Histone H1 Preferentially Binds to Superhelical DNA Molecules of Higher Compaction

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ABSTRACT In chromatin, the physiological amount of H1 is one molecule per nucleosome or, roughly, one molecule per 200 bp of DNA. We observed that at such a stoichiometry, H1 selectively binds to supercoiled DNA with $|\sigma| \geq 0.012$ (both negative and positive), leaving relaxed, linear, or nicked DNA molecules unbound. When negative and positive DNA topoisomers of varying superhelicity are simultaneously present in the binding mixture, H1 selectively binds to the molecules with highest superhelicity; less supercoiled forms are gradually involved in binding upon increasing the amount of input protein. We explain this topological preference of H1 as the consequence of an increased probability for more than one H1-DNA contact provided by the supercoiling. The existence of simultaneous contacts of H1 with both intertwined DNA strands in the supercoiled DNA molecules is also inferred by topoisomerase relaxation of H1-DNA complexes that had been prefixed with glutaraldehyde.

INTRODUCTION

Linker histone H1 and its variants play a pivotal role in chromatin compaction. Although their exact location in chromatin is still unknown, it is agreed that both entry and exit strands of DNA at the nucleosome receive some protection upon binding of a single linker histone molecule (for recent reviews see Zlatanova and van Holde, 1996; Pruss et al., 1995). In accordance with the concept that one molecule of protein can bind to two DNA strands, crystallographic studies of the linker histone H5 demonstrated that its globular domain may provide at least two potential regions of contact with DNA (Ramakrishnan et al., 1993). Furthermore, site-directed mutagenesis has now clearly shown that these two regions do in fact make such contact (Goytisolo et al., 1996). Bavykin et al. (1990) suggested that in condensed chromatin H1 is localized close to the center of the linker DNA and is rearranged on DNA sequences adjacent to the core particle during chromatin decondensation. Other observations show that transcription activation is associated with unfolding of chromatin fiber, mediated by a reduced H1 complement (Zlatanova and van Holde, 1992; van Holde and Zlatanova, 1996). What are the mechanisms whereby regional redistribution, depletion, or enrichment of H1 in specific chromatin regions is induced and maintained? Numerous studies aimed at answering this question have used free DNA as a model substrate for H1 binding in chromatin (Zlatanova and Yaneva, 1991).

Electrophoresis (De Bernardin et al., 1986), sedimentation (Böttger et al., 1976, 1981; Singer and Singer, 1978; D'Anna et al., 1979; Vogel and Singer, 1975a), and filter-binding experiments (Vogel and Singer, 1975a,b, 1976; Yaneva et al., 1990) have demonstrated a preferential interaction of H1 with superhelical DNA and suggested that the extent of interaction may be related to the degree of superhelicity. Moreover, a biphasic response of H1 binding to titration with ethidium bromide (EthBr) was observed in sedimentation (Böttger et al., 1981; Vogel and Singer, 1975b) and filter-binding experiments (Vogel and Singer, 1975b), suggesting that H1 binds preferentially to both negatively and positively supercoiled DNA, as compared to the relaxed form. However, no convincing explanation for this preference has been forthcoming.

To more closely examine the extent of selectivity of H1 for the topological state of DNA, we have carried out experiments under competitive conditions, i.e., when different DNA forms are simultaneously present in the binding mixture. The experiments were performed at low salt concentrations, because the selective binding to supercoiled DNA seems to be more pronounced under such conditions (De Bernardin et al., 1986). Our results demonstrate that H1 selectively binds to those DNA molecules in the mixture that have the highest number of superhelical turns and shows, at most, a limited discrimination between negative and positive topoisomers of comparable superhelical density. We interpret these results to mean that the plectonemically coiled superhelix provides a highly favorable environment for H1 binding, by allowing for more than one independent contact between the double helix and the globular domain of H1.

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MATERIALS AND METHODS

Purification of plasmid DNA

Plasmids pBR322 and pUC19 were purified on CsCl density gradients (Sambrook et al., 1989). DNA containing a limited number of nicks per molecule was prepared by digestion with deoxyribonuclease I (DNase I) (0.068 u/μg DNA) in the presence of EthBr (Clark and Felsenfeld, 1991). Linear DNA was prepared by a single-site restriction nuclease digestion of

the plasmids followed by phenol/chloroform extraction and ethanol precipitation. Partially relaxed topoisomers of pBR322 with a specific linking difference (superhelical density) σ ranging from 0 to about ± 0.02 were prepared by treatment of plasmids with wheat germ topoisomerase I (Promega) in the presence of different amounts of EthBr (Singleton and Wells, 1982) or $MgCl_2$. Relaxed products were gel analyzed and appropriate samples were combined to obtain populations of topoisomers spanning different ranges of supercoiling density. To obtain a mixture of negative and positive topoisomers, the procedure of Jackson (1995) was used. *Escherichia coli* AS19 cells (gift from Dr. L. Liu) were transformed with pUC19. Cells were treated with novobiocin and plasmid DNA fractionated in a CsCl gradient in the presence of 1 mg of EthBr/ml. DNA from appropriate gradient fractions was collected and purified by butanol and phenol extractions and ethanol precipitation.

Purification of linker histones

H1 and H5 were purified from chicken erythrocytes under nondenaturing conditions from NaCl extracts of purified nuclei (Banchev et al., 1991). The globular domains of H1 and H5 were obtained from a trypsin digest of the proteins, as described by Krylov et al. (1993).

DNA binding assay

Mixtures containing 25–75 μg of DNA/ml and different amounts of protein (see legends to figures) were prepared at room temperature in a buffer containing 20 mM 2-(*N*-morpholino)ethanesulfonic acid-KOH, pH 6.6, 1 mM Na_2EDTA, and 22.5 μg of bovine serum albumin/ml. After 15 min of incubation, samples were analyzed by gel retardation.

Gel electrophoresis

Electrophoretic fractionation of purified DNA and DNA-protein complexes was performed in 1% agarose gels using Tris-acetate buffer (Sambrook et al., 1989) at room temperature and 2.5 V/cm. For two-dimensional (2D) electrophoretic analysis, samples were first separated under the above conditions. The gels were then soaked in 2.5 μg of chloroquine/ml (for pBR322) or 2.0 μg of chloroquine/ml (for pUC19) for 2 h, rotated by 90°, and reelectrophoresed in the second dimension in the presence of the same concentration of chloroquine (Bowater et al., 1992). Scanning was performed on an EPSON ES-1200C scanner using Adobe Photoshop 3.0 program (Adobe Systems, Mountain View, CA). For Western analysis, material from the gel was transferred to nitrocellulose filters after digesting DNA by DNase I directly in the gel (Ivanchenko and Zlatanova, 1996) and stained with anti-H1 antibody (gift from Drs. J. Yaneva and S. Zacharieva).

Topoisomerase I relaxation of H1-DNA complexes

H1-DNA complexes were prepared and fixed with 0.08% of glutaraldehyde overnight at room temperature. Fixed samples were freed from the glutaraldehyde by ethanol precipitation and washing twice with 70% of ethanol, and relaxed with topoisomerase I for 6 h at 37°C in a buffer containing 50 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$, 10 mM dithiothreitol, and 25 μg of bovine serum albumin/ml.

RESULTS

H1 strongly prefers supercoiled DNA to linear or nicked DNA; among supercoiled molecules, H1 prefers the molecules with the highest superhelicity

Plasmids pBR322 and pUC19 were purified from bacteria at a superhelical density $\sigma \approx -0.06$, which corresponds to ~27 superhelical turns for a DNA molecule of the size of pBR322 and to ~17 superhelical turns for pUC19 (Bates and Maxwell, 1993). The level of superhelicity was determined by electrophoretic separation in long 2D gels, using a ladder of topoisomers (Bowater et al., 1992) as a standard (not shown).

Mixtures containing roughly equal amounts of supercoiled, linear, and nicked DNA were prepared, incubated with H1, and analyzed by agarose gel electrophoresis (Fig. 1, *left*). At low input protein/DNA ratios, only the supercoiled DNA was retarded by the protein. At the highest input ratios, linear and nicked DNA also showed a slight retardation. This result raises the following question: is the difference in retardation due to a difference in the level of binding of H1 to the DNA forms, or is the supercoiled DNA simply more retarded at a given level of binding? Similar experiments by De Bernardin et al. (1986) using Coomassie blue staining failed to show evidence for H1 binding to nicked DNA in competition with supercoiled DNA. However, these samples contained only about 10% of nicked circles and no linear DNA. To provide a more definitive answer to the above question, the material from the agarose gel in Fig. 1 was transferred to nitrocellulose membranes and stained with anti-H1 antiserum (Fig. 1, *right*). The intensity of staining on the retarded supercoiled DNA increased with increasing input of protein; at the same time, only a faint reaction was observed on the retarded linear and nicked DNA forms, and this only at the highest H1/DNA ratio used. No H1 staining was ever observed on nonretarded DNA bands. Thus the extent of retardation was

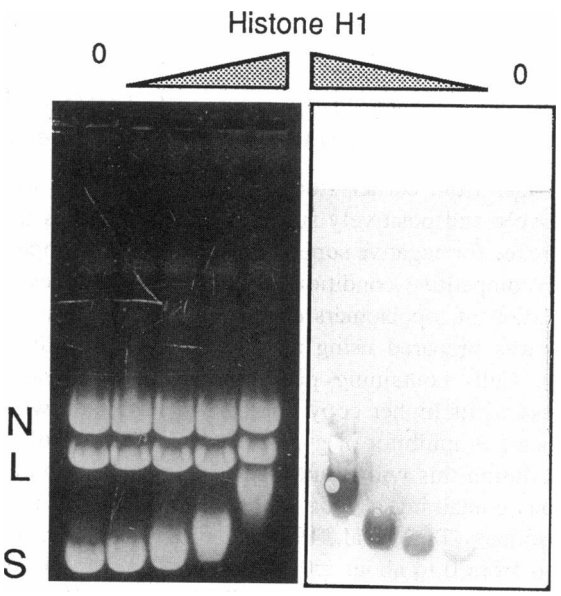

FIGURE 1 Selectivity of H1 binding to highly supercoiled DNA. H1 was incubated with a mixture of supercoiled (S), nicked (N), and linear (L) pBR322, and the products of interaction were analyzed by agarose gel electrophoresis. (*Left*) Gel stained with EthBr; (*right*) staining with anti-H1 antibody. H1/DNA ratios (in molecules of H1 per bp) were 0, 1/600, 1/300, 1/150, and 1/75 in consecutive lanes from left to right in the EthBr-stained gel, and from right to left in the Western blot.

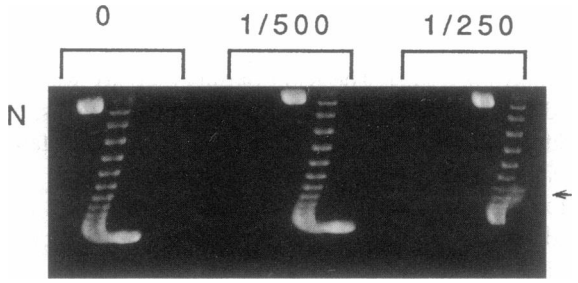

FIGURE 2 Interaction of H1 with negatively supercoiled DNA. Complexes formed with pBR322 topoisomers with σ ranging from 0 to ~ -0.06 are analyzed on 2D gels by using 2.5 μg of chloroquine/ml in the second dimension. The arrow denotes retarded topoisomers. N denotes the position of nicked DNA. H1/DNA ratios are given above the brackets.

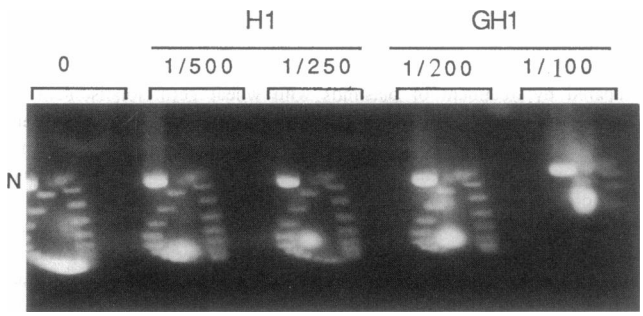

FIGURE 3 Interaction of H1 and its globular domain (GH1) with a mixture of negative and positive topoisomers of pUC19, with σ from 0 to $\sim \pm 0.06$. The right-handed arc of the pattern is formed by topoisomers that were positive in the first dimension of electrophoresis; the left-handed arc is formed by topoisomeres that were negative. The slowest band connecting the two arcs represents the relaxed topoisomer; the most supercoiled topoisomers are not well resolved in the first dimension of electrophoresis. Chloroquine, adding positive supercoils to closed DNA molecules, increases the migration of positive topoisomers and decreases the migration of negative topoisomers, permitting their resolution in the second dimension. The bright spot between the two arcs is formed by retarded H1/DNA complexes. N denotes the position of nicked DNA. H1/DNA ratios are given above the brackets.

directly related to the amount of bound protein. Furthermore, because the three DNA bands were of roughly equal intensity, the Western blots demonstrated a dramatically higher affinity of H1 for supercoiled DNA.

A broadening of the retarded DNA bands was detectable upon H1 titration, suggesting unequal loading of the DNA molecules with protein. Such a heterogeneity of binding is likely a consequence of heterogeneity in superhelicity of the supercoiled DNA molecules in the plasmid population.

To study the extent of preference of H1 for DNA of varying superhelicity, an artificial population of pBR322 topoisomers was used in H1 binding experiments. As Fig. 2 shows, the topoisomers with the highest number of supercoils ($\sigma \approx -0.05$ and higher) were the only ones to be retarded at physiological H1/DNA ratios (one molecule H1 per 250 bp).

The preference of H1 for supercoiled DNA does not depend strongly on the sign of the supercoils

It is clear from earlier studies that H1 will bind to both negatively and positively supercoiled DNA. But is there a preference for negative supercoils versus positive supercoils under competitive conditions? To address this question, a population of topoisomers containing both kinds of molecules was prepared using an in vivo approach (Jackson, 1995). Cells containing pUC19 (this plasmid was used because of its higher copy number) were treated with novobiocin, an inhibitor of gyrase; under conditions of active transcription this will result in production of plasmid populations containing a wide range of negative and positive topoisomers (Tsao et al., 1989). A mixture of topoisomers with σ from 0 to about ± 0.06 was titrated with H1 or the globular domain of H1 (Fig. 3). With increased input protein, topoisomers with less superhelical turns were more and more involved in the retardation; under these circumstances no evident preference for either topoisomer was observed (especially evident for GH1; see the 1/100 bp ratio in Fig. 3). Because the globular domain of H1 shows a preference for supercoiled DNA similar to that of the intact histone, the preference must reside in some structural feature of the globular domain and does not depend on the histone tails.

Estimation of the preference of H1 for negatively supercoiled DNA based on its DNA unwinding effect

If the binding of a protein to DNA produces a change in the twist or writhe of the molecule, then it follows from simple thermodynamic arguments that the topological state must influence the binding (see, for example, Bates and Maxwell, 1993; Vologodskii and Cozzarelli, 1994). Specifically, a ligand that unwinds DNA should bind preferentially to a molecule with negative supercoils compared to an unconstrained molecule (nicked or linear), because the binding of the ligand will reduce the unfavorable (negative) supercoiling. Binding to a relaxed closed circle and positively supercoiled molecules will be even less favorable, because the result of binding is the creation of energetically unfavorable (positive) writhing. In accord with this, nonhistone protein HMG1, known to unwind DNA by $\sim 60°$ per molecule bound (Sheflin and Spaulding, 1989; Sheflin et al., 1993), preferentially binds to negatively supercoiled DNA as compared to nicked or positively supercoiled plasmids (Sheflin et al., 1993). We have recently reported that each molecule of H1 unwinds DNA by $\sim 10°$ (Ivanchenko et al., 1996a). Should this lead to a significant preference for negative supercoils? To answer this question we perform a simple calculation. If binding of one molecule produces a change in twist $\Delta\theta$, then the remaining closed plasmid must experience a compensatory change in superhelix density $\Delta\sigma$,

$$\Delta\sigma = -\frac{\Delta\Theta/360}{N/\gamma}, \qquad (1)$$

where N is the number of base pairs in the plasmid, and γ is the number of base pairs per turn of DNA (10.4). This change in σ will correspond to an additional increment in free energy in the binding reaction, which can be calculated from the expression for supercoiling free energy (Vologodskii and Cozzarelli, 1994),

$$G_{sc} = KRTN^2\sigma^2/\gamma^2, \quad (2)$$

where K is a constant for a particular plasmid and is proportional to $1/N$. It also depends on ionic strength (Vologodskii and Cozzarelli, 1994). Differentiating Eq. 2 to get ΔG_{sc} for small $\Delta\sigma$, we get

$$\Delta G_{sc} = 2KRTN^2\sigma\Delta\sigma/\gamma^2. \quad (3)$$

We have estimated K at low ionic strength as $1700/N$, using data of Vologodskii and Cozzarelli (1994). Combining Eq. 1 and Eq. 3 yields

$$\Delta G_{sc} = -\frac{2 \times 1700RT}{10.4 \times 360}\sigma\Delta\Theta, \quad (4)$$

or at 25°C,

$$\Delta G_{sc} = -5.4 \times 10^2\sigma\Delta\Theta, \quad (5)$$

which corresponds, at $\sigma = -0.06$, and $\Delta\theta = -10°$, to $\Delta G_{sc} = -0.32$ kcal/mol. This is the free energy "advantage" in H1 binding to a plasmid with $\sigma = -0.06$, as compared to binding to a relaxed plasmid ($\sigma = 0$), and corresponds to a ratio of only 1.7 in favor of the former. We conclude, therefore, that for a plasmid population with $|\sigma| \leq 0.06$, this small contribution to the free energy of binding cannot explain the strong preference for negatively supercoiled DNA compared to linear and nicked forms (Fig. 1), or the selectivity for neighboring negative topoisomers differing only slightly in σ (Fig. 2). Furthermore, preferential binding to positive topoisomers over more relaxed forms (Fig. 3) certainly cannot be explained in this fashion. On the other hand, it should be noted that the sixfold greater unwinding produced by HMG 1 (Sheflin and Spaulding, 1989; Sheflin et al., 1993) can easily explain why this protein preferably binds to negatively supercoiled DNA, even at low values of $|\sigma|$ (Sheflin et al., 1993).

The preference of H1 for topoisomers decreases for low states of superhelical density

To define the lowest σ value at which the corresponding topoisomer would be selectively bound, the same physiological H1/DNA ratios (one H1 molecule per 100–300 bp) were tested with several more relaxed populations of pBR322 (Fig. 4). No selective retardation of particular topoisomers was observed at $|\sigma| \leq 0.010$ (0–4 turns) (Fig. 4 *A*). With samples containing topoisomers having slightly larger σ (± 0.012, about five superhelical turns), the selectivity for particular topoisomers was more pronounced, as demonstrated by the disappearance of topoisomers +5 and −5 from their initial position in the gel at all three H1/DNA ratios tested (Fig. 4 *B*). The position of the retarded complexes is difficult to assess with these relatively relaxed populations, possibly because of partial overlapping with the free DNA bands. Such a preference of H1 for pBR322 molecules of superhelical densities in this range over the more relaxed forms was reported elsewhere (Krylov et al., 1993).

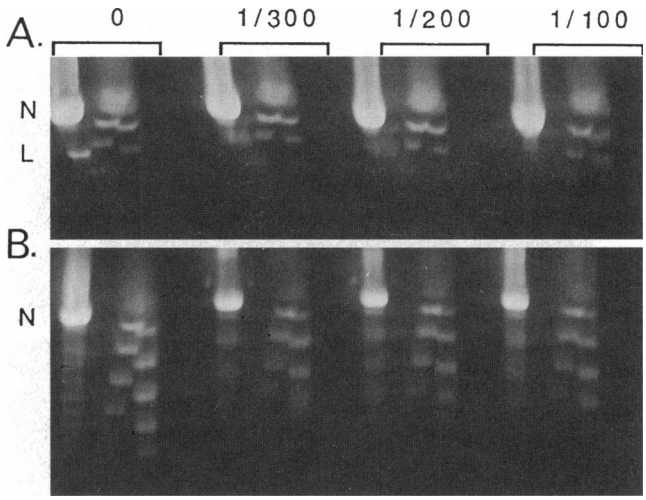

FIGURE 4 Interaction of H1 with mixtures of negative and positive topoisomers at low and moderate superhelical densities. The ranges of superhelical densities are from 0 to ± 0.007 (*A*) and from 0 to ± 0.01 (*B*). H1/DNA ratios are given above the brackets. N denotes the position of nicked DNA, and L denotes the position of linear DNA.

Cross-linking of H1 to DNA prevents relaxation by topoisomerase I

Bina-Stein and Singer (1977) reported previously that binding of H1 limited the relaxation of pBR322 by topoisomerase I and interpreted their data as indicating stabilization of preexisting superhelical turns by H1 binding. To test whether such an interpretation was correct, we prepared a mixture of topoisomers containing from 0 to ~9 positive or negative turns (σ from 0 to ± 0.02). This superhelical density is low enough to provide good resolution of neighboring topoisomers in 2D gels; at the same time such a mixture contains topoisomers with $|\sigma| \geq 0.012$, to which H1 selectively binds at moderate protein/DNA ratios. H1 was added to the mixture at different ratios, and the samples were fixed with 0.08% of glutaraldehyde and then relaxed by topoisomerase I under conditions that lead to complete relaxation of free DNA. A parallel set of samples was relaxed without fixing the protein to DNA. Eukaryotic topoisomerase I relaxes both negatively and positively supercoiled DNA, giving rise to a narrow distribution of topoisomers centered around the fully relaxed molecule. Because the relaxation and electrophoretic separation were performed under different environmental conditions, the center of the observed distribution corresponded to ~ +1.6 instead of 0 superhelical turns (see Shure and Vinograd, 1976; Wang, 1969; for an explanation of this shift). As Fig. 5

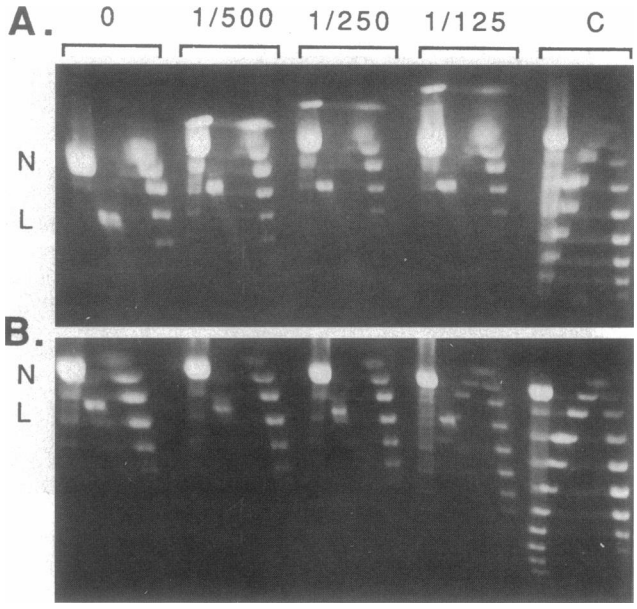

FIGURE 5 Relaxation of H1/DNA complexes by topoisomerase I. (*A*) Relaxation products of nonfixed samples; (*B*) relaxation products of samples after fixation with glutaraldehyde. H1/DNA ratios are shown above the brackets. C shows the control DNA before relaxation. N and L denote the position of nicked and linear DNA, respectively.

A shows for the nonfixed material, almost identical relaxation was achieved in the control reaction and in the relaxation mixtures containing H1 (compare sample "0" with the three other samples). The slight shift to negative topoisomers upon increasing the amount of added H1 can be explained by the unwinding effect of H1 (Ivanchenko et al., 1996a,b), which is almost negligible at the protein/DNA ratios used here. However, with an increased amount of H1 in the fixed samples, the relaxation that could be attained gradually decreased (Figs. 5 *B* and 6), as evidenced by the presence of topoisomers with more and more remaining superhelical turns. A slight gradual shift to more positively supercoiled products was also observed in fixed reactions (Fig. 6).

DISCUSSION

When H1 was added at a physiologically relevant ratio to a mixture of DNA molecules of different topological state, H1 was observed to bind almost exclusively to the molecules with the highest superhelical density (negative or positive), leaving the nonconstrained (nicked or linear) and the less supercoiled molecules unbound. This selectivity was lost at $|\sigma| \leq 0.012$. The preference for supercoiled DNA cannot be explained by the unwinding properties of H1. Our results on these points confirm, extend, and quantitate some earlier studies (see Introduction).

The question remains as to the reason for this topological preference of H1. A possible explanation, at least for the results with highly superhelical topoisomers, could be torsion-induced formation of single-stranded regions to which H1 might preferably bind. However, we do not favor this possibility, because we were not able to detect any preference for H1 binding to single-stranded DNA molecules over the same molecules after annealing to their double-stranded form (not shown). Other kinds of non-B DNA structures that may form in highly supercoiled pBR322, such as cruciforms, B-DNA/Z-DNA junctions, triplex sites, etc. (for a review, see Palecek, 1991), should also be considered. Indeed,

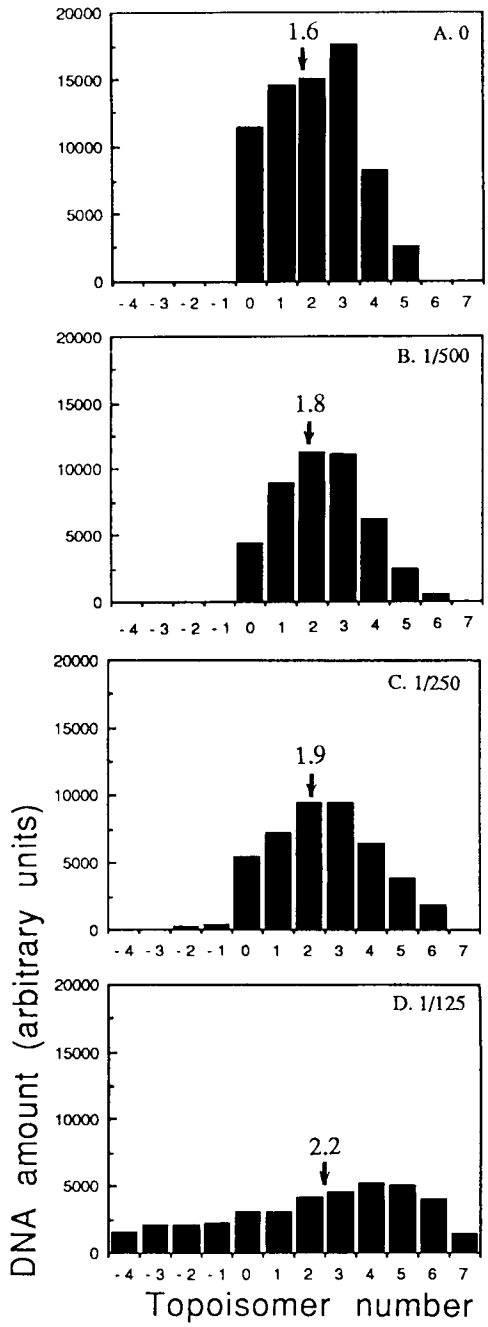

FIGURE 6 Scan of samples from Fig. 5 *B*, comparing the products of topoisomerase I relaxation at different H1/DNA ratios. The arrows and numbers above them represent the centers of topoisomer distribution. The statistical centers of distribution were determined as the point at which the sum of the material present in all proceeding successive topoisomers reaches 50% of the total material present in each pattern.

a preference of H1 for four-way junction DNA (a structure present at the base of cruciforms in supercoiled plasmids) was previously reported from our laboratory (Varga-Weisz et al., 1993, 1994). However, such structures should be sensitive to cleavage by single-strand-specific nucleases such as S1 or P1; no such cleavages occur in pBR322 at superhelical densities less negative than −0.03 (~13 superhelical turns) (Ivanchenko et al., 1996b; Carnevali et al., 1984). At the same time, the preferential binding of H1 is still detectable, even at $|\sigma| \sim 0.012$ (Fig. 4 *B*), suggesting no direct correlation with the presence of non-B DNA.

De Bernardin et al. (1986) speculated that the preferential binding sites for H1 are likely to be along the closely intertwined duplex strands in supercoiled DNA because the negative charge density in such a structure is higher than in a single duplex DNA. However, such an electrostatic effect also seems insufficient to explain the preference for topoisomers with higher superhelical density, because the same level of preference is manifested by the purified globular domain of H1 (Fig. 3), which carries much less positive charge than the intact molecule (Clark and Thomas, 1986).

Our explanation for the selectivity proposes that the possibility for multiple H1-DNA contacts is strongly enhanced in plectonemically wound supercoiled plasmids. This hypothesis is supported by the results of topoisomerase I relaxation of H1-DNA complexes after fixation with glutaraldehyde (Fig. 5 *B*). In the presence of H1, some of the DNA turns could not be relaxed, suggesting that H1 braided the two DNA strands together. This effect of H1 cannot be seen in nonfixed samples (Fig. 5 *A*), suggesting that the double H1-DNA contacts are not strong enough to prevent the relaxation by topoisomerase, or that the equilibrium between bound and unbound states permits eventual relaxation.

Based on Monte Carlo simulations, Vologodskii et al. have recently predicted the effect of supercoiling on some protein-DNA interactions (Vologodskii et al., 1992; Vologodskii and Cozzarelli, 1996). Relaxed DNA molecules are expected to be irregular in form, with occasional random foldovers in projection. However, by $\sigma \geq -0.03$, the molecules are all plectonemically wound. Moderate and high supercoiling is calculated to increase the probability of juxtaposition of two DNA sites by about a factor of 100, and this effect is almost independent of ionic strength. This effect of supercoiling may be even higher for a protein like H1, which, in general, does not require specific binding sites on the DNA strands and can explain the strong preference of H1 for both negative and positive topoisomers having $|\sigma| > 0.03$ (Figs. 1–3). A second effect of supercoiling is predicted to result from notable changes in the relative angular orientation of the intertwined DNA duplexes, even at very low supercoiling ($\sigma = -0.01$) (Vologodskii and Cozzarelli, 1996). If the binding surfaces on the H1 molecule are relatively fixed in spatial orientation (Ramakrishnan et al., 1993), the change in the angular orientation of the DNA strands provided by low levels of supercoiling may contribute to the free energy of H1-DNA binding. In accordance, we observe a preference of H1 for negative and positive topoisomers at σ as small as ±0.012 (Fig. 4 *B*).

As judged by cryoelectron microscope images (Adrian et al., 1990), the effective diameter of intertwined superhelices of naturally supercoiled plasmids is ~12 nM at low ionic strength (10 mM Tris-HCl, 1 mM EDTA). This is still about four times the size of the globular domain of H1 (~2.9 nM; Aviles et al., 1978). However, when a molecule of H1 binds to a given (random) site of a supercoiled plasmid, the positive charges of the molecule should neutralize the phosphates in the DNA, allowing the opposing intertwined strands to approach each other even more closely, further increasing the probability of braiding the DNA strands together. If the most important requirement for stable binding of H1 is the possibility of more than one simultaneous H1-DNA contact, this may explain not only the preference for supercoiled DNA, but also the formation of "tramline"-like complexes involving two parallel linear DNA duplexes braided together by H1 molecules, observed in other laboratories (Clark and Thomas, 1986, 1988; Thomas et al., 1992; Draves et al., 1992; Goytisolo et al., 1996).

It has recently been reported (Pruss et al., 1996) that the globular domain of the linker histones is asymmetrically located inside the gyres of DNA in a nucleosome reconstituted on a 5S rDNA sequence. Such a location would allow interaction with only one DNA duplex. The present results on the interaction of histone H1 with naked DNA would support a location nearer the entry/exit point of the nucleosomal DNA, where linker histones can simultaneously bind to both DNA duplexes (Zlatanova and van Holde, 1996). Whether the location reported for the 5S gene represents a special case remains to be directly tested with other defined nucleosomes employing the type of footprinting experiments used by Pruss et al. (1996).

In the perspective of our explanation for the selectivity, the shift to positive relaxation products produced by H1 in fixed samples (Figs. 5 *B* and 6) is not unexpected. As can be seen in Figs. 3–5, positive topoisomers migrate faster in gels than their negative counterparts, suggesting higher compaction; such more compacted molecules may provide better substrates for H1 binding. Positively supercoiled DNA was also shown to be significantly more flexible than negatively supercoiled DNA (Selvin et al., 1992) and thus may be a better substrate for simultaneous binding at multiple sites. Such factors may outweigh the slight preference that the small unwinding produced by H1 should give to negatively supercoiled DNA.

Finally, we are tempted to speculate that regional redistributions of H1 in chromatin (Bavykin et al., 1990), as well as the reduction of H1 complement during transcription (Zlatanova and van Holde, 1992), may be modulated, among other things, by changes in the proximity and the angular distribution of the DNA strands to which H1 simultaneously binds.

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