Preferential and Cooperative Binding of Histone I to Chromosomal Mammalian DNA

(mammalian DNA–histone I interaction/preferential binding sites/nitrocellulose-filter assay)

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ABSTRACT There is a strong preferential binding of histone I to lymphocyte DNA as compared to Escherichia coli DNA when large DNA fragments (2 × 10^6 daltons) are used. The binding of histone I to lymphocyte DNA and to E. coli DNA is cooperative. The distribution of preferential binding sites has been investigated on fragmented DNA. Most of the 2 × 10^6 dalton fragments were found to have at least one preferential histone I binding site, whereas most of the 2 × 10^6 dalton fragments have none.

Specific DNA–protein interactions are most probably involved in generating the structure of the chromosome and regulating the activity of the genes. There is a large amount of experimental evidence which points to the acidic chromosomal proteins as likely candidates for specific gene regulators (1). Histones seem to bind less specifically to DNA than acidic chromosomal proteins (2). Histone I, present in almost all eukaryotic chromosomes and responsible for their insolubility under physiological conditions, has been implicated as a participant in the folding of the chromosomes (3, 4). If this is the case, histone I may be expected to interact in a preferential way with certain regions of DNA to generate a specific folding pattern. In this paper experimental evidence for such preferential DNA–histone I interactions will be given.

MATERIALS AND METHODS

After stimulating bovine lymphocytes with Phytohemagglutinin P (DIFCO) and labeling with [4H]thymidine for 64 hr, cell nuclei were treated with RNAse and pronase. Escherichia coli H 560 (thy–) was labeled with [14C]thymine and crude DNA was isolated essentially according to Thomas (5). The following steps were identical for both mammalian and bacterial DNA. DNA was adsorbed onto hydroxyapatite in 2 M NaCl, 5 M urea, 0.15 M potassium phosphate (pH 6.8) and washed three times with the same buffer. DNA was eluted with 0.5 M potassium phosphate (pH 6.8) and freed from residual proteins by phenol extraction. After gel filtration on Bio-Gel A-5m in 0.15 M NaCl, 0.01 M Tris·HCl at pH 7.5, 0.001 M EDTA, the DNA was fractionated by sucrose gradient centrifugation. The small molecular weight DNA (< 8 × 10^6) was produced by sonication and then separated on a sucrose gradient. The molecular weight of the fractionated DNA was calculated from its sedimentation coefficient \( s_{20,w} \) according to Studied (6) using 32P-labeled fd replicative form DNA (a gift from Dr. H. Schaller’s laboratory) as reference. When the fractionated DNA samples were analyzed on a second sucrose gradient, the peaks formed showed a Gaussian distribution. In each sample there is a range of fragment sizes which, however, is rather narrow, e.g., 76% of our 2 × 10^6 dalton sample is composed of DNA pieces of sizes between 0.8 and 4.4 × 10^6 daltons. The specific activity of the bovine DNA was 7000 ^4H cpm/μg; that of E. coli DNA was 1000 ^14C cpm/μg. Chromatin was mainly obtained from bovine lymph nodes, but also from bovine liver and HeLa cells. The chromatin was isolated from nuclei (7), dialyzed against 2 M NaCl, 5 M urea, 0.01 M sodium phosphate (pH 6.8) and mixed with hydroxyapatite (8, 9). Under these conditions nucleic acids and acidic nuclear proteins are adsorbed onto hydroxyapatite but histones are not. In most experiments the histone fractions were generous gifts from S. Mrzoeck. Histone I was separated from other histones on Bio-Gel P-10 (10), and analyzed for purity by electrophoresis on sodium dodecyl sulfate (11) and acetic acid–urea gels (12). After the second chromatography, histone I was the only visible band on the gels. Histone concentration was determined spectrophotometrically by taking \( A_{280} = 4.25 \) for 1 mg/ml of histone solution (13). In most of the filter binding experiments histone I, in a stock solution of 1.0–10.0 μg/ml in buffer A (0.15 M NaCl, 0.01 M Tris·HCl at pH 7.5, 0.001 M MgCl₂, 0.0001 M EDTA, and 40 μg bovine serum albumin per ml) was added to a solution of 1 ml that contained DNA and buffer A. After incubation for 15 min at 25° the reaction mixture was filtered through Sartorius nitrocellulose membrane filters (no. SM 11308, size 22 mm, pore size 0.15 μm) which had been pretreated with KOH (14, 15). The flow rate was 0.1 ml/sec. The filters were washed three times with 0.7 ml of buffer A, dried, and the radioactivity was monitored in a liquid-scintillation spectrometer. All values given are the mean of three experiments. The standard deviation was never greater than 20%. After KOH treatment of the filters, only 0.5–1% of protein–free DNA was retained.

RESULTS AND DISCUSSION

The filter binding assay, employed in the study of various other DNA–protein interactions (16–18), has been used for these studies of DNA–histone I interaction. Whereas native DNA is not retained on nitrocellulose filters, histone I and DNA–histone I complexes are. With increasing amounts of histone I added to a solution of DNA, an increasing proportion of the DNA is retained on the filter. This direct assay was not found to be useful in the detection of binding specificity. No significant differences have been observed when DNA of E. coli is compared to DNA of bovine lymphocytes. However, if the two DNA species are added simultaneously (competition) in equal amounts, one labeled with ^4H and the other with ^14C, more mammalian DNA is retained on the filter (Fig. 1). This finding indicates that histone I binds preferentially to lymphocyte as compared to E. coli DNA.

If histone I is responsible for the folding of the chromosome, one might expect that in vitro the folding of mammalian DNA with the aid of histone I would be a size-dependent process.
Therefore, we looked for a DNA size dependence of the DNA-histone I interaction. Histone I, given the chance to choose between DNA fragments of different sizes, favors the bigger molecules (Fig. 2). When equal masses of lymphocyte [3H]DNA of defined size (940,000 daltons) and unlabeled DNA (of the same origin but of varying molecular weights) are mixed with histone I, a remarkable increase in the DNA binding capacity for histone I is observed as the size of the fragments is raised from $3 \times 10^6$ to $2 \times 10^6$ daltons. No change of such magnitude can be detected with DNA from E. coli. Small lymphocyte DNA pieces ($2 \times 10^6$ daltons) bind histone I molecules only by a factor two to three times more efficiently than small E. coli DNA of the same size. However, lymphocyte DNA fragments with a molecular weight of $2 \times 10^6$ daltons were found to associate with histone I at least 15 times more strongly than E. coli DNA also of $2 \times 10^6$ daltons. This was measured in additional competition experiments (Fig. 3). If we say (labeled) DNA fragments bind or associate n times more efficiently or strongly than other (unlabeled) DNA fragments with histone I, we mean that n times as much (by weight) unlabeled DNA is needed in order to compete for 50% of the labeled DNA. Thus, large mammalian DNA fragments compete much better for histone I than small fragments. As the experiments discussed in this paragraph show, the binding of histone I to mammalian DNA is both cooperative and preferential. We favor the idea that the large molecules may fold back on themselves, for example, by forming double-stranded hairpins.

One interpretation of these results is that there are preferential binding sites on the mammalian DNA which are distributed at intervals of approximately 0.5 to $2 \times 10^6$ daltons. The sites would, therefore, not be present on each small lymphocyte DNA piece which thus would not bind histone I markedly better than small E. coli DNA fragments. However, a majority of the large lymphocyte DNA pieces would possess the putative site(s) and, therefore, bind histone I much better than large E. coli fragments.

If there are preferential binding sites on the mammalian DNA, as discussed above, they should be separable by breaking or cutting the DNA to small fragments. Therefore, lymphocyte DNA was extensively sheared by sonication ($2 \times 10^6$ daltons) and mixed with a limiting amount of histone I on a
Histone I (0.2 μg) was added to 1 μg of ³H-labeled lymphocyte DNA (9 × 10⁵ daltons) and 1 μg of ¹⁴C-labeled E. coli DNA (same size) in 1 ml of 5 mM Tris-HCl that contained different NaCl concentrations. The reaction mixtures were filtered (first filters). The filters were then adjusted to 0.15 M NaCl by addition of 4 M NaCl and filtered again (second filters). In these competition experiments ¹⁴C and ³H radioactivity was determined and used to calculate the absolute amount of lymphocyte and E. coli DNA retained on the filters.

preparative scale, so that 20% of the DNA is retained on filters. This DNA, isolated from the filter, bound histone I two to three times more efficiently than the remaining 80% of the DNA. The simplest explanation is that we enriched preferential binding sites since they are not present on all DNA fragments of 2 × 10⁶ daltons. The melting temperature of the retained DNA was 5°C lower (which corresponds to 12% more A + T) and renameling of the DNA (Cot plot) indicated a lack of highly repetitive DNA fragments (G + C-rich), in agreement with Sponar and Sormova (19).

To examine the distribution of the preferential binding sites on large DNA fragments, we incubated lymphocyte DNA (2 × 10⁶ daltons) with a limiting amount of histone I. After filtration, the filtrate (50% of total DNA) was then compared in a competition experiment with total ³H-labeled lymphocyte DNA (same size) for histone I binding capacity. The total DNA associated with histone I bound only 1.5 times more strongly than the 30% of the DNA in the filtrate. This change in the binding capacity is small if we consider that a 2 × 10⁶ dalton lymphocyte DNA fragment binds histone I at least 15 times more strongly than an E. coli DNA fragment of the same size which does not contain preferential binding sites (as discussed above). This result suggests that most of the 2 × 10⁶ dalton DNA fragments contain at least one preferential binding site.

If we assume (a) that all free DNA can pass through the filter and (b) that all histone I molecules are bound to DNA which, as a consequence, are retained by the filter, we can calculate from the linear part of the filter binding experiments in Fig. 1 that a DNA fragment of 2 × 10⁶ daltons contains about one histone I molecule per 70 base pairs. That the first assumption is correct follows from the fact that no DNA is retained (Fig. 1) when no histone I is added. The second assumption, which corresponds to experimental observations by Olins (20), has been shown to be correct in control experiments with sucrose gradients in which ¹⁴C-labeled histone I association to DNA has been investigated. These experiments have shown that at 0.15 M NaCl more than 90% of the labeled histone I sediments as a DNA–histone complex with a sedimentation coefficient much greater than that of free DNA.

The retention of DNA–histone complexes at different NaCl concentration is shown in Fig. 4 and Table 1. At low salt (0.01 M NaCl) very little DNA is retained on the filter. Increasing the NaCl concentration results in the retention of more complexes and the maximum is reached at 0.08–0.15 M NaCl. (Note that the filter retention of a mixture of histones lacking histone I is different; it is lower and inversely related to the ionic strength and does not indicate a preferential association with the mammalian DNA.) If the NaCl concentration is further raised, less DNA is retained. The inability to form complexes at about 0.5 M NaCl coincides with the dissociation of histone I from the chromosome under these conditions.

Whereas complexes formed at 0.15 M NaCl are retained on filters, complexes formed at lower ionic strength pass through the filters. That the DNA passing through the filter is indeed associated with histone I is indicated by the retention of the same DNA after it was adjusted to 0.15 M NaCl in the filtrate (Table 1). This observation is surprising, since histone I is retained by nitrocellulose filters at low ionic strength.

Finally, the preferential binding of histone I to mammalian DNA, which we have described, cannot be explained by contamination of the DNA preparations with single-stranded nucleic acids, though it associates very effectively with them (Fig. 5). In contrast to native DNA, single-stranded nucleic acids change their binding capacity for histone I very little when their molecular size is altered (unpublished observation). Denatured DNA binds histone I about eight times more efficiently than 1 × 10⁶ dalton DNA, but definitely not better than 2 × 10⁶ dalton DNA (Fig. 5). Moreover, experiments done in the presence of an endonuclease from Neurospora crassa did not change the specificity of histone I binding. This batch of Neurospora crassa endonuclease was a gift from Dr. H. Schaller and was found to have a 1000-fold higher scission rate on single-stranded than on double-stranded DNA.
Furthermore, it has been ruled out that the observed specificity of DNA–histone I interaction is an artifact produced by sonication. When fragments prepared by sonication of DNA are compared in the filter binding test with fragments of unsonicated DNA of the same size (0.5 to 2.0 × 10^6 daltons), no difference in behavior has been observed. In summary, the preferential binding described in this paper seems to be due to a property of native mammalian DNA.

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