HEPARIN CAUSES A REDISTRIBUTION OF CHROMOSOMAL PROTEINS

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1. Introduction

Polyanions are known to cause structural changes in isolated nuclei and chromatin [1–9]. Ultrastructurally, these changes are seen as thinning and extension of chromatin fibrils [4–6] which correlate with physical studies showing a reduced sedimentation velocity in at least a portion of the treated chromatin [7,9]. It has long been known that histones and polyanions form strong complexes in solution [10] and although it has been suggested that polyanions exert their effects by direct interaction with one or more histones in nuclei and chromatin [1–6, 11–13], there is relatively little supporting evidence. Berlowitz et al. [12] and Miller et al. [5] have reported selective binding and extraction of histones by polystyrene sulfonate. Heparin binding to chromasomal proteins was shown by Kitzis et al. [14] but no binding specificity was reported in whole chromatin.

To study the specificity, if any, of the polyanion effect on rat liver chromatin, we have used the altered sedimentation behavior of treated chromatin to monitor the heparin effect and have fractionated heparin-treated chromatin by differential centrifugation. Analyses of the fractions obtained indicate that the concentration-dependent change in sedimentation behavior after heparin treatment is associated with a complex redistribution of chromosomal proteins. Of particular interest is the highly consistent partition of the H1 histone group and two non-histone proteins of the high mobility group.

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2. Materials and methods

2.1. *Isolation of chromatin*

The 78 000 × *g* fraction of rat liver chromatin was prepared as previously described [15]. A combined 3500 × *g* and 78 000 × *g* chromatin fraction was obtained by centrifuging the supernatant from a 500 × *g* 5 min centrifugation at 78 000 × *g* for 60 min. Chromatin pellets were resuspended by homogenization in ice-cold 5% (w/v) sucrose and used immediately.

2.2. *Heparin incubation and differential centrifugation*

Heparin incubations were carried out in 5% sucrose at 0°C for 30 min at a DNA concentration of 1 mg/ml determined spectrophotometrically [15]. After incubation, the samples were centrifuged at 100 000 × *g* for either 90 min or 18 h. In some experiments, the 90 min supernatants were centrifuged for an additional 18 h. Following centrifugation, the resulting pellets were homogenized in 5% sucrose and aliquots of each pellet and supernatant were taken for both SDS–gel electrophoresis and the determination of optical densities after dissolution in 1% SDS (final).

2.3. *SDS–Polyacrylamide gel electrophoresis*

SDS–Gel electrophoresis was performed on 10 cm, precast 12% polyacrylamide gels in 0.205 M Tris, 0.205 M acetic acid, 0.1% SDS at an operating pH of 6.1 for 7 h at 4 mA/gel. The samples were diluted 1:1 with a sample buffer containing a 1:5 dilution of running buffer, 30% (w/v) sucrose, 10% (v/v) β-mercaptoethanol, 0.025% bromophenol blue, and 4% SDS and applied directly to the gel. The gels were fixed, stained and destained by the method of Weber and Osborn [16]. The gels were scanned on a Gilford Model 2400

recording spectrophotometer with a Gilford Model 2410 Linear Transport.

2.4. *Reagents and materials*

Heparin (sodium salt, 160 J-A units/mg) was purchased from Sigma Chemical Company, St. Louis, Mo. Precast 12% gels and electrophoresis grade SDS were obtained from Bio-Rad Laboratories, Rockville Centre, NY. All other reagents were certified grade from Fisher Scientific Company.

3. Results

3.1. *The effect of heparin on the sedimentation behavior of chromatin*

The addition of heparin to chromatin induces the formation of a more slowly sedimenting fraction. The amount of this fraction produced is dependent on the heparin concentration as is evident from table 1. From the 90 min data, it is clear that the heparin effect is dramatic, since over 60% of the chromatin remains in the supernatant fraction at the lowest concentration used. Even after 18 h centrifugation there is a substantial amount of material which fails to sediment to the bottom of the tube.

To partially characterize the slowly sedimenting fractions, the relative nucleic acid and protein contents were estimated by the ratio of absorbances at 260 nm and 280 nm. The 90 min supernatants exhibited ratios essentially indistinguishable from the untreated chromatin with $A_{260/280} = 1.75$. Although the 18 h supernatants are slightly enriched in protein as demonstrated by an average ratio of 1.73, the results are compatible with the characterization of these fractions as some form of deoxyribonucleoprotein.

3.2. *SDS–Polyacrylamide gel electrophoresis*

Evaluation of the protein composition of the heparin-treated chromatin fractions by SDS–gel electrophoresis shows a complex redistribution of chromosomal proteins between the various fractions which affects both the histones and non-histone proteins. The effects to be described have been seen at all heparin levels used, but the magnitude of change is greater at higher concentrations. We have selected an intermediate heparin concentration of 120 μg/430 μg DNA to demonstrate the observed changes.

Representative gel scans of the fractions obtained after incubation with heparin and centrifugation at 100 000 × *g* for 90 min are shown in fig.1. The 90 min pellet (middle frame) is dramatically enriched in non-histone proteins, especially those of an intermediate mobility. Although the combined 3500 × *g* and 78 000 × *g* chromatin preparation was used as starting material in this experiment, similar changes are seen when only pure 78 000 × *g* chromatin is used.

The gel patterns in fig.2 represent the fractions isolated after 18 h centrifugation at 100 000 × *g*. The effect on the intermediate mobility non-histone proteins is not as readily apparent; however, there is a substantial alteration in the relative amounts of the nominal H1 histone group and two of the high mobility group non-histones. There is a preferential association of the two non-histones migrating immediately behind H1 (middle frame, arrows) with the sedimenting fraction, and a relative increase in the amount of protein migrating in the H1 region in the supernatant fraction.

Table 1
The effect of heparin on the sedimentation behavior of chromatin

μg Heparin/430 μg DNA	% Recovered A_{260} nm			
	90 min centrifugation[a]		18 h centrifugation[b]	
	Pellet	Supernatant	Pellet	Supernatant
0	83	17	95	5
60	35	65	81	19
120	15	85	68	32
240	7	93	46	54

[a]Average of 4 experiments
[b]Average of 2 experiments

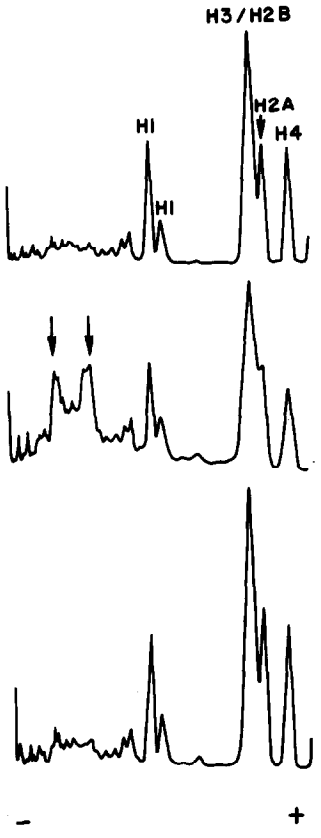

Fig.1. SDS–Gel electrophoresis of fractions obtained after heparin treatment (120 μg/430 μg DNA) and centrifugation at 100 000 × *g* for 90 min. Lower frame, untreated chromatin; middle frame, 90 min pellet; upper frame, 90 min supernatant. Region between arrows is referred to in the text as an intermediate mobility range of non-histones.

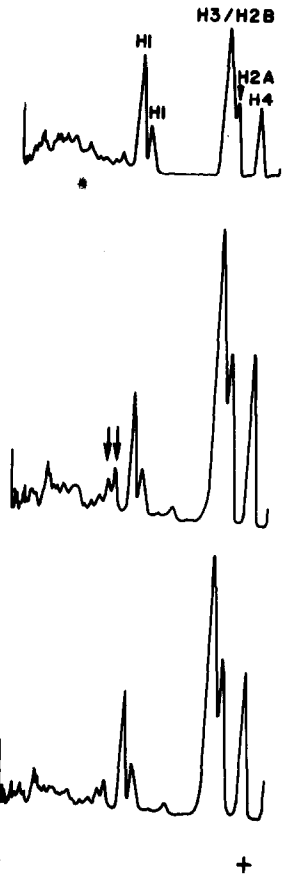

Fig.2. SDS–Gel electrophoresis of fractions obtained after heparin treatment (120 μg/430 μg DNA) and centrifugation at 100 000 × *g* for 18 h. Lower frame, untreated chromatin; middle frame, 18 h pellet; upper frame, 18 h supernatant. Arrows, two high mobility group non-histones (see text).

This relative partition of the apparent H1 group and the two high mobility group non-histones was also present in fig.1, but is even more striking in fig.3. These latter samples were prepared by centrifuging heparin-treated chromatin for 90 min and recentrifuging the supernatants (fig.1, top frame) for an additional 18 h. In this preparation there is also an apparent preferential association of histone H4 with the pellet fraction. We have observed the H4 effect in 18 h supernatants at high heparin levels and in recentrifuged 90 min supernatants, but it appears to be a variable result. One further effect of some consistency is the disappearance from the supernatant fractions of the small band migrating between the H1 group and the remaining four histones. This band appears to correlate with the H1° (fl°) histone described by Panyim and Chalkley [17].

4. Discussion

Our data indicate that the effect of heparin on chromatin is more complex than selective histone binding and extraction. We find that the change in sedimentation behavior of heparin-treated chromatin shown previously by us [9] and others [7] is associat-

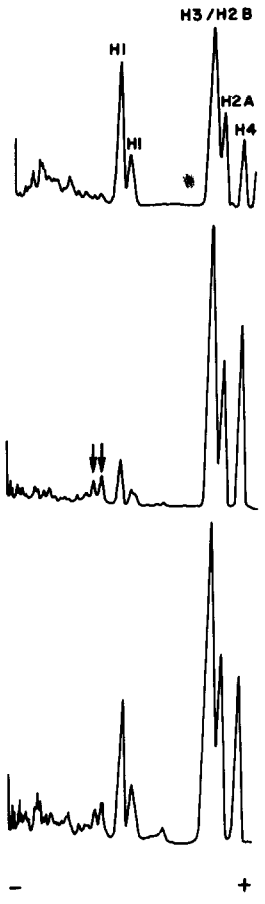

Fig.3. SDS–Gel electrophoresis of fractions obtained by recentrifuging the 90 min supernatant of heparin-treated chromatin (120 μg/430 μg DNA) for an additional 18 h. Lower frame, untreated chromatin; middle frame, recentrifuged pellet; upper frame, recentrifuged supernatant. Arrows, two high mobility group non-histones (see text).

ed with a significant redistribution of chromosomal proteins between the fractions obtained by differential centrifugation. It appears likely that heparin produces a gradient of chromatin strand populations with differing protein complements and sedimentation rates. The most rapidly sedimenting fractions show gross alterations in the non-histone content, but these changes are obscured at long sedimentation times. We have interpreted this result as a dilution effect produced by the sedimentation of another population of chromatin strands which is depleted in non-histone proteins. The changes in the relative amounts of the H1 group and the two high mobility group non-histones is, however, highly consistent and even exaggerated at longer sedimentation times, perhaps indicating that this effect is of greater conformational significance.

When chromatin is incubated with tritiated heparin and fractionated as described, we find that more than 97% of the label is associated with the non-sedimenting fractions and that it appears to be largely free in solution as demonstrated by gel chromatography (data not shown). This suggests that either the strength of binding is very weak or very little heparin is actually bound. In view of the complexity of these several effects, we believe that the mechanism of heparin action may reside in its ability to disrupt the interactions between chromosomal proteins which may, in turn, alter the associations of the proteins with DNA. Particularly suggestive is the correlation of changes in the H1 histone group and the high mobility group non-histones with altered sedimentation behavior. Smerdon and Isenberg [18] have recently reported specific complex formation between certain H1 subfractions and the HMG 1 and HMG 2 proteins. Whether our results reflect alterations of these or similar interactions cannot be determined from the present data, but such a mechanism would be an attractive explanation for some of the structural changes produced in chromatin by polyanions.

Acknowledgements

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