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Dependence of the linking deficiency of supercoiled minichromosomes upon nucleosome distortion

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ABSTRACT

The contribution from each nucleosome to the linking number of minichromosome DNA depends on two factors. These are the wrapping number, ω, which is the number of times the DNA wraps about the axis of the nucleosome; and the winding number, Φ, which is the number of base pairs on the nucleosome divided by the helical repeat of the DNA. If the nucleosome is distorted with DNA surface contacts being preserved, Φ remains unchanged. The wrapping number may still change, however, depending on the extent of the distortion. For example, if the usual cylindrical shape of the nucleosome is deformed into an ellipsoid while preserving the equatorial radius, then the wrapping number will increase. We apply these concepts to minichromosomes torsionally stressed by supercoiling with, for example, DNA gyrase. We analyze the experimental result that the maximum amount of supercoiling obtained by gyrase treatment of minichromosomes is the same as that of naked DNA. In particular, we show that this phenomenon can be explained by a relatively slight distortion of the nucleosome core while maintaining the surface contacts of the DNA on the core.

INTRODUCTION

The primary structural form of eucaryotic DNA is that of a sequence of nucleosome-wrapped segments separated by histone H1-associated linker regions (1). This elementary structure is especially amenable to study in minichromosomes, which consist of relatively short covalently closed circular DNAs complexed with histone octamers (2-4). The covalent closure permits advantage to be taken of the conservation of the linking number (5,6) in the analysis of DNA structural alterations that accompany changes in the number of octamers bound following equilibration with topoisomerases. In a relaxed minichromosome, no change in the linking number takes place upon incubation with a topoisomerase, provided that no nucleosomes are removed from the DNA. In a torsionally strained minichromosome, in contrast, the linking number changes under the same conditions.

The effect of increased torsional strain in minichromosomes has been the subject of a recent investigation (7) in which synthetic minichromosomes, constructed by reconstituting complexes of core histones with closed circular plasmid

pBR322, were treated with DNA gyrase. The surprising result was that the maximum amount of supercoiling that could be obtained by gyrase treatment was the same for the minichromosome as for naked DNA. A possible explanation for this result, as suggested by the authors, is that, in contrast to relaxed minichromosomes, the DNA wrapped on histone octamers is no longer immobilized at high levels of supercoiling. In the present paper we offer an alternative explanation of this result. In particular, we show that slight deformations of the histone core particle itself can account for the results. The DNA may remain immobilized on the core particle, with the points of attachment preserved, but the octamer itself may undergo a relatively slight geometric distortion due to the gyrase-induced supercoiling.

ANALYSIS

The Linking Number of Surface Wrapped DNA

We have shown previously (6,8) that the linking number of closed DNA constrained to lie on a surface divides into two components. These are the winding number, Φ, the number of base pairs divided by the average helical repeat as measured in the reference frame of the surface; and the surface linking number, SLk, the sum of the writhe and that part of the twist induced solely by the surface geometry. In simple cases in which the DNA is planar or is plectonemically supercoiled, SLk = 0 and Lk = Φ. If the DNA is toroidally wrapped ω times about a protein complex, SLk = $\pm\,\omega$ and Lk = $\pm\,\omega + \Phi$. Here the sign is (+) if the wrapping is right-handed and (–) if left-handed. We next apply these concepts to minichromosomes.

The Relaxed Minichromosome

We first analyze the relaxed minichromosome, in which the complex of core histones and closed circular plasmid DNA has been treated with a topoisomerase. In this case the two components of the linking number Lk are easily obtained (6,9). The winding number of the entire DNA is $\Phi = N/h$, for a DNA of N base pairs and an average helical repeat of h base pairs per turn. The surface linking number is equal to the wrapping number of the DNA on each histone octamer, ω, multiplied by the number of octamers, m. The value of ω is 1.8 for a single nucleosome crystal (10-12). The linking number is then

$$Lk = -\omega m + N/h \qquad [1]$$

where the sign of the first term is negative due to the fact that the wrapping is left-handed. Eq. 1 may be interpreted more precisely by dividing the minichromosome into core regions and linker regions. We denote by N_c and h_c the number of base

pairs and the average helical repeat of the DNA on a core particle, and denote by N_l and h_l the corresponding quantities for a linker region. Then Eq. 1 may be rewritten

$$Lk = m[-\omega + N_c/h_c + N_l/h_l] \quad [2]$$

Since the entire structure is relaxed, we take the helical repeat h_l to be equal to that of the relaxed histone-free DNA, denoted h_o (7).

The Supercoiled Minichromosome

If the relaxed minichromosome is supercoiled, each of the three components of the linking number in Eq. 2 may change. In addition, a new tertiary structure-dependent term may be required. First, the linker helical repeat h_l can change significantly from the value for relaxed DNA, depending on the maximum superhelix density that is attained (6). The spatial nature of the supercoiling, whether plectonemic, toroidal or a combination of the two, will also determine the contribution of the linker regions to SLk. If the supercoiling introduced is entirely plectonemic, as in free DNA (13), the resulting change in SLk is zero. If τ toroidal coils are introduced by the supercoiling, then SLk changes by $\pm\tau$, the sign being positive if right-handed and negative if left-handed. Second, it is possible that the DNA is partially lifted off the surface of the histone core particles and supercoiled; in that event both the wrapping number, ω, and the average helical repeat, h_c, can change, depending on the maximum superhelix density attained. The third possibility, which we introduce and examine in detail here, is that the structure of the histone octamer is itself changed. Even without lifting the DNA off the surface, as we show in the next section, ω can change as a result.

All the geometric quantities defined above can in principle change as a result of supercoiling, and we denote these by a superscript asterisk. For the supercoiled minichromosome, the formula for the linking number is obtained by extension of Eq. 2:

$$Lk^* = m[-\omega^* + N_c/h_c^* + N_l/h_l^*] \pm \tau \, . \quad [3]$$

The first and second kinds of structural changes mentioned above have been considered previously for gyrase-induced supercoiling (7).

The Supercoiled Minichromosome with no Detachment of DNA

In this section we discuss the linking number Lk* of the supercoiled minichromosome, subject to the condition that the DNA remains attached to the surface of the histone core octamer. We consider separately the linker and core regions. First, we consider changes in Lk associated with the linker regions, as-

suming that these regions behave like naked DNA. For supercoiled DNA free of any bound protein, the equation of the linking number is straightforward. Written in terms of the superhelix density, the linking number, Lk_f^*, of a supercoiled naked DNA is given by the formula

$$Lk_f^* = (1 + \sigma)\, Lk_0 = (1 + \sigma)\, N/h_0\,, \qquad [4]$$

where Lk_0 is the linking number of the same DNA relaxed, N is the number of base pairs in the DNA, and σ is defined by the equation $\sigma = (Lk_f^* - Lk_0)/Lk_0$. Applying this to the linker regions, the net contribution to Lk from the linker regions is then $m(1 + \sigma)N_l/h_0$. Thus the sum of the linker-associated terms in Eq. 3, $[mN_l/h_l^* \pm \tau]$, is equal to $(1 + \sigma)mN_l/h_0$. This equality is true regardless of the distribution between SLk and Φ. This provides a major simplification in the analysis, since it is unnecessary to know the nature of the coiling of the linker region in the minichromosome.

Next, we consider the changes in Lk associated with the core regions. The winding number in these regions is unchanged (6), as long as the attachment site contacts are preserved. The helical repeat of core DNA is also necessarily unchanged, thus $h_c^* = h_c$. The term $-\omega^* m$ takes into account the change in SLk in the core regions. Thus, the linking number of the supercoiled minichromosome is

$$Lk^* = m[-\omega^* + N_c/h_c + (1 + \sigma)\, N_l/h_0\,], \qquad [5]$$

where ω^* is, as before, the wrapping number of the DNA around the distorted histone core.

Comparison of Supercoiled Free DNA with Supercoiled Minichromosome

In this section we compare the supercoiling of free DNA to that of the same DNA formed into a minichromosome complex. In order to compare linking numbers, we note that $N = m(N_c + N_l)$, so that for the free DNA

$$Lk_f^* = (1 + \sigma)m(N_c + N_l)/h_0. \qquad [6]$$

The difference between the linking numbers of supercoiled free DNA and supercoiled minichromosomal DNA can be ascertained by subtracting Eq. 6 from Eq. 5.

$$Lk^* - Lk_f^* = m[-\omega^* + N_c(1/h_c - (1 + \sigma)/h_0)]. \qquad [7]$$

Eq. 7 shows, as expected, that the properties of the linker DNA do not enter into the difference in linking numbers. This is, of course, due to the assumption that

the linker regions behave like naked DNA.

An interesting special case, which occurs upon supercoiling by gyrase, is that in which Lk^* is the same for free DNA as for minichromosomal DNA. In this case $Lk^* - Lk_f^* = 0$, and Eq. 7 can be solved to obtain the wrapping number.

$$\omega^* = N_c [1/h_c - (1 + \sigma)/h_o] \quad [8]$$

This expression yields the wrapping number ω^* that would be required in order to satisfy two conditions. First, the presence of histone octamers does not change the superhelix density obtained in a given closed circular DNA. Second, the DNA contacts on the histone octamer (14,15) remain intact. Another way of stating this is that ω^* represents the amount of wrapping necessary so that the superhelix density in the core regions of the DNA is equal to the superhelix density caused by the action of the enzymes in the linker regions. We next review how ω^* is changed as the nucleosome core particles are distorted.

Change in the Wrapping Number ω^* due to Histone Core Distortion

The usual geometric model for the histone core is that of a cylinder of revolution of radius 4.3 nm and height 5.04 nm (16). The nucleosomal DNA wraps 1.8 times in a left-handed helical fashion about the cylinder with an average pitch ($2\pi p$) of 2.8 nm (16). We showed previously (17) that for relaxed minichromosomes small distortions of the cylindrical surface of the nucleosome into other surfaces of revolution, such as ellipsoids or hyperboloids, can account for significant changes in the wrapping number. If the deformation is to an ellipsoid, ω^* is numerically greater than 1.8; and if the deformation is to an hyperboloid, ω^* is numerically less than 1.8.

A straightforward way to express the extent of the deformation of a cylinder into an ellipsoid or hyperboloid is in terms of the cross section at the top or bottom of the surface relative to that at the center. Before distortion, all cross sections along the long axis have the same radius as that of the central cross section. After deformation the cross sections increase symmetrically in area from the center to the top and bottom regions if the deformation is to a hyperboloid, and decrease if the deformation is to an ellipsoid. If we denote the radius of top cross section by r_{top}, then the ratio of r_{top} to the fixed central radius (4.3 nm) provides a measure of the extent of the distortion. Since the DNA is assumed to be of constant length, if r_{top} is less than 4.3 nm (the ellipsoid case), ω^* will be greater than 1.8; and if r_{top} is greater than 4.3 nm (the hyperboloid case), ω^* will be less than 1.8. Figure 1 shows the deformation for the ellipsoidal case. Figure 2 presents a plot of ω^* versus r_{top} for the deformations that preserve volume or

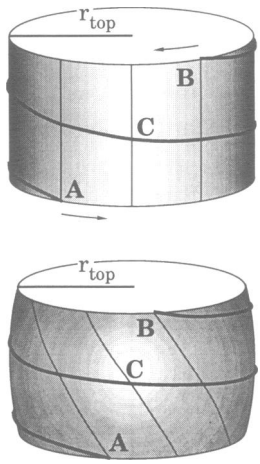

Figure 1: Surface distortion of a cylindrical nucleosome to an ellipsoidal nucleosome. The upper portion of the figure depicts the generally accepted picture of the unstressed cylindrical nucleosome. The radius r_{top} is shown and for this case is approximately 4.3 nm. The axis of the DNA is seen to wrap about the surface 1.8 times in a left-handed manner, with a pitch of approximately 2.8 nm. The lower portion of the figure shows the nucleosome distorted to an ellipsoidal shape, with the height and central radius of the nucleosome unchanged. The distortion can be envisioned as a slight rotational displacement in the direction indicated by the arrows. The magnitude of this displacement is greatest at the top and bottom and decreases toward the central region. Thus, for example, the vertical lines through the points A, B and C in the upper figure are distorted to the curved lines in the lower figure. The value of r_{top} has decreased by 7% from the upper to the lower figure.Here the vertical pitch distance between DNA strands is approximately 2.6 nm, although the actual surface distance is slightly larger due to the elliptical shape. In this case the DNA wraps nearly 1.9 times about the ellipsoidal nucleosome. Exact values for r_{top} versus wrapping number SLk are plotted in Figure 2.

height of the nucleosome. The range of ω^* depicted encompasses a maximum range of approximately 15% distortion.

APPLICATIONS

Application to Gyrase-induced Supercoiling

We now address the specific case in which gyrase is used to supercoil both free DNA and minichromosome DNA. It was found experimentally that Lk_f^* and Lk_f are nearly identical, permitting use of Eq. 8, and that the maximum superhelix density that can be obtained (7) is approximately $\sigma_m = -0.10$. The wrapping number ω changes to a new value, ω_m^*, as a result of nucleosome distortion. This new value is determined by the requirement that the superhelix density on the

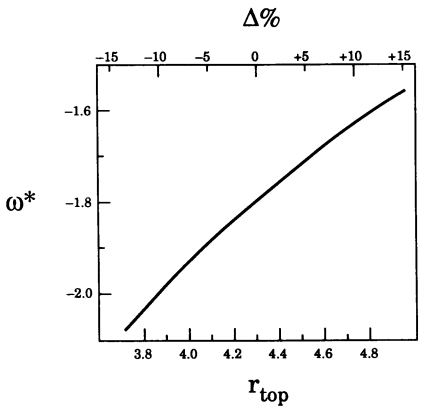

Figure 2: The wrapping number ω* of a distorted nucleosome. The values of ω* are presented as a function of the radius of the top of the solid, r_{top}, or the percent of change in that radius from the cylindrical radius, Δ%, where Δ% = 100 x (r_{top}/4.3 - 1). The distortions induced by gyrase supercoiling are ellipsoidal and are shown in the leftmost portion of the Figure, varying from 0% to approximately −14%. The distortion curve shown is that which arises from those distortions that preserve either height of the nucleosome or volume.

core regions be the same as that induced in the linker regions. We set $\sigma = \sigma_m$ and, using Eq. 8, obtain

$$\omega_m^* = N_c [1.0/h_c - 0.9/h_o]. \quad [9]$$

We take h_o to be 10.5 bp/turn for the magnesium salt of DNA (18-20), h_c to be 10.1 ± 0.1 bp/turn (21,22), and N_c to be 146 bp. The calculated value of ω_m^* is then 1.942 ± 0.146. Referring to Figure 2, the extent of core distortion required varies from 0% at the lower limit of h_c to 14% in the ellipsoidal direction at the upper limit of h_c. On the average, the %Δ is 7% ± 7%. This extent of distortion is comparable to that obtained in previous calculations of the nucleosome distortion needed to account for the temperature dependence of the helical repeat in relaxed minichromosomes, ±5% (17). In the present case the core distortion presumably arises from the torque about the nucleosome long axis generated by gyrase-associated supercoiling, as indicated by the arrows in Figure 1. Since the central H3-H4 tetramer is more rigid that the H2A-H2B terminal dimers, as discussed above, it seems reasonable that the distortion should be towards an ellipsoid. Thus the straight lines through the points A, B and C in the upper part of Figure 1 are distorted to the corresponding curved lines in the lower portion. The exact trajectories of the curved lines can be constructed on the basis of two assumptions. First, the height

of the cylindrical and ellipsoidal nucleosomes are assumed to be the same; and second, the helical pitches, although different, are constant in each case (17).

We emphasize that the above result applies in the event that the specific contacts between the DNA and the histone octamer are preserved during supercoiling of the minichromosome. This is an experimental question that can be answered by, for example, the appropriate nuclease digestion experiment. It is clear that large DNA structural perturbations are not permitted, since nucleosomes do not form with A (23,24) or Z (25) DNAs or with poly(dA) · poly(dT) tracts (23). Nucleosomes do, however, form with DNAs of a variety of defined sequences (D.M. Crothers, private communication). The addition of certain drugs, leading to a structure more complex than that considered here, apparently does lead to alteration of the DNA/octamer structure without disruption (26). In the event that the DNA contacts on the nucleosome are not preserved, and the DNA is allowed to rotate freely, Eq. (9) may be employed to obtain the necessary values of h_c^* or of N_c such that $\omega_m^* = \omega$. The result is

$$h_c^* = \frac{h_o}{\left[\frac{\omega h_o}{N_c} + 0.9\right]} \qquad [10]$$

Thus, an increase in the helical repeat of the nucleosome wrapped DNA by 0.1 bp/turn would also account for the experimental results. The possibilities that the DNA either rotates on the nucleosome or is partially displaced during gyrase treatment were considered earlier (7).

Biological Applications

The concept of nucleosome distortion has possible general application to the structure of DNA-protein complexes, both *in vitro* and *in vivo*. Thus, DNA is commonly torsionally strained in procaryotic nucleoprotein complexes (27,28). Several reports have recently appeared suggesting that eucaryotic DNA is also subject to torsional stress under some circumstances. For example, SV40 minichromosomes from the nuclei of infected cells are sensitive both to staphylococcal nuclease at 0° (29) and to single strand-specific nuclease S1 (30). Treatment with eucaryotic type I topoisomerase eliminates this sensitivity (30), suggesting that the SV40 minichromosome is not completely relaxed in the natural state. Similarly, about half of the minichromosomes produced following injection into *Xenopus laevis* oocytes appear to be supercoiled to an extent greater than can be accounted by nucleosome winding alone (31,32). The presence or absence of torsional strain might well have biological significance as a control mechanism, as suggested by a recent report that the rate of topoisomerization of DNA by type I topoisomerases is extremely slow when the DNA is relaxed (33).

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