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Effects of DNA supercoiling on the topological properties of nucleosomes

(DNA gyrase/minichromosomes/chromatin)

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ABSTRACT **In the nucleosome core particle, at least 145 base pairs of DNA are bound to the histone octamer in a superhelical conformation. We have asked what effect the presence of these particles has on the ability of DNA gyrase to supercoil DNA. Synthetic minichromosomes, constructed by reconstituting complexes of core histones with the closed circular plasmid pBR322, were treated with various amounts of DNA gyrase. We have found that the maximum level of supercoiling that is attainable is nearly identical for protein-free plasmids and for plasmids half-saturated with core histones, even though supercoiling does not result in a loss of histones from the complex. It appears that, at sufficiently high levels of supercoiling, the core particle is disrupted in such a way that the DNA bound to histones is no longer constrained.**

Although there is considerable evidence linking the biological function of prokaryotic DNA to its topological state, the role of DNA supercoiling in eukaryotes is more obscure. Data from transfection experiments in eukaryotic cells suggest that expression of genes introduced on plasmids depends upon the presence of intact closed circular DNA (1). Other evidence suggests that chromatin domains, either genomic (2) or extrachromosomal (3), may exist in some circumstances in torsionally stressed states; it has been argued that such states are associated with expression of genes within the domain. In every case, however, the assessment of the role of supercoiling is complicated because of the presence of histones, which alter torsional stress when they bind to topologically isolated DNA domains.

In the earliest experiments designed to measure the topological effects of histone binding, complexes of core histone octamers and relaxed closed circular DNA were incubated with topoisomerase I, and the resulting linking number change (ΔL) was measured after the DNA had been freed of protein (4). A recent determination by this method gives a value of $\Delta L = -1.0$/core particle (5). The binding of a histone octamer thus has a topological effect equivalent to the unwinding of one turn of DNA duplex. Since the final linking number measurements were made after treatment with topoisomerase I, the observed values refer to a DNA–protein complex that is under no torsional stress.

DNA within the nucleus is not necessarily in such a relaxed state, however. As noted above, there is some evidence that certain regions of the genome are under stress. This could in principle arise either through the removal of histone octamers from a topologically isolated domain or by the action of a putative DNA gyrase. In either case, the way in which the stress is distributed through the domain will be determined by the properties of the DNA in the remaining nucleosomes of the domain. Something is known of the effect of bound histone octamers on the torsional properties of the surrounding DNA when the DNA is relaxed (6), but there have been no measurements of the properties of DNA within nucleosomes associated with plasmids that are superhelically stressed.

In this paper, we examine the effect of the binding of core histones on the topological properties of closed circular DNA at high levels of superhelical stress. Histone octamers were reconstituted onto plasmids, and the complexes were treated with DNA gyrase. We find, surprisingly, that the largest negative change in linking number that can be attained is about the same in the synthetic minichromosomes as it is in protein-free plasmids. This shows that, at high levels of superhelical stress, histone octamers no longer serve to immobilize DNA in the same way as they do in relaxed minichromosomes.

METHODS

DNA gyrase A and B proteins were prepared and characterized as described (7). Core histones were isolated by hydroxylapatite column chromatography by a modification of the procedure of Simon and Felsenfeld (8). NaDodSO$_4$/polyacrylamide gel electrophoresis showed that all four histones were present in equal stoichiometric amounts and were undegraded. Histone concentrations were determined using the extinction coefficients given by Stein (9). Supercoiled plasmid pBR322 DNA was isolated by the alkaline NaDodSO$_4$ method and was banded twice in CsCl/ethidium equilibrium density gradients. Avian erythrocyte nicking-closing extract was prepared from adult chicken erythrocytes as described (10).

Histone–DNA reconstitution was performed by the salt/urea step dialysis method (11) at 50 μg of DNA per ml and the desired concentration of histones. After dialysis an aliquot of the reconstitute was treated with nicking-closing extract to assay the extent of reconstitution. The number of bound octamers was determined from the linking number of the resulting protein-free plasmid.

Gyrase reactions were carried out at a DNA concentration of 37 μg/ml under conditions slightly modified from those previously described (7). The reaction mixture contained 35 mM Tris·HCl (pH 7.5), 4 mM $MgCl_2$, 1.8 mM spermidine hydrochloride, 24 mM KCl, 0.14 mM Na_3EDTA, 5 mM dithiothreitol, 6.5% (wt/vol) glycerol, 1.4 mM ATP, 9 μg of tRNA per ml, and 0.36 mg of bovine serum albumin per ml. To this was added phospho*enol*pyruvate to a final concentration of 10 mM and 0.1 μg of pyruvate kinase per ml as an ATP regenerating system. The reaction was carried out for 4 hr at 25°C. Because gyrase A protein is always present in excess of gyrase B, units are given in terms of gyrase B. The reaction was terminated by the addition of EDTA, and an aliquot was electrophoresed on a 10 × 17 cm, 0.3% gel of GTG agarose (FMC; Rockland, ME) in 10 mM Tris, pH 7.5/1 mM EDTA at 4°C for 2.5 hr at 100 V; the gel was then stained with ethidium bromide. Another aliquot was treated with nicking-closing extract, deproteinized by treatment with

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proteinase K/LiOAc/NaDodSO$_4$, extracted with phenol/chloroform, and precipitated with ethanol. The remainder of the reaction was deproteinized as above without prior nicking-closing extract treatment.

These deproteinized DNA samples were electrophoresed on a 10 × 17 cm, 1.2% agarose gel in 40 mM Tris/30 mM NaH_2PO_4/1 mM EDTA for 15 hr at 40 V with the indicated concentration of chloroquine diphosphate. Samples were routinely analyzed in two gels containing different concentrations of chloroquine diphosphate to distinguish positively from negatively supercoiled DNA. Gels were soaked in 3 changes of buffer, stained 10 min with ethidium bromide (5 μg/ml) in 90 mM Tris borate/3.5 mM EDTA, and destained extensively. Photographic negatives were quantitated by scanning on a Hoefer GS300 scanning densitometer.

RESULTS

We used DNA gyrase to supercoil circular pBR322 DNA that had been complexed with core histones. The histone–DNA complexes were prepared by dialysis of a mixture of the components against graded concentrations of NaCl and urea (see *Methods*); this procedure was designed to result in maximum formation of nucleosome cores. We first examined the effect of various amounts of gyrase on a complex containing an average of 15 nucleosome cores per molecule (Fig. 1). An ATP regenerating system was present in these experiments (see *Methods*). Increasing gyrase concentrations resulted in increased levels of supercoiling until a maximum level was reached. Beyond that point, there was a slight decrease in supercoiling, perhaps reflecting the presence of sufficient gyrase to overwhelm the system for regenerating ATP. Although more gyrase was required to reach maximum supercoiling in histone–DNA complexes than in the protein-free plasmid controls, the superhelix density at this maximum was about the same for the complex as for naked DNA (Fig. 1). In both cases, the superhelix density at the maximum of the gyrase titration was $\sigma \approx -0.10$.

We repeated this experiment with complexes containing various ratios of histone to DNA. Typical results are shown in Fig. 2 for complexes with 7 and 15 octamers per plasmid. The corresponding linking numbers for the 15-octamer complex are given in Table 1, which also provides data from a similar analysis of plasmids carrying 21 octamers. Over a 3-fold range of histone loadings, there is only a small change in the maximum superhelix density. This shows that the extent to which gyrase supercoils the complex cannot reflect merely the relative amounts of linker versus nucleosomal DNA. A different result is obtained with a complex containing 24 octamers: this complex is completely resistant to supercoiling (Fig. 2), presumably because there is no continuous stretch of histone-free DNA long enough to accommodate the enzyme, which occupies about 120 base pairs (bp) when it binds to DNA.

We determined the number of histone octamers bound in each complex by treating the complex with a crude topoisomerase I preparation from avian erythrocytes (see *Methods* and ref. 4). The superhelical density of the resulting DNA reflects the number of histone octamers originally bound: each properly formed nucleosome core induces one negative superhelical turn. This is the method used by Morse and Cantor (6) in determining the number of bound nucleosomes. It does not count any histones that might be bound in a way that does not affect topological properties. We used the same method to determine the number of histone octamers remaining bound to the plasmid after the gyrase reaction. As shown in Fig. 3, the supercoiling attained following topoisomerase I treatment is the same for a given complex before and after exposure to gyrase. This shows that the action of the gyrase does not result in any permanent displacement of histone octamers. The result was confirmed by electrophoresing the complexes themselves before and after exposure to gyrase (data not shown). As expected, complexes containing a higher ratio of histone to DNA migrate more slowly, but gyrase treatment has no effect on the mobility of a given complex, indicating that the complex retains its integrity.

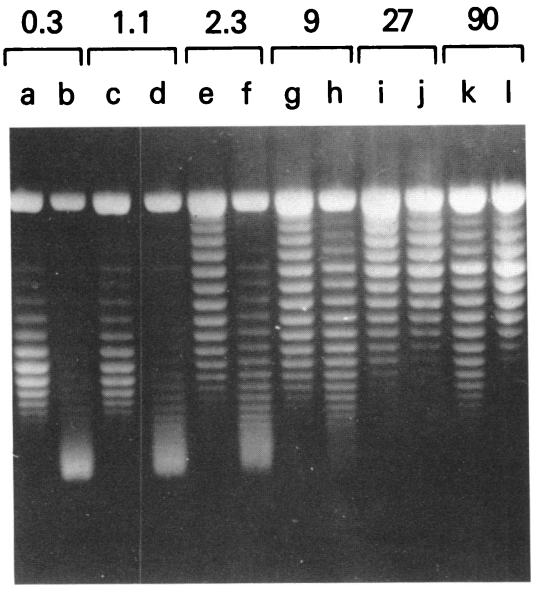

FIG. 1. Effect of various gyrase concentrations on supercoiling of protein-free pBR322 (lanes a, c, e, g, i, and k) and pBR322 carrying an average of 15 histone octamers (lanes b, d, f, h, j, and l). Units of gyrase per μg of plasmid DNA are given at the top of each pair of lanes. Supercoiling reactions are described in the *Methods*. After supercoiling, the plasmids were freed of protein. The electrophoretic gel contained chloroquine at 300 μg/ml. Under these conditions, all of the samples shown are migrating as positively supercoiled DNA, as determined by comparison with a second gel containing less chloroquine.

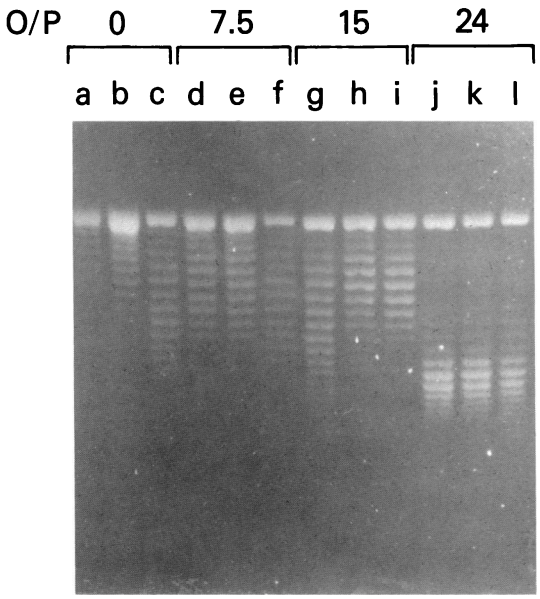

FIG. 2. Supercoiling of pBR322 as a function of bound histone octamers. O/P is the number of octamers per plasmid. For each histone-to-DNA ratio, supercoiling was carried out at three gyrase concentrations (units per μg of plasmid DNA): 9 units (lanes a, d, g, j); 27 units (lanes b, e, h, k); and 90 units (lanes c, f, i, l). The electrophoretic gel contained chloroquine (300 μg/ml).

Table 1. Linking number differences induced by DNA gyrase

*n**	Gyrase, units/μg of DNA	ΔL	
		DNA only	DNA + histones
15	0.3	−36.7	—
	1.1	−37.5	—
	2.3	−40.2	—
	9	−40.0	−38.7
	27	—	−41.7
	90	−39.3	−41.2
21	27	−40.0	—
	90	—	−38.3

*n, Number of nucleosomes.

DISCUSSION

When DNA gyrase acts on closed circular DNA molecules, the maximum level of supercoiling that is achieved is determined by the balance between the driving force of the reaction and the increasing amount of free energy required to further reduce the linking number as the superhelix density σ becomes more negative. This value (12) is about −0.10 for protein-free plasmids, as confirmed by the results shown in Fig. 1. It seemed reasonable to suppose that when nucleosome core particles are present, the part of the DNA wrapped around the octamer to form the core particle might be immobilized in such a way as to resist deformation. If that were true, only the linker DNA that connects core particles might respond to the action of gyrase by altering its twist and writhe.

This expectation was further supported by the observations of Morse and Cantor (6) concerning the ability of DNA in minichromosomes to undergo temperature-dependent changes in conformation. Under the ionic conditions these authors employed, protein-free closed circular DNA undergoes an untwisting of about 0.011°/bp per degree Celsius when the temperature is raised. Morse and Cantor found that each histone octamer appears to prevent about 230 bp of DNA from undergoing the normal thermally induced change. Thus the amount of DNA apparently immobilized in these experiments exceeds the 165 bp contained in the two nucleosomal turns or the 145 bp found in the core particle and includes linker DNA as well.

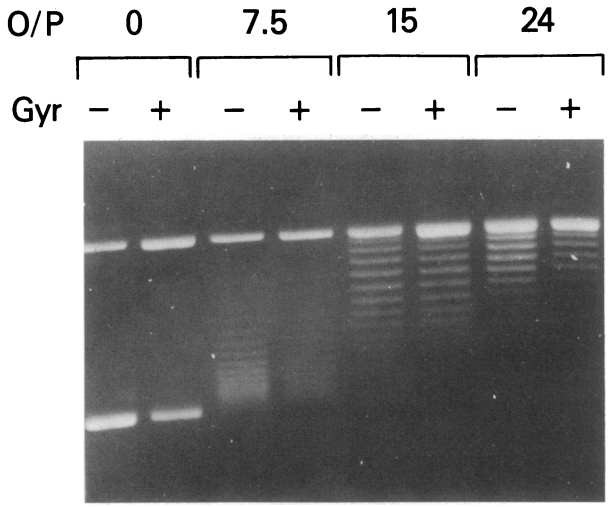

FIG. 3. Control showing that action of gyrase has no effect on the stability of the DNA–histone complex. Complexes of histone octamers with pBR322, with octamer-to-plasmid ratios (O/P) given at the top, were either incubated with (+) or without (−) gyrase and then incubated with topoisomerase I to relax the complexes. The plasmids were freed of protein and electrophoresed (see *Methods*) on a gel containing chloroquine (20 μg/ml). Under these conditions, the DNA in the first six lanes is positively supercoiled and that in the last two lanes (24 octamers per plasmid) is negatively supercoiled.

The results we present here show that at high levels of supercoiling the DNA of the nucleosome behaves in a completely different and unexpected manner. When DNA gyrase acts on reconstituted minichromosomes, the maximum level of supercoiling is identical to that observed when the protein-free plasmid is treated with gyrase. With the exception of the one reconstituted complex that is so loaded with histones as to be totally resistant to gyrase, all complexes can be supercoiled to nearly the same extent regardless of the number of nucleosomes present. In the simplest interpretation, this implies that all of the DNA wound around the histone octamer or in the linker is free to alter its twist and writhe as though it were protein-free.

This behavior is not necessarily inconsistent with the observations of Morse and Cantor, which were made under conditions in which the minichromosomes were relaxed. Presumably the energy of stabilization of the intact nucleosome core is sufficient to resist perturbations at low levels of superhelical stress but not when the minichromosome is highly supercoiled, a possibility envisioned by these authors (6).

If the DNA of the nucleosome were immobilized under our conditions, what values of the linking number should we have expected? Assume that each particle involves two superhelical turns (165 bp) of DNA and a contribution of −1.0 to the linking number (this corresponds to a local superhelix density of about −0.06) and that this DNA is resistant to changes in writhe or twist. Assume also that the remaining DNA of the plasmid, not complexed to histones, behaves like free plasmid DNA with respect to gyrase action. If there are n nucleosomes on a plasmid 4363 bp in length, then the expected linking number change would be $\Delta L = -1.0n + (4363 - s{\cdot}n)\sigma_m/10.5$, where s is the mean number of base pairs immobilized on the nucleosome core's surface (165 in this case) and σ_m is the maximum superhelix density attained by the action of gyrase on free DNA, which is −0.097 (Table 1). The expected values of ΔL for 0, 5, 10, 15, and 21 nucleosomes are −40.2, −37.6, −35.0, −32.4, and −29.3, respectively, which are quite different from the experimental values in Table 1 and Fig. 2. Somewhat different calculated values, varying from −40.2 to −33.1, are obtained if it is assumed that the structure contributing a linking number increment of −1.0 immobilizes 145 bp of DNA. If, on the other hand, we use $s = 230$ as suggested by the data of Morse and Cantor (6) for relaxed minichromosomes, then the predicted value of ΔL is −16.7 when 21 nucleosomes are present. None of the predicted values for any value of s from 145 to 230 bp is consistent with our observation of $\Delta L = -38.3$ for maximally supercoiled minichromosomes with 21 bound histone octamers (Table 1).

How can one account for an average superhelix density of about −0.10 for a maximally supercoiled minichromosome? This could arise because, at sufficiently high superhelical stress, DNA bound to histones is able to alter its conformation freely either by twisting on the octamer surface or by unfolding the complex. Perhaps the entire complement of DNA within the particle behaves in this way, but it is also possible that only a certain fraction, near the ends, can break loose from the surface of the core particle so that it effectively becomes part of the linker DNA (see for example refs. 13–15). Our results would then suggest that the portion of DNA remaining bound to the core must have an average superhelix density close to −0.1. As noted above, the measured value of the linking number increment for the binding of a histone octamer to relaxed DNA is −1.0, and if the measurement refers to an immobilized 230 bp length of DNA, as the results of Morse and Cantor (6) suggest, the

superhelix density averaged over 230 bp is about −0.046. Alternatively, it has been argued from structural data (16) that the 145 bp contained in the core particle would contribute a linking number of −1; this gives a superhelix density, averaged over 145 bp, of −0.07. If we were to assume that the linking number contribution remains constant with further unwinding of the core particle DNA from the ends, then it would be necessary to reduce the immobilized DNA to 105 bp to attain a superhelix density of −0.1. The assumption of a constant linking number contribution with unwinding from the ends requires postulation of a distinctly nonuniform path for the DNA on the core particle surface. Crystallographic studies (17) show that the path of the backbone on the core particle surface is indeed nonuniform, and detailed nuclease digestion studies reveal that the twist of the bound DNA also varies. On the other hand, it would require a rather large nonuniformity (probably in twist), for which there is no evidence, to generate average local superhelix densities as large as −0.1 within the central 105 bp of the core particle. Models in which the DNA is merely unwound from the ends of the core particle, therefore, seem difficult to support.

It is also possible, in principle, that the minichromosome is capable of folding into some higher order structure with a writhe that contributes just enough to the linking number to compensate for the presence of the histone octamers. This seems unlikely, since it would require higher order structures that vary with octamer loading to provide varying degrees of compensation.

The most probable explanation of these observations thus involves the twisting of some considerable fraction of core DNA. Perhaps during this process the DNA remains bound to an intact histone octamer, but this would lead to mismatches between negatively charged phosphodiester groups and positively charged sites on the octamer surface. It seems more likely that the twisting would require partial disruption of the core particle, either by unfolding of the octamer or liberation of DNA from the surface in such a way as to allow the DNA to twist and writhe freely.

The existence of a eukaryotic gyrase, analogous to the prokaryotic enzyme, has not yet been demonstrated unequivocally, although evidence consistent with gyrase activity has been reported (3). Our results suggest that if such an enzyme is present in eukaryotes, its ability to supercoil DNA might not be affected by the presence of histone octamers, at least in those situations where histone H1 is absent and higher order structure disrupted. In that case, the level of supercoiling attained in a topologically isolated domain might be governed, as it is in bacteria, by a steady state involving the supercoiling action of the putative gyrase and the relaxing action of another topoisomerase. In eukaryotes, however, the potential exists to increase superhelical stress also by the local removal of histone octamers. If all of these mechanisms are at work within the nucleus, it will be rather difficult to predict the resulting local superhelix density.

It is not clear what effect intermediate levels of superhelical stress would have on core particle structure. However, our results raise the possibility that sufficiently high levels of superhelical stress within the nucleus may lead to partial disruption of the compact form of the nucleosome.

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