The effect of H1 histone on the action of DNA-relaxing enzyme

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ABSTRACT

The action of DNA-relaxing enzyme on H1-DNA complexes was investigated. Complexes of superhelical and relaxed closed circular duplex DNA with H1 were treated with mammalian relaxing enzyme, deproteinized, and electrophoresed on agarose gels. At relatively low ratios of H1 to superhelical DNA, molecules of superhelical density intermediate between those of the starting material and relaxed DNA, the normal product, were generated. At relatively high H1 histone concentrations (H1:DNA greater than 0.4 w/w), the superhelical DNA was not relaxed. Further, no superhelical turns were introduced into relaxed closed duplex DNA at any concentration of H1 tested. Thus, the binding of H1 histone to DNA prevents the action of the relaxing enzyme. Moreover, H1 histone does not appear to unwind the DNA duplex upon binding. The implications of these observations and the previously demonstrated specificity of H1 histone for superhelical DNA are discussed in relation to the structure of chromatin.

INTRODUCTION

The fundamental structural unit of chromatin fibers in eukaryotic nuclei appears to consist of 200 base pairs of DNA associated with histones (1-5). Approximately 140-160 base pairs are associated with a complex of histones H2A, H2B, H3 and H4 (6-9) and the additional residues appear to be associated with H1 histone (10, 11). These elementary particles have been named nu bodies or nucleosomes (2, 12). The DNA constrained in the nucleosomal particles is compacted 6-7 fold at 0.15 M NaCl (13) and a number of structural models describing the possible course of the DNA duplex around the histone core have been proposed (14-17).

H1 histone interacts with protein free superhelical DNA (18-21). The extent of interaction of SV40 DNA with H1 histone depends on the degree of superhelicity (19, 20) and increases as the number of negative or positive superhelical turns increase. This dependence was demonstrated by two different approaches. First, DNA molecules of varying negative and positive superhelicity were generated by titration with ethidium bromide and their

interaction with H1 was studied (19). Second, closed circular DNAs of varying negative superhelical densities were prepared by sealing nicked circular duplexes with polynucleotide ligase in the presence of varying concentrations of ethidium bromide, and, after removal of the dye, it was shown that the percent of DNA bound at a given H1 concentration varies with superhelical density (20). There are several possible explanations for the dependence of the interaction on superhelical density. The binding of H1 histone might unwind the DNA duplex thereby removing superhelical turns. In that case the conformational free energy contained in the superhelical turns would favor the binding reaction (22, 23). Alternatively, the interaction might result from specificity for particular structural features of supercoiled DNA. Germond et al (24) demonstrated that the formation of each nucleosome (composed of DNA and histones H2A, H2B, H3 and H4) introduces a torsional constraint equivalent to an unwinding of one turn of the DNA duplex. In an effort to distinguish between the two possible mechanisms described above for the interaction between H1 and superhelical DNA we carried out experiments analogous to those of Germond et al. (24). We studied the products of the action of mammalian DNA-relaxing enzyme (24-27) on complexes of superhelical and relaxed closed circular duplex SV40 DNA and H1 histone at several H1 histone to DNA ratios.

MATERIALS AND METHODS

The relaxing enzyme from 3×10^9 LA9 cells was prepared by the method of Germond et al. (24). We are grateful to Dr. W. McBride for providing the cells. One μ l of relaxing enzyme preparation converted 40 μ g of native superhelical SV40 DNA (DNA I) to relaxed closed circular duplex DNA (DNA I^R) in 15 minutes at 22°. SV40 DNA I was purified from Hirt supernatant fractions as previously described (18) or from purified virions by a slightly modified standard procedure (28). In general a narrower distribution of superhelical densities was observed with DNA I obtained from purified virions than was observed with DNA obtained from Hirt supernatant fractions, as previously reported (29). The SV40 DNA isolated from virions has about 24-26 negative superhelical turns (27, 30). DNA I^R was obtained by incubating DNA I under our standard reaction conditions (20) [0.1 M NaCl, 0.05 M Tris-HCl, pH 7.8, 1 mM EDTA, and 100 μ g/ml bovine serum albumin (Sigma)], in the presence of relaxing enzyme at 22°. The reaction was terminated by the addition of sodium dodecylsulfate (1% final concentration). DNA containing an average of about 15 negative superhelical turns (DNA I⁻¹⁵) was prepared by incubating DNA I (78 μ g of DNA in a final volume of 1.4 ml)

under the same conditions but in the presence of 0.034 μg of ethidium bromide per μg of DNA (27). The DNA I^{r} and DNA I^{-15} were subsequently purified by phenol extraction, ethanol precipitation, and extensive dialysis against 1.5 mM NaCl, 0.15 mM sodium citrate, 1 mM EDTA, pH 7.0.

The H1-DNA complexes were prepared by incubating 2-3 μg of DNA in 0.1 ml at various ratios of H1 to DNA in the standard assay mixture for 10 minutes at 22°. The H1-DNA complex was then incubated with one μl of relaxing enzyme for an additional 25 minutes. The reaction was stopped by addition of sodium dodecylsulfate, as above, and the mixture was extracted with phenol. The aqueous phase was extracted with ether and the DNA was subsequently precipitated with two volumes of ethanol. Keller showed (31) that it is possible to resolve covalently closed superhelical DNA of different superhelical densities by agarose gel electrophoresis. DNA I exhibits a higher mobility than does DNA I^{r} and closed circular duplexes of intermediate superhelical densities migrate between DNA I and DNA I^{r} . We characterized the DNA product after treatment with H1 histone and relaxing enzyme by electrophoresis on 1% agarose slab gels (31) using the buffer system [36 mM Tris-HCl, 30 mM NaH_2PO_4 , 1 mM EDTA, pH 7.7] described by Germond and co-workers (24). A one cm cushion of 6% acrylamide, 0.1% bisacrylamide in the same running buffer was formed at the bottom of the slabs prior to addition of agarose. The acrylamide gel prevented the sliding of the agarose gel between the glass plates. The gels were run for 2 hr prior to application of the samples. After ethanol precipitation the DNA samples were dissolved in the electrophoresis buffer containing 10% glycerol and 0.05% bromophenol blue. Electrophoresis was carried out for 9 hrs at room temperature at 60 volts. The gels were stained with 0.5 $\mu\text{g}/\text{ml}$ of ethidium bromide (32) in the same buffer for 30 minutes. Fluorescent DNA bands were detected by illuminating the gel with an ultraviolet transilluminator (Ultra-Violet Products, Inc.) and were photographed (31).

Formation of H1-DNA complexes was measured by the nitrocellulose filter binding assay, as previously described (18-20).

RESULTS

Effect of relaxing enzyme on complexes of H1 with superhelical DNA

DNA I was associated with H1 histone at different H1 to DNA ratios. The complex was subsequently treated with relaxing enzyme. Thereafter the reaction mixture was deproteinized and the DNA was subjected to agarose gel electrophoresis. In the absence of H1 histone, treatment of DNA I with relaxing enzyme results in the formation of DNA I^{r} (Figure 1,H), as expected

Figure 1: Agarose gel electrophoresis of DNA I after treatment with DNA relaxing enzyme at various H1 histone to DNA ratios. The concentration of DNA was kept constant (20 $\mu\text{g/ml}$). All other components and the procedures are described in the text.

A) DNA I marker, untreated, contaminated with some nicked DNA.

In B through H, the ratio of H1 to DNA I (w/w) was: B) 0.7, C) 0.52, D) 0.36, E) 0.29, F) 0.26, G) 0.13, H) 0.0.

(33, 34). When H1 histone is added to DNA I the amount of DNA I converted to DNA I^r is decreased and DNA molecules with superhelical densities between that of DNA I and DNA I^r accumulate (Figure 1, D-G). Above an H1 to DNA ratio of about 0.4 to 0.5 (w/w) the DNA I is completely resistant to the action of the relaxing enzyme (Figure 1, B, C). Similar results were obtained obtained when DNA I⁻¹⁵ was used (Figure 2).

We investigated the possibility that the H1 was inactivating the DNA-relaxing enzyme. In the experiment shown in Figure 3 a concentration of H1 histone affording complete inhibition of relaxing enzyme action (H1:DNA, 0.5, w:w) was chosen. H1 and DNA were allowed to associate as usual and then the mixture was treated with relaxing enzyme. Thereafter, additional DNA was added to certain reaction mixtures and incubation was continued. Finally, all the samples were deproteinized and electrophoresed. In those samples which received the additional DNA, the activity of the relaxing enzyme was again apparent (Figure 3C, D and F).

Effect of relaxing enzyme on H1-DNA I^r complexes

Varying concentrations of H1 histone were incubated with DNA I^r and the complex was then treated with relaxing enzyme. As expected, the

Figure 2: Agarose gel electrophoresis of DNA I⁻¹⁵ after treatment with relaxing enzyme at various H1 histone to DNA ratios. From left to right the H1 to DNA I⁻¹⁵ ratios (w/w) were 0, 0.1, 0.2, 0.3, 0.35 and 0.4, respectively. The last channel at the right is the DNA I⁻¹⁵ marker.

DNA I^r starting material (Figure 4G) contains a distribution of molecules having a mean value of zero superhelical turns (33,34). The agarose gel electrophoresis of the DNA after deproteinization reveals that in the presence of H1 histone, the relaxing enzyme does not introduce detectable superhelical turns into the relaxed DNA (Figure 4). The apparent increase in the number of turns in the product with increasing concentrations of H1 histone (Figure 4A-D) represents increasing protection of the turns present in the starting material. This is demonstrated by Figure 4F and G: the center of distribution of the DNA molecules in the starting material (Figure 4G) has the same mobility as the center in the product obtained at the highest H1 concentration (Figure 4D and 4F are the same sample run on different gels). H1 histone does stabilize the few superhelical turns present in the starting material (compare Figure 4, F and G).

Figure 3: Agarose gel electrophoresis of DNA I treated with DNA-relaxing enzyme at constant H1 concentration with the subsequent addition of extra DNA. H1 histone and DNA I were associated at 10 μg per ml of H1 and 20 μg per ml of DNA I in a total volume of 100 μl , for 15 min at 22°. DNA-relaxing enzyme was then added and incubation continued for 15 min. Subsequently, additional DNA I was added to the indicated reactions and incubation was continued for 10 additional minutes. The reaction was stopped and reaction mixtures were treated as described in Materials and Methods. A) No H1, no additional DNA; B) no additional DNA; C) 2 μg additional DNA; D) 4 μg additional DNA; E) untreated DNA I marker; F) the same as D; G) the same as E. F) and G) were on a separate gel and 1 μg of the DNA was electrophoresed. In A) through E), 0.5 μg of DNA was electrophoresed.

H1-DNA complex formation as measured by the filter binding assay

The concentrations of DNA used to study the effect of H1 on relaxing enzyme activity are considerably higher than the concentrations used previously in the filter binding assay (18-20). Therefore the interaction of H1 with superhelical DNA was studied by the nitro-cellulose filter assay at concentrations of DNA comparable to the concentrations used in the above experiments (Figure 5). The specificity for interaction with DNA I compared to DNA I^r observed previously at low DNA concentrations is apparent.

DISCUSSION

Superhelical DNA interacts with H1 histone in a way that is dependent on the superhelicity and independent of the sign of the superhelical turn: both negative and positive superhelices interact preferentially compared to relaxed DNA (19, 20). In order to elucidate the nature of the reaction between H1 histone and superhelical DNA we have treated the H1 histone-

Figure 4: Agarose gel electrophoresis of DNA I^r treated with relaxing enzyme at varying H1 to DNA I^r ratios. The DNA I^r was at a constant concentration of 20 µg/ml. The reaction conditions are described under Materials and Methods. The first channel at the left (A) had no H1 added. The subsequent channels correspond to H1 to DNA I^r ratios (w/w) of B) 0.2; C) 0.35; D) 0.5. Channel E is the DNA I marker. Channel F and G are from a separate gel. F is the same as D; G is the starting DNA I^r.

DNA complexes with an extract containing DNA relaxing activity and characterized the topological state of the resulting DNA. In analogy to the experiments of Germond and coworkers (24), if the interaction of H1 histone with DNA unwound or coiled the DNA duplex, one would expect a) superhelical turns to remain after treatment of H1-DNA I with DNA relaxing enzyme and subsequent removal of the protein and b) superhelical turns to be generated by the action of DNA relaxing enzyme on the H1-DNA I^r complex and subsequent removal of the protein.

Experiments documented in Figure 1 and Figure 2 demonstrate that, in the absence of H1 histone, the DNA I present in the reaction mixture is converted to DNA I^r (Figure 1 H). Addition of H1 to DNA I results in the accumulation of DNA molecules with superhelical density distributed between DNA I^r and the starting SV40 DNA I (Figure 1 G). Further increase of the H1 to DNA I ratio results in a decrease of the amount of DNA I^r with a

Figure 5: Nitrocellulose filter binding assay for DNA I (●) and DNA I^r (○) with H1 histone. The DNA was kept at a constant concentration of 28 g/ml and titrated with increasing amounts of H1 histone. All the other components are described under Materials and Methods. The binding assay was previously described in detail (18).

concomitant increase in the quantity of DNA having an increasing number of superhelical turns (Figure 1G-D). At an H1- DNA I ratio greater than 0.4 all the superhelical turns present in the DNA I are stabilized against the action of relaxing enzyme (Figure 1D). Similar results are obtained when DNA having an average of 15 negative superhelical turns is used. The interaction of H1 with DNA I⁻¹⁵ stabilizes all the preexisting superhelical turns, but no DNA having a higher superhelical density than the starting material is generated (Figure 2). Figure 4 indicates that when DNA I^r-H1 complexes are treated with the relaxing enzyme no detectable increase in the number of negative superhelical turns is obtained. The above observations suggest that H1 histone, upon interaction with closed circular DNA duplex, does not introduce any superhelical twists onto the DNA but fully stabilizes the preexisting superhelical turns. Stabilization of the preexisting superhelical turns could result from interaction of H1 with particular structural features of supercoiled DNA. At relatively high H1 concentrations stabilization may result, in part, from incipient aggregation induced by H1 since rapidly sedimenting H1-SV40 DNA complexes have been observed at high H1/DNA ratios (21). Intramolecular crosslinking of DNA by H1 as well as intermolecular crosslinking of DNA into a network like structure could result in an inhibition of relaxation of the superhelical turns through stabilization of the supercoiled structure.

Since the DNA is compacted 6-7 fold in the nucleosome (13) one may predict that segments of double helical DNA come in close proximity with one another. Topological similarities between such a structure and that of a protein-free superhelical DNA in solution may exist. The properties of the specific interaction of H1 with protein-free superhelices (18-20) therefore suggest that H1 might interact with the coiled (14, 16) or kinked (15, 17) DNA on the outside of the nucleosome thus stabilizing the preexisting turns. Recently several groups of investigators (6, 10, 11) have obtained evidence suggesting that while 150 to 170 base pairs may be involved in the basic nucleosome structure another 30 to 50 base pairs may be associated with the nucleosome in a complex involving H1 histone. The fact that H1 appears to be associated with a particular additional length of DNA suggests that interaction may occur near the point where DNA emerges from its association with the basic nucleosome core. Since H1 does not distinguish between positive and negative superhelical turns, the sign of the pitch of a coiled DNA, or the direction of a kink, would not be a determining factor in the binding of H1, and might in fact differ from one nucleosome to another.

An alternative explanation for the observations reported here is that H1 histone could interact with the relaxing enzyme and thereby inhibit the enzyme from fully relaxing the superhelical DNA. The result in Figure 3 indicates that whereas at an H1 to DNA ratio of 0.5 no relaxed DNA was generated by the action of the relaxing enzyme, subsequent addition of DNA I results in a redistribution (19) of H1 on the DNA molecules and allows the enzyme to react with the unprotected superhelical turns. It should be noted that interference with DNA-relaxing activity occurs at H1 histone concentrations that are similar to those required for complex formation as measured by the nitro-cellulose technique. Furthermore, partial interference, at relatively low H1 concentrations, results in the accumulation of DNA molecules containing superhelical densities intermediate between those of DNA I and DNA I^R, not a simple decrease in the rate of conversion of DNA I to DNA I^R. These observations indicate that H1 does not inhibit the relaxing enzyme irreversibly. A reversible interaction between H1 and the enzyme can not be ruled out at this time.

Finally, the results presented here suggest certain speculative considerations concerning the function of H1 histone in, for example, DNA replication or transcription. H1 histone may control the ability of DNA-relaxing enzyme to unwind DNA duplexes preliminary to the replication of a segment of DNA. There are conformational attributes of H1 histone that are involved in

the specific interaction of H1 with superhelices, in particular, a globular structure near the middle of the polypeptide (35). It is possible that alterations in the conformation of H1, perhaps resulting from known modifications of amino acid side chains (see reference 36 for review), influence the ability of the DNA-relaxing enzyme to act.

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