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Interaction of histones H1 and H1° with superhelical and linear DNA

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By using direct competition experiments, the binding of histone H1AB (a mixture of H1A and H1B) and H1° to superhelical and linear DNA forms was studied. Mouse liver H1 isohistones and plasmid pαGD containing part of the 5′ flanking and part of the coding sequence of the mouse α-globin gene in pUC18 were used as partners in the binding reaction. The competition experiments were performed by direct mixing of the histone with labelled supercoiled DNA (at 125 mM NaCl and at a histone/DNA ratio of 1.0) and addition to the mixture of increasing amounts of cold competitor DNA, either supercoiled or linear. The radioactivity of the complex formed was determined by filter binding. The results show that both histones H1 and H1° possess a strong binding preference for supercoiled DNA forms. Thus, histone H1° resembles the regular somatic set of histone H1 and not the other differentiation-specific histone H5 studied thus far.

Histone H1; Histone H1°; Linear DNA; Supercoiled DNA; H1/DNA interaction

1. INTRODUCTION

The belief that in chromatin, histone H1 interacts primarily with DNA has led to intensive studies of artificial H1/DNA complexes as model systems. The interest in such complexes was constantly fed by the existing correspondence between the salt-induced compaction of the nucleosomal fiber and the transition from non-cooperative to cooperative binding that is observed under similar salt conditions.

One of the features of the interaction between histone H1 and naked DNA which has attracted considerable attention is the preference of H1 for supercoiled DNA forms. The first reports of Vogel and Singer [1] and Singer and Singer [2] on filter-binding experiments have led to the conclusion that H1 possesses a higher affinity for superhelical than for relaxed circular or linear DNA. Later, however, this conclusion was questioned by several authors. Thus, for instance, Knippers et al. [3] considered the apparent preference for supercoiled DNA as a consequence of the fact that at the ionic strength at which the original experiments of Vogel and Singer were performed (100 mM NaCl), the cooperativity of binding of H1 to DNA was lowered by the superhelicity of DNA which led to retention of more supercoiled molecules on the filters. Iovcheva and Dessev [4] also argued that the efficiency of complex formation between histone and different forms of DNA could not serve as a reliable measure of affinity under the conditions used. It is clear that in order to distinguish any preference that H1 may have for one form of DNA over the other, direct competition experiments are needed [5] of the kind performed by Iovcheva and Dessev [4].

The aim of our work was to reinvestigate the binding of H1 to superhelical and linear DNA forms by the use of direct competition experiments. In addition, we compared the behavior of H1°, the differentiation-specific subfraction of H1 [6–8] with that of H1. The idea behind this comparison was to see whether the different mechanism of action of the two histone H1 subtypes might be somehow connected to the way they interact with differently supercoiled DNA.

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; PMSF, phenylmethylsulphonylfluoride; SDS, sodium dodecylsulphate

2. MATERIALS AND METHODS

2.1. *Isolation and purification of histones H1 and H1°*

Histones H1AB and H1° were obtained by 5% $HClO_4$ extraction of crude mouse liver nuclei followed by gel filtration on BioGel P100 [9].

2.2. *Isolation of the different DNA forms*

Plasmid pαGD was kindly provided by Dr I. Pashev (Institute of Molecular Biology); it was obtained by subcloning the *Pst*I-*Bam*HI 174 bp fragment of plasmid pML2 [10], containing the mouse α-globin gene, into pUC18. pαGD contains 45 bp of the 5′ flanking and 129 bp of the coding sequence of the α-globin gene. Radioactive pαGD was obtained following in vivo labelling with [^{3}H]thymidine with a specific activity of about 1500 cpm/μg DNA. Total plasmid preparations were used as supercoiled DNA forms (see section 3). Linearization of the plasmid was achieved by treatment with *Eco*RI.

2.3. *Binding reactions and filter retention assays*

The concentration of DNA was determined spectrophotometrically from the absorbance at 254 nm and that of the histone by weighing

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the dry protein powder and dissolving it in a specified volume of H_2O. The H1/[^{3}H]DNA complex was formed by direct mixing of the two components in binding buffer (in mM, 125 NaCl, 10 Tris-HCl, pH 7.5, 1 EDTA, 1 DTT and 0.1 PMSF) at a ratio 1:1 (w/w). The mixture was incubated at room temperature for 1 h and filtered through nitrocellulose filters according to [11]. In some cases the bound DNA was eluted from the filters with 1.5 ml 0.1% SDS, 1 mM EDTA for 2 h at room temperature. The extract was deproteinized and DNA analyzed by electrophoresis. For the competition experiments, the cold competing DNA (supercoiled or linear) was added directly following the mixing of the histone with the labelled DNA and further treated as above.

3. RESULTS AND DISCUSSION

3.1. *Choice of the components of the binding reaction*

For a long time it was believed that histone H1 interacts with DNA nonspecifically, the nature of the interaction being purely electrostatic. The work of Diez-Caballero et al. [12], Berent and Sevall [13] and Pauli et al. [14] has indicated that H1 might interact specifically with specific regions of eukaryotic genes. We have also obtained evidence that H1 possesses some selectivity of interaction with different regions of the mouse α-globin gene ([15], Zlatanova et al., in preparation). Thus, for instance, while the pure prokaryotic plasmid pUC18 was not significantly retained on nitrocellulose filters by H1 binding, its derivative pαGD, containing part of the 5′ flanking and part of the coding sequence of the human α-globin gene was bound to the filter by H1 (Fig. 1A). Additional experiments were performed to demonstrate directly that it was the eukaryotic insert in pαGD that was responsible for the retention of the recombinant plasmid on the filter. To that end, pαGD was treated with appropriate restrictases so as to release the insert from the plasmid body and the mixture of the two fragments was passed through filters following incubation with H1. The analysis of the DNA bound to the filter revealed that it was only the insert that was retained (Fig. 1B). On the basis of these experiments, it was decided to perform our competition experiments with pαGD.

In order to achieve as great a fidelity of interaction as possible for an in vitro reconstitution, it was logical to use H1 isohistones from the same species. This consideration determined the use of the mouse histones.

3.2. *Characterization of the DNA and histone preparations*

As the aim of this work was to compare the binding of isohistones of the lysine-rich class to DNA forms of different conformation, it was essential to use: (i) pure protein fractions not contaminated with each other; and (ii) DNA preparations containing exclusively or predominantly either the superhelical or the linear form. The purity of the H1 subfractions used is illustrated in Fig. 2A and that of the different DNA forms in Fig. 2B. As can be seen, the procedure used for H1 subfractionation gives electrophoretically pure

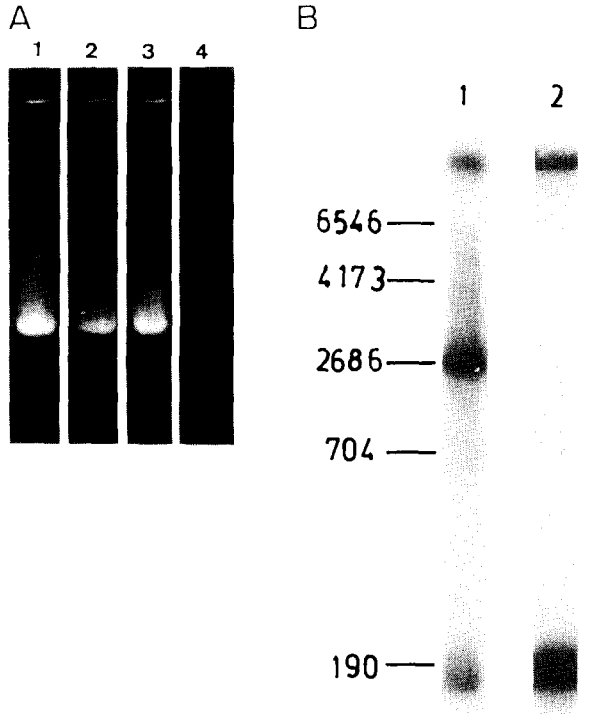

Fig. 1. Electrophoretic analysis of DNA retained onto nitrocellulose filters by histone H1 binding. (A) Comparison between the parental plasmid pUC18 and its derivative pαGD: lane 1, control plasmid pαGD; lane 2, DNA eluted from the filter following incubation of the same plasmid with histone H1; lane 3, control plasmid pUC18; lane 4, DNA eluted from the filter following incubation of the same plasmid with histone H1. (B) Autoradiography of the DNA fragments retained on the filter by H1 binding. Plasmid pαGD was digested with *Pst*I-*Bam*HI, the resulting fragments were ^{32}P-labelled at the 5′ end and separated on an agarose gel (lane 1); the mixture of the fragments was allowed to react with histone H1, the bound fragments were eluted from the filter and analyzed by agarose gel electrophoresis (lane 2). Only the eukaryotic insert (174 bp) was retained on the filter; the plasmid body (2686 bp) was not visible. Fragment length markers are denoted on the side.

fractions. The preparation used as supercoiled DNA contained about 90% of the DNA in the fastest moving band, i.e. that of the population of supercoiled forms. The remaining 10% was about equally distributed between the relaxed circular and the linear forms. No efforts were made to additionally enrich the plasmid preparation in supercoiled forms via, for instance, electroelution of the superhelical band from agarose gels, as preliminary experiments have indicated that the resulting preparation contained comparable proportions of the nonsuperhelical forms. The linear DNA preparation was obtained by complete digestion with a restriction enzyme which possessed one recognition site in pαGD (Fig. 2B). In this way, the molecular masses of the supercoiled and linear forms were the same; this avoided complications connected with the existence of preference of H1 for DNA molecules of higher molecular mass.

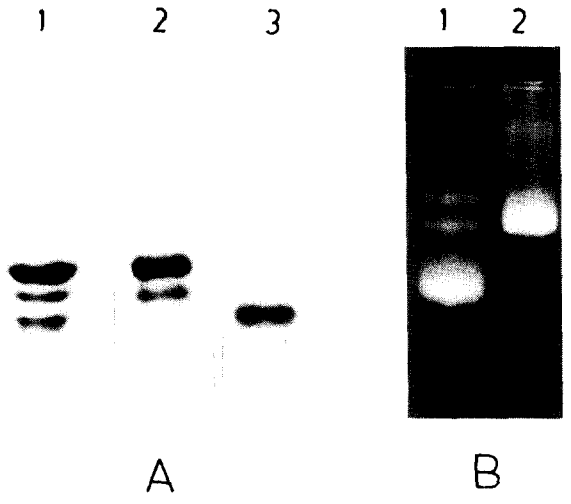

Fig. 2. Electrophoretic characterization of the histone and DNA preparations used in the competition experiments. (A) Polyacrylamide electrophoretic gel showing the purity of histones H1AB (lane 2) and H1° (lane 3) obtained by gel filtration; lane 1, initial crude H1 preparation loaded on the BioGel P100 column. (B) Agarose gel with superhelical (lane 1) and linear DNA (lane 2). For details see section 2.

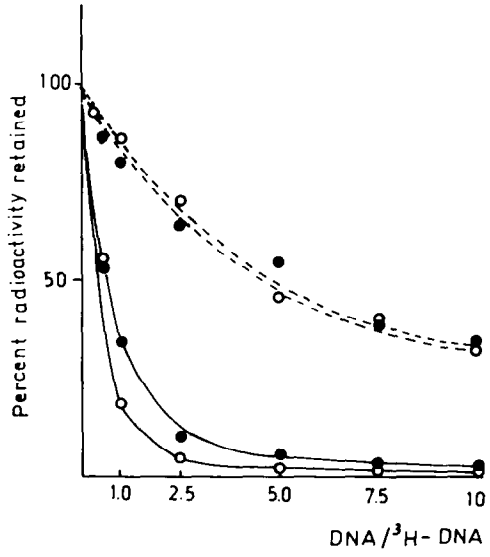

Fig. 3. Competition experiment for estimation of the relative affinities of histones H1AB and H1° to supercoiled and linear DNA. Around 7500 cpm (5 μg) of supercoiled [^{3}H]DNA were loaded on the filters. The amount of radioactivity retained in the control samples (DNA plus histone H1AB or H1° without cold competitor DNA) was considered as 100%. Each point is the mean of three independent experiments. Solid line, competition with supercoiled DNA; broken line, competition with linear DNA. (●) Histone H1AB, (○) histone H1°.

3.3. *Conditions of the filter retention assay*

As the degree of retention of DNA by proteins on filters depends on the ionic strength, it was necessary to find optimal conditions for performing the assay. In accordance with some previously published data (e.g. [3]), it was found that DNA was best retained at 120 mM NaCl. At this ionic strength both H1 and H1° bind to DNA in a cooperative way (Yaneva et al., in preparation). The other parameter important in filter binding assays was the input histone/DNA ratio. A ratio of 1.0 was used since control experiments have indicated that the lower input ratios used by most of the other authors (usually around 0.4) led to a much lower retention of the DNA. In addition, this ratio is the one of equivalence (all negative charges of DNA can be neutralized by the positive charges of the histone [16]). Under these conditions about 30–35% of the input labelled DNA was retained on the filter in the absence of competing DNA.

3.4. *Competition experiments*

The experiments aimed at estimating the relative affinity of H1 and H1° to supercoiled and linear DNA were performed in the following way: the labelled supercoiled DNA was mixed with the respective H1 isohistone and increasing amounts of cold competing DNA (from 0- to 10-fold excess over the labelled one) were added to the reaction mixture. The radioactivity retained on the filter was measured with the results shown in Fig. 3. It is evident that the supercoiled DNA was an extremely strong competitor in the binding reaction of the supercoiled form and both H1 and H1°; already at a competitor/labelled DNA ratio of 1.0 the retention of the DNA label on the filters dropped to 20–30% and was practically 0 at ratios ≥ 5. At the same time, however, the competition by the linear DNA was much smaller (Fig. 3). Both proteins, H1 and H1°, behaved in the same way.

4. CONCLUDING REMARKS

The results of the direct competition experiments performed in this study show that: (i) H1 possesses a strong binding preference for supercoiled DNA forms as compared with their linear counterparts of the same molecular mass; and (ii) histone H1°, in contrast to H5 [4], is indistinguishable with respect to its preference for supercoiled DNA. These facts mean that the functional differences observed between H1 and H1° do not involve different affinities of the proteins to differently supercoiled DNA, constrained in the distinct loop domains of the eukaryotic genome.

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