Protection of Deoxyribonucleic Acid from Nuclease Action by Histones

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The observation that four of the five main histone proteins present in chromatin do not occur as separate monomeric units, but form an oligomeric structure with equimolar amounts of all these histones,¹ has placed research in this field in a new phase of development. Only the very lysine-rich histone F1 seems to function independently from other histones,² while the histones F2A1, F2A2, F2B and F3 form a complex, which apparently has an octameric structure containing two copies of each histone.¹ When chromatin is subjected to the action of DNase, units consisting of the histone octamer and the DNA about 200 nucleotides long are released.³ The histone octamer is thought to protect the regions of DNA which are associated with it, while DNase is free to cut DNA between the histone complexes.

The arrangement of different histones in the oligomer needs to be investigated further. We have approached this problem by studying the capacity of different histones to protect DNA from nuclease action. The idea behind these experiments was that the histone species that protect DNA best are those that interact physically with DNA in the octamer, while those that protect DNA poorly are possibly not in direct association with DNA. The special properties of histones make these types of reassociation experiments reasonable. Histones are relatively small proteins and for this reason they apparently can, under proper conditions, return easily to their native conformation, which they lose in acidic conditions during purification. This is suggested by some recent studies showing that the complex formation *in vitro* between purified individual histones and also between histones and DNA gives a product identical with, or at least similar to, the product formed *in vivo*.^{4,5}

Methods. Both DNA and histones were prepared from calf thymus tissue. DNA was isolated according to the method given by Schwander and Signer.⁶ The total histones

were extracted with 0.2 N H₂SO₄ from the nuclei, which were prepared by slightly modifying⁷ the procedure of Blobel and Potter.⁸ This acid extraction was preceded by three washings of the nuclei with 0.14 M NaCl–0.05 M NaHSO₃. The histones were precipitated from the clarified acidic extract with five volumes of ethanol, and the precipitate obtained was washed several times, first with ethanol and then with ether.

The different histone proteins were isolated according to "method 2" of Johns.⁹ Four fractions, F1, F2A, F2B, and F3, were obtained. The fraction F2A was then separated into two subfractions, F2A1 and F2A2, by precipitating F2A2 with acetone from an acidic solution.¹⁰ The lysine-rich histone F1 was purified further by the method of Oliver *et al.*¹¹

The composition of the histone preparations was analyzed by polyacrylamide gel electrophoresis according to Johns.¹²

The digestion of DNA by DNase in the presence or absence of histones was assayed as follows: DNA plus different histone proteins and DNase were dissolved each separately in 0.1 M CH₃COONa buffer, pH 5.0 with 0.005 M MgSO₄. The concentration of DNA was 0.04 mg/ml. A 0.5 ml solution containing varying amounts of histone was added to 1 ml of DNA solution, and the mixture incubated at 37°C for 10 min. After this preincubation, 0.5 ml of enzyme solution (50–100 units/ml) was added, and the reaction allowed to continue at 37°C for 7 min. The reaction was stopped with 0.5 ml of 3 M CCl₃COOH. The precipitate was removed by centrifugation, and the amount of UV-absorbing material in the supernatant was measured in a spectrophotometer at 260 nm. Electrophoretically purified, pancreatic DNase I was purchased from Merck, Darmstadt. The amount of enzyme which gave a 0.001 O.D. unit rise in absorption at 260 nm in 1 min, under the experimental conditions employed with DNA as the substrate, was defined as one enzyme unit.

Protein content was determined according to Lowry *et al.*,¹³ and DNA content by a diphenylamine method.¹⁴

Results. The main histone species were purified from calf thymus. Fig. 1 shows an electrophoretic analysis of these preparations

Fig. 1. Polyacrylamide gel electrophoresis of histone preparations. Gel 1, whole histone; gel 2, F1; gel 3, F2A1; gel 4, F2A2; gel 5, F2B; gel 6, F3.

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Fig. 2. Protection of DNA by histones from enzymatic digestion by DNase I. In order to allow complex formation between DNA and histones, the DNA was preincubated with different histone fractions at 37°C for 10 min prior to subjection to DNase action. O, whole histone; ●, F1; ■, F2A1; □, F2A2; △, F2B; +, F3.

on polyacrylamide gels. Each histone fraction appears to be relatively free of the other histones and nonhistone proteins. These preparations were used to investigate the capacity of histones to protect DNA against DNase digestion.

Increasing amounts of total histones, or each histone protein separately, were incubated with the same amount of calf thymus DNA for 10 min, in order to allow the complex formation between DNA and histones to occur. After preincubation, the susceptibility of the DNA in these complexes to DNase I was examined. As is shown in Fig. 2, the DNA was protected to varying degrees from enzymatic digestion by DNase, the extent of this protection being dependent on the histone employed. The lysine-rich histone F1 gave the best protection, while the arginine-rich histone protein F2A1 had the weakest effect. The F2A2 histone, which is a slightly lysine-rich protein, inhibited DNase action somewhat more effectively than the histones F2B and F3. The DNA protection capacity of the total histones was very similar to that of F2A2.

Discussion. It is obvious that the histone F1 is directly bound to DNA in chromatin, because it does not appear to interact with the other histones.² Its strong inhibitory effect on DNase activity can be well understood on this ground. On the other hand the histone F2A1 may not interact with DNA to any great extent, but rather with other histones in the octamer, because it protected DNA much less than the other histones of the complex. Some interactions with DNA may, however, take place, because the F2A1 protein also has basic terminal segments, which apparently

are the primary sites of interaction of the individual histones with DNA.¹⁵ The histones F2A2, F2B and F3 may all interact directly with DNA, because they all protected DNA fairly effectively almost to the same extent. The histone F2A1 may function as a protein which holds the different histones of the complex together. This is also supported by the crosslinking experiments of Thomas and Kornberg,¹ which demonstrated that F2A1 is apparently bound to all three other histones of the complex.

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