Z=1}^{N_Z}\left[\sum_{r_Z=1}^{r_Z(max)} M(n_Z,r_Z)e^{-G(n_Z,r_Z,\alpha)/RT}\right] \tag{11}$$

Here $r_Z(max)$ is the maximum number of Z-form runs, which is

$$r_Z(max) = \begin{cases} N_Z-n_Z+1, \text{ if } n_Z > [(N_Z+1)/2]; \\ n_Z, \textit{ otherwise.} \end{cases} \tag{12}$$

Here (but not elsewhere) the square brackets denote the greatest integer function.

The midpoint of transition occurs where the aggregate population of all transformed states equals the population of the untransformed state at equilibrium. By applying equation 3 in the present context one finds that this midpoint occurs at that value of the linking difference α where

$$e^{-G(0,0,\alpha)/RT} = \sum_{n_Z=1}^{N_Z}\left[\sum_{r_Z=1}^{r_Z(max)} M(n_Z,r_Z)\, e^{-G(n_Z,r_Z,\alpha)/RT}\right]. \tag{13}$$

Using the expressions for the free energies and residual deformations given by equations 8 and 9, one finds after algebraic manipulation that the midpoint of transition in this case happens at that value of α where

$$\sum_{n_Z=1}^{N_Z}\left[\sum_{r_Z=1}^{r_Z(max)} M(n_Z,r_Z)\, e^{-[2bn_Z+ar_Z+K\alpha y+(Ky^2/2)]/RT}\right] = 1, \tag{14}$$

with

$$y = n_Z A + 2r_Z\theta. \tag{15}$$

The midpoints of the B-Z transition have been calculated from these equations as functions of the coefficient K of the residual linking difference. This has been done for domains of

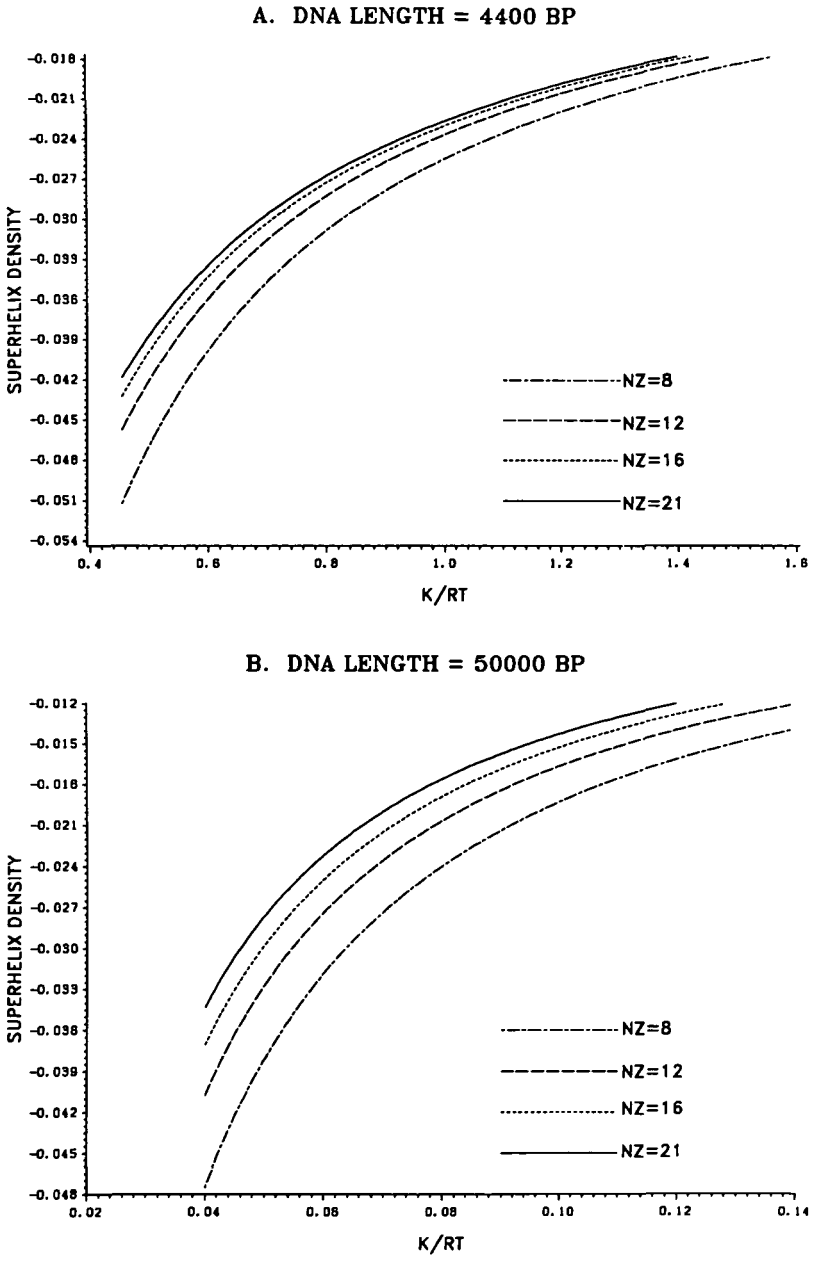

Figure 1: This figure shows the superhelix density at which the midpoint of the B-Z transition described in the text occurs as a function of the coefficient K of the residual free energy. This calculation has been performed for two representative lengths N of the DNA domain and for four sizes NZ of Z-susceptible site (measured in dinucleotide repeat units), as shown in the labels.

Midpoints of Transition				
	N = 4400 bp		N = 50,000 bp	
Tertiary Structure	free	fixed	free	fixed
K/RT	.685	1.53	.060	.135
$NZ = 8$	-.0353	-.0179	-.0318	-.0143
$NZ = 12$	-.0321	-.0170	-.0274	-.0124
$NZ = 16$	-.0308	-.0167	-.0249	-.0114
$NZ = 21$	-.0301	-.0165	-.0232	-.0107

Table 1: This table shows the superhelix densities where the midpoint of the B-Z transition has been calculated to occur at equilibrium according to the methods described in the text. This is done for two domain lengths N and, in each case, for four lengths NZ of the Z-susceptible site (NZ measured in dinucleotide repeat units). For each length N the transition midpoint is found for two cases, where the tertiary structure is entirely unconstrained and where it is rigidly fixed. The entry K/RT is the free energy coefficient of residual linking in each case. For each length N the lower value of this coefficient occurs in the column corresponding to the unconstrained tertiary structure.

length $N = 4400$ bp and $N = 50{,}000$ bp, sizes characteristic of plasmids and loop domains respectively, which contain a single Z-susceptible site whose length is either $N_Z = 8$, 12, 16, or 21 dinucleotide repeat units. In this calculation the values of a, b and θ are used which were deduced (45) from the experimental data of Peck and Wang (37). The results of these calculations are displayed in Figure 1, where the midpoints of transition are expressed in terms of the superhelix density $\sigma = \alpha/Lk_0$ (also known as the specific linking difference). The results displayed in this figure demonstrate that any increase in the apparent stiffness K of a DNA domain to residual linking decreases the superhelical deformation where transition occurs. Large differences in K can greatly alter the onset of transition.

The two cases of greatest interest occur when the tertiary structure of the domain is either entirely unconstrained, corresponding to solution conditions, or where its tertiary structure is rigidly fixed, as may occur *in vivo*. The computed results for these situations are given explicitly in Table 1. When the domain is unconstrained, α_{res} can be freely partitioned between torsional and writhing deformations so as to minimize the free energy involved. In this case the appropriate value of K to use at the substantial superhelicities where transitions occur is $K = 3015RT/N$ (45). In contrast, consider a molecule whose writhing is entirely constrained by other factors, such as recent experiments indicate is the case for genomic DNA in association with nucleosomes (22). Now the entire residual linking difference must be expressed as ΔTw_{res}, so the correct value of K to use in this case is the one which applies to torsional deformations of DNA, which is $K = 6730RT/N$. Table 1 below gives the values of K and the midpoints of transition for the two domain lengths and four susceptible site lengths N_Z, expressed in terms of the superhelix density. These results show that this transition takes place at half the superhelical deformation in rigidly fixed domains as in unconstrained ones. If one uses the value of K that has been measured near the relaxed state, $K = 2220RT/N$, this difference becomes even greater.

DISCUSSION

The central conclusion of the present analysis is that superhelical conformational transitions will occur at less extreme deformations in DNA domains whose tertiary structure is externally constrained than they will in domains which are free to deform, other factors remaining fixed. This suggest that DNA constrained within nucleosomes *in vivo* could experience a rich repertoire of conformational transitions at slight negative superhelicities. Precisely which transitions are possible will depend upon on the base sequence of the DNA involved. Previous analyses have shown that superhelical transition behavior is extremely intricate at equilibrium (28,30,50). Certain transitions may occur only in a narrow range of linking difference, with the reversion of some transitions coupled to the occurrence of others. This suggests that conformational transitions could provide delicate mechanisms by which DNA superhelicity might modulate physiological function *in vivo*.

These results will be quantitatively accurate provided that the relaxed DNA conformations, the energetics of transitions and the torsional stiffness all are the same for DNA whose tertiary structure is constrained as they are for molecules free in solution. However, it is not known at present whether this is the case for DNA that is complexed into nucleosomes. For this reason the conclusions presented here must be considered to be strictly qualitative in character.

In this analysis a quadratic free energy has been ascribed to the residual linking difference α_{res}. For DNA domains free in solution which are near the relaxed state this law is known to be accurate (32). When α_{res} must be expressed entirely as twist, a quadratic free energy also is known to pertain. In other circumstances an effectively quadratic free energy has been deduced, which may be the best-fitting approximation to a more complicated free energy law (37). This approximation will have no significant effect on the calculated midpoints of transitions so long as one remains within the range of α_{res} where the approximate law has been shown to hold, as is the case in the present analysis.

A more precise treatment of the energetics of α_{res} is complicated by the fact that the linking difference is a topological constraint whereas the various torsional and flexural deformations by which it is manifested are geometric, not topological, in character. Moreover, the magnitudes of the total flexural and torsional deformations and the free energies associated to them are all extensive quantities, which α, α_{res} and Wr are not. Except under conditions where the DNA's tertiary structure is fixed by external constraints, neither α nor α_{res} can be expressed as an integral along the molecule of any intensive parameter. A more rigorous analysis of the energetics of residual linking may be feasible, based on an extension of the treatment of mechanical equilibria presented in (51). However, such a calculation would be very complicated to perform and is certainly beyond the needs of the present paper.

The influence of DNA confinement within a gel on superhelical transitions also may be analyzed by the methods described here. In this case the constraint imposed on the DNA tertiary structure by the gel network precludes conformations in which the molecule is widely spread. In particular, the relaxed conformations accessible to molecules in solution are not possible when the DNA is confined in a gel. Because these low energy structures are precluded, relaxed molecules must adopt higher energy conformations having re-entrant tertiary struc-

tures within the gel network. This means that the effective free energy of residual linking is higher near the relaxed state for molecules in a gel than for identical molecules in solution, other factors being fixed. When experimental information becomes available regarding the magnitude of this free energy change, quantitative analyses of superhelical transitions in gel conditions of the type developed in this paper will be possible. The qualitative effect of this type of tertary structural restriction will be to disfavor transitions which act to relax the DNA. This means that higher superhelicities would be required to drive such transitions in a gel than in solution. This matter becomes important because many experiments detect superhelical transitions when the DNA is in a gel. Indications that DNA structure and transition behavior in gels may differ significantly from those in solution have been reported previously (31,52). This may account in part for the different values of K found for relaxed DNA in solution (32) and for superhelical DNA under gel conditions (45).

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