Differences among Subfractions of H1 Histone in Retention of Linear and Superhelical DNA on Filters*

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Four kinds of rabbit thymus H1 histone differ among themselves in their ability to retain DNA on nitrocellulose filters. This is true for linear, or superhelical DNA, but the order of effectiveness of the different H1 histones depends on the physical conformation of the DNA. For linear DNA the binding efficiencies of the H1 histones are: RTL2 > RTL3 > RTL4 > RTL1. This order of effectiveness parallels the effectiveness of the H1 histones previously found for the condensation of linear DNA as observed by circular dichroism and viscosity. The binding efficiencies of the various histones toward superhelical DNA were: RTL4 > RTL3 > RTL1 > RTL2. The variation in amino acid sequence between different rabbit thymus H1 histones might thus introduce structural variations in nucleohistone fibers and perhaps in chromatin.

Histone H1 is generally thought to be involved in the condensation of chromatin. Only a modest amount of attention has been given, however, to the possibility of multiple degrees of condensation that might depend upon selective interactions between various kinds of DNA and H1 histones.

Selectivity in the binding of DNA by H1 histone has been studied by measuring the quantity of various DNAs retained by the histone on nitrocellular filters. Renn showed in competition experiments (1) that base composition affects selectivity. Singer and her coworkers have reported that histone H1 has a higher affinity for supercoiled DNA than for linear DNA (2-5). Vogel and Singer showed that H1 is 2 to 3 times more efficient in binding circular, supercoiled DNA (SV40 Form I) to nitrocellulose filters than it is in binding either relaxed circles (SV40, Form II) or fulllength linear DNA (SV40, Form III) (2). Poly-L-lysine with an average molecular weight of 23,000 was unable to cause accumulation of SV40 DNA (I) on nitrocellulose filters at the low ratios of protein to DNA where histone H1 was effective (4). This difference between H1 histone and polylysine has been taken (4) to indicate that the reaction of H1 and superhelical DNA depends on more complicated structural aspects of H1 than simply its polycationic nature.

The studies mentioned above were carried out with unfractionated mixtures of H1 histones, but this class of histones is generally heterogeneous in amino acid sequence, even when obtained from a single animal. The structural diversity of H1 histone within single organisms has been proven by peptide mapping (6), sequence studies (7), and immunology (8). These differences are not to be confused with varying degrees of phosphorylation (9) since the level of phosphorylation in most mature tissues is trivial (10). It is clear, then, that the multiplicity of H1 histones must impart a compositional heterogeneity to chromatin, even apart from phosphorylation. The question arises whether that compositional heterogeneity generates conformational variations within chromatin. Since the relative proportions of H1 subfractions are correlated with the terminally differentiated phenotype (11, 12), hormonal induction (13), mitotic index (14), and embryonic development (15), such conformational variations might even have functional significance. A plausible basis for the introduction of structural heterogeneity into chromatin by a multiplicity of H1 histones was revealed in our recent studies on complexes formed between T7 DNA and individual H1 histone subfractions (16). The H1 histones differed among themselves in their ability to condense this linear DNA, as measured with circular dichroism and viscosity.

The variation among H1 histones in their ability to condense linear DNA might be taken along with the salt-dependent selectivity of H1 toward (A + T)-rich DNA and the preferential binding of a particular conformation (superhelix) of DNA to suggest that particular three-dimensional amino acid distributions in H1 histone are critical for the binding and condensation of DNA. If this suggestion is true, then it is likely that the differences observed in their condensation of linear DNA would not be the same for the binding and condensation of supercoiled DNA. This likelihood was borne out in the experiments about to be reported, which showed that H1 histones differed among themselves in their ability to bind superhelical DNA to nitrocellulose filters, but that their differences did not match those observed in the case of linear or relaxed DNA.

MATERIALS AND METHODS

Nitrocellulose filters, 13 mm in diameter, were punched from sheets of nitrocellulose obtained from Schleicher and Schuell (type B6, 0.45 µ pore size). Filters, 25 mm in diameter, were purchased precut from Schleicher and Schuell (type B6).

The 13-mm filters were soaked overnight in assay dilution buffer (0.01 M citrate, pH 7, 0.1 mM EDTA, NaCl to the desired concentration). Any filters that did not wet immediately were discarded. In assays with SV40 DNA (I) and SV40 DNA (II and III), the salt concentration was 0.1 M NaCl with T7 DNA the ionic strength was varied from 0.01 to 0.2 M NaCl. In a typical assay, the desired amount of histone was diluted to 90 µl with distilled water immediately before use. Ten microliters of DNA containing 0.1 to 0.3 µg of DNA were added to 100 µl of incubation buffer (2 times dilution buffer). The histone was placed on the filter with no vacuum applied. The reaction was started by adding the DNA in incubation buffer. After 2 min at room temperature the vacuum was applied to give a filtering rate of 0.8 ml/min. (A preincubation of 2 min was chosen on the basis of kinetic studies that showed no increase in DNA binding...
occurred with longer times.) After the sample passed through, the filter was washed with 1 ml of dilution buffer, dried, and counted in toluene-based scintillation fluid. Total volume of the assay was 200 μl; final DNA concentration was 1 to 2 pg/ml. Total DNA was determined by spotting an aliquot of DNA on a presoaked filter, drying, and counting. For the binding data on T7 DNA, all points are the mean of at least three experimental determinations. In the binding data of SV40 DNA (I) and SV40 DNA (I and III), all points are the mean of six experimental determinations. Preparations of SV40 DNA (I and III) were prepared from SV40 DNA by relaxing the supercoiled DNA with DNase I. The reaction was carried out in an incubation mixture containing 48 mM Tris-HCl, pH 8.2, 5.3 mM MgCl₂, 4.8 mM β-mercaptoethanol; 0.05 mg of DNase per nmol of SV40 DNA (I) was incubated to ethanol; 0.05 mg of DNase per nmol of SV40 DNA (I) and SV40 DNA (I and III), all points are the mean of at least three experimental determinations.

Preparation of SV40 DNA (II and III)—SV40 DNA (II and III) was prepared from SV40 DNA (I) by relaxing the supercoiled DNA with DNase I. The reaction was carried out in a incubation mixture containing 48 mM Tris-HCl, pH 8.2, 5.3 mM MgCl₂, 4.8 mM β-mercaptoethanol; 0.05 mg of DNase per nmol of SV40 DNA (I) was incubated at 30°C for 15 min. The reaction was stopped by chilling and adding EDTA, pH 7.0, to 0.075 mM. After dialysis with 2 volumes of water, the number of nicks per SV40 DNA was determined with the filter-binding assay. The preparation of SV40 DNA (II and III) used contained 1.25 nicks per DNA molecule; assuming a Poisson distribution of random cleavage, it was left as a mixture of nicked, relaxed circles and full-length linear SV40 DNA.

Contamination of SV40 DNA (I) with nicked, relaxed circles was determined by use of a filter-binding assay, measuring the amount of single-strand DNA which binds to nitrocellulose after alkaline treatment of the DNA. Ten microliters of the DNA to be tested were added to 200 μl of 10 mM Tris, pH 8. Two-hundred microliters of 0.3 M K₂HPO₄-KOH, pH 12.4, were added, mixed, and left at room temperature for 3 min. The solution was neutralized by adding 100 μl of 1 M KH₂PO₄-HCl, pH 7.0, followed by 200 μl of 5 mM NaCl, 0.05 mM Tris, pH 8. The entire volume was filtered through a 25-mm nitrocellulose filter presoaked in 1 M NaCl, 0.05 mM Tris, pH 8. The flow rate was no faster than 10 μl/min. The filter was washed with 0.3 M NaCl, 0.03 M citrate, pH 7, dried, and counted. Only DNA which originally existed as nicked, relaxed circles would remain single stranded and bind to the filter. The background was usually about 3%. Contamination was always less than 10%.

RESULTS

Differences between subfractions of rabbit thymus H1 histone were exhibited in the efficiency with which they bound T7 DNA to nitrocellulose filters in 0.1 M NaCl (Fig. 1). RTL2 and RTL3 appear to be the most effective in binding T7 DNA to the filter, and RTL1 the least effective. The differences between subfractions seem quite significant since each point in Fig. 1 represents six experimental determinations that showed an average deviation of ±6% from the mean.

As the salt concentration was lowered to 0.01 M NaCl, DNA retention was increased 4- to 5-fold, but the binding was nonspecific in the sense that there was no difference from one subfraction of H1 to the next. Upon increasing the ionic strength to 0.2 M NaCl, differences between subfractions were much the same as they were at 0.1 M NaCl, and the efficiencies of binding were slightly reduced. These observations are parallel to those of Singer and Singer who showed that the selective binding of superhelical DNA by unfractionated H1 was maximal between 0.1 M and 0.2 M NaCl while nonspecific retention of relaxed DNA simply increased sharply as the ionic strength was lowered.

Differences among H1 histones, seen in their interaction with linear DNA, were also observed when they formed complexes with superhelical SV40 DNA (I) (Fig. 2). However, the order of effectiveness of the H1 subfractions in binding DNA to the filters was not the same for the two kinds of DNA. RTL4 was the most efficient in binding supercoiled DNA, RTL2 was the least efficient, and RTL1 and RTL3 were intermediate. At H1/DNA = 0.2, RTL4 bound twice as much supercoiled DNA to the filter as did RTL2.

The difference in the order of effectiveness of H1 histone subfractions in their binding of T7 DNA and SV40 DNA (I) was due to superhelicity and was not simply caused by differences between the two DNAs in size or base composition. This was shown by allowing RTL2 and RTL4, the two most divergent subfractions when binding Form I of SV40 DNA, to interact with SV40 DNA that had been relaxed with DNase I to give a mixture of Forms II and III. As expected (Table I) neither histone bound the relaxed forms of SV40 DNA as well as they did the supercoiled form. More to the point, however, was that the 2-fold difference in retention power between RTL2 and RTL4 toward the supercoiled SV40 DNA (I) was

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**Table 1**

<table>
<thead>
<tr>
<th>Histone/DNA Retention (%)</th>
<th>RTL2</th>
<th>RTL4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Form I</strong></td>
<td><strong>Form II/III</strong></td>
<td><strong>Form I</strong></td>
</tr>
<tr>
<td>0</td>
<td>2.7</td>
<td>3.3</td>
</tr>
<tr>
<td>0.05</td>
<td>4.3</td>
<td>11.2</td>
</tr>
<tr>
<td>0.1</td>
<td>12.5</td>
<td>17.1</td>
</tr>
<tr>
<td>0.2</td>
<td>25.3</td>
<td>22.6</td>
</tr>
<tr>
<td>0.5</td>
<td>57.9</td>
<td>25.5</td>
</tr>
</tbody>
</table>

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**Fig. 2. Interaction of supercoiled SV40 DNA with subfractions of H1 histone.** Standard reaction mixtures contained 0.1 M NaCl, 1.2 μg/ml of [³H]SV40 (I) DNA. ○, RTL1; △, RTL2; Δ, RTL3; ●, RTL4.
no longer evident in their binding of relaxed SV40 DNA to the filters.

**DISCUSSION**

The principal conclusion to be drawn from the present observations is that the various H1 histones from rabbit thymus differ among themselves in their interactions with DNA, as expressed in the retention of DNA on nitrocellulose filters. They differ whether the DNA is supercoiled or not, but the pattern of differences depends on the physical form of the DNA.

The differences demonstrated in the present work between various rabbit thymus H1 histones, in their ability to retain T7 DNA (linear) on nitrocellulose filters, were correlated with differences we saw earlier in their ability to condense this DNA. Indeed there is a parallel between the order of retention efficiencies and the order of effectiveness in condensation as measured by both viscosity and circular dichroism. For example, RTL3 was the most efficient at retaining T7 DNA on filters, and T7 DNA/RTL3 complexes (10) were the least viscous and showed the most distorted DNA circular dichroic spectra. In complementary fashion, complexes of T7 DNA and RTL1 had the highest intrinsic viscosity (almost twice that of T7 DNA/RTL3 complexes) and the least distorted spectra (16), while RTL1 was shown in the present work to be the least effective in retaining T7 DNA on filters.

Among other things the correlation between the present results with linear DNA and the previous ones could mean that a condensation or aggregation process is a significant factor in the filter-binding assay. Indeed ultracentrifugal studies revealed that H1 histone forms aggregates when it is factor in the filter-binding assay. Indeed ultracentrifugal studies revealed that H1 histone forms aggregates when it is complexed with DNA. D’Anna et al. (18) reported that supercoiled DNA formed heterogeneous aggregates of sedimentation coefficient \( >1500 \) S, in addition to a complex of 140 S and perhaps another at 32 S where the naked DNA sedimented. Somewhat similarly, Singer and Singer (19) found cooperative formation of H1/DNA complexes (presumably with sedimentation coefficients including both 140 S and \( \geq 1500 \) S) that sedimented to the bottom of sucrose gradients. Both relaxed and supercoiled DNA formed heavy aggregates, although the two forms of DNA may well have produced different degrees of aggregation or condensation. Singer and Singer (17) showed that the preferential retention of superhelical DNA on filters was more dependent on a peptide fragment from residues 73 to 106 of the H1 histone than on any other peptide fragment they tried. This region represents a major part of the globular (20) hydrophobic core of the molecule.

Since the globular part of the histone seemed important in selective retention of superhelical DNA on filters (17), it was surprising to find that H1 histones differed in their effectiveness at retention of SV40 DNA (1); the globular regions of the H1 subfractions are essentially identical in amino acid sequence (21, 22). Apparently, the sequence variable regions of the subfractions affect the globular regions, either by changing their conformations or by modulating the coalescence of one globular region with another. Singer and Singer (17) presented data in which a peptide fragment of residues 73 to 212 retained even more SV40 DNA (1) than intact H1 did on an equimolar basis. Our interpretation of these data is that the NH\(_2\)-terminal region (residues 1 to 72) of intact H1 histone must moderate the DNA retention, each subfraction to a different extent, depending on its amino acid sequence.

An interplay between various regions of the H1 molecule has been postulated before on the basis of fluorescence depolarization (23) and circular dichroic spectra (24, 25). Measuring DNA binding of H1 histone fragments by fluorescence depolarization, Glotov et al. (23) found that a very tight binding of the COOH-terminal segment (residues 73 to 212) was moderated by the NH\(_2\)-terminal segment. They also showed that the COOH-terminal fragment allowed aggregation of the protein-DNA complexes while the NH\(_2\)-terminal region did not.

Our overall view of the interaction of DNA and H1 histone is that a direct, electrostatic binding between protein and nucleic acid is followed by a condensation of the H1-DNA complexes, moderated to different extents by the sequence variable cationic regions of H1. While complexes of H1 histone and DNA can scarcely be considered models of chromatin (which contains numerous other components) the variation among H1 histones in their power to condense nucleoprotein fibers reveals their potential for the imposition of conformational heterogeneity in chromatin.

**REFERENCES**