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DISSOCIATION AND LOOSENING OF CHROMATIN BY HEPARIN IN A MEDIUM OF PHYSIOLOGICAL IONIC STRENGTH

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UDC 612.173.015.4.014.46:615.273.53

KEY WORDS: chromatin; heparin; polyanion.

Natural polyanions play a very important role in the function of the genetic apparatus in eukaryotic cells. As a rule it is considered that polyanions can induce dissociation of repressor histone molecules from DNA, and can thereby influence gene expression [13]. Some workers, while not ruling out the dissociating effect of polyanions on chromatin, distinguish their modifying effect on the structure of chromosome fibrils [13]. Structural reorganization of chromatin by polyanions is not disputed, although some of its details are not yet clear. However, experimental confirmation of the dissociating ability of polyanions in a medium of physiological ionic strength is not to be found in the literature. We know [1] that dissociation of the histones of chromatin under the influence of tRNA or DNA, taking place in a solution of low ionic strength, is considerably weakened in intensity by an increase in the salt concentration in the medium. The presence of 40 mM NaCl or 1 mM $MgCl_2$ is sufficient to completely suppress dissociation of all histones except H1 by polyanions [1].

The object of this investigation was to study the possibility of dissociation of chromatin proteins under the influence of the natural polyanion heparin in medium with physiological ionic strength (0.15 M NaCl). Under these conditions evidence could be obtained on the loosening effect of heparin on the compact fibrils of chromatin isolated from cells.

EXPERIMENTAL METHOD

Chromatin was isolated from calf thymus. The minced tissue was homogenized in 0.75 M NaCl plus 0.025 M EDTA-Na_2, pH 8.0, and washed five times in the same medium and twice in 0.15 M NaCl plus 0.7 mM Na-phosphate buffer, pH 7.0. Dispersions of chromatin in the last medium with equal DNA concentration (C_{DNA} = 80 μg/ml, $C_{protein}/C_{DNA}$ = 1.1 to 1.3) were mixed with equal volumes of heparin solutions (from Polfa, Poland) in order to obtain mixtures with different heparin/DNA ratios. The heparin solutions were prepared by diluting the mother solution, obtained by dissolving a definite quantity of the dry preparation in water and subsequent adjustment of the ionic strength of the medium to physiological with concentrated salt solution. Mixtures of deoxyribonucleoprotein (DNP) and heparin were centrifuged for 1.5 h or 12 h at 114,000g (Beckman L2-65B centrifuge, USA). Concentrations of DNA [6] and protein [9] were determined in the supernatant. The presence of heparin did not affect the determination of DNA. The contribution of heparin was allowed for during determination of the protein concentration (1 g heparin corresponds to 0.008 g protein), assuming that all the heparin added to the suspension was present in the supernatant. Since the stoichiometry of binding of histones with heparin is characterized by a histone/heparin ratio by weight of 2.5:1 [7], this assumption is not essential for the conclusions drawn above. The composition of histones in the DNP-heparin mixtures was determined by electrophoresis [8, 6]. Densitograms of the gels were obtained on a Gilford spectrophotometer at 600 nm.

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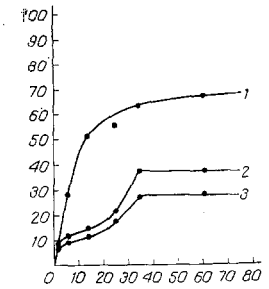

Fig. 1

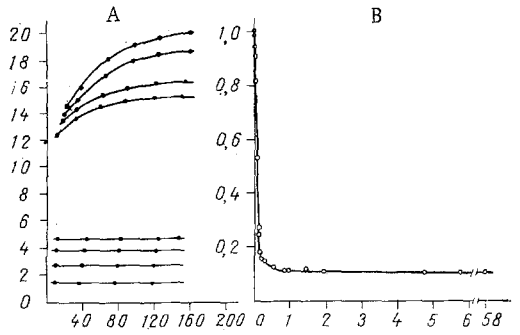

Fig. 2

Fig. 1. Quantitative analysis of protein and DNA remaining in supernatant after centrifugation of heparin–chromatin mixtures with different heparin/DNA ratios in medium of physiological ionic strength. Abscissa, heparin/DNA ratio in mixtures; ordinate, percentages of DNA and protein remaining in supernatant after centrifugation of mixtures. 1) Protein after centrifugation for 1.5 h, 2) protein after centrifugation for 12 h, 3) DNA after centrifugation for 1.5 h. Characteristics of mixtures: C protein/C_{DNA} = 1.1; C_{DNA} = 40 μg/ml.

Fig. 2. Structural modification of chromatin by heparin in medium of physiological ionic strength. A) Reduced viscosity of chromatin suspension as a function of time in absence (below) and presence (above) of heparin, at four shear intensities. Abscissa, time (in sec) after introduction of suspensions for analysis into viscometer. Ordinate, reduced viscosity η_{sp}/C_{DNA} (in dl/g). Characteristics of mixture: $C_{protein}/C_{DNA}$ = 1.3; C_{DNA} = 30 μg/ml; $C_{heparin}/C_{DNA}$ = 1. High values of viscosity correspond to low shear intensities; B) effect of heparin on turbidity of chromatin suspension ($A_{350\ nm}$). Abscissa, heparin/DNA ratio in DNP–heparin mixtures; ordinate, optical density A_{350} nm. Characteristics of mixtures: $C_{protein}/C_{DNA}$ = 86 μg/ml.

Immediately before measurement of viscosity and turbidity the chromatin suspension was exposed for 90 sec on an ultrasonic disintegrator (MSE) to disperse the aggregates present. The viscosity of the suspensions was measured on an Ostwald capillary viscometer at four shear intensities: τ = 1.126; 0.77; 0.477; and 0.232 dyne/cm². The turbidity of the suspensions was measured on a Hitachi differential spectrophotometer, optical density being measured at 350 nm.

EXPERIMENTAL RESULTS

The results of analysis of the protein and DNA concentrations in supernatants obtained after centrifugation of chromatin–heparin mixtures for 30 min and 12 h at 114,000g in medium of physiological ionic strength (0.15 M NaCl plus 0.7 mM Na-phosphate buffer, pH 7.0), are given in Fig. 1. As the proportion of heparin in the mixtures increased, solubilization of protein and DNA also increased, evidently to reach saturation in the presence of high heparin concentrations in the mixtures. After centrifugation for 1.5 h up to 27% of DNA and up to 66% of protein of the original chromatin remained in the supernatants.

It can be suggested that a certain structurally heterogeneous chromatin fraction, rich in protein, remained in the supernatant. In the presence of heparin the fibrils of this fraction acquired sedimentation resistance, possibly as a result of their easier loosening compared with other chromatin fibrils. Differences in the degree of loosening of the chromosomal fibrils may be associated with differences – quantitative and also, possibly qualitative – in their protein composition.

Preparations of chromatin in medium of physiological ionic strength $DNP_{0.15}$ were aggregatively unstable, like preparations in medium of high ionic strength with 0.7 M NaCl ($DNP_{0.7}$) [4, 5] and this was reflected in an increase in their viscosity measured at low shear inten-

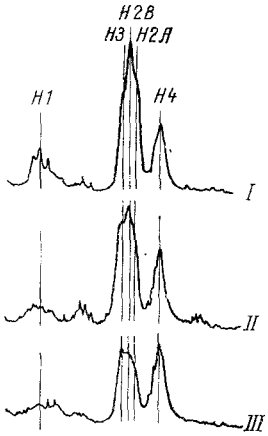

Fig. 3. Electrophoretic analysis of proteins remaining in residues after centrifugation of heparin–chromatin mixtures in medium of physiological ionic strength with different heparin/DNA ratios. Heparin/DNA ratios in mixtures: I) control (DNP without heparin); II) 12.5, III) 60.5.

sities (τ). At sufficiently high values of τ preventing aggregation, $DNP_{0.15}$, like $DNP_{0.7}$ [3] was a stable system (Fig. 2a). However, on addition of heparin the viscosity of $DNP_{0.15}$ rose abruptly and then increased more slowly. This was not due to the formation of more stable aggregates, for heparin also causes a decrease in the turbidity of the suspension (Fig. 2b), i.e., a decrease in the volume of the elements of the dispersed phase scattering light [2]. The explanation of the decrease in turbidity by dispersion of DNP aggregates existing in the presence of heparin is unacceptable because it is difficult to attribute the stability of these aggregates to the action of ultrasound and, moreover, as was stated above, aggregative instability of DNP systems is expressed as an increase in viscosity whereas disaggregation of DNP fibrils must lead to a decrease in viscosity. Accordingly, the only acceptable explanation of the data in Fig. 2 can be loosening of the structure of the DNP fibrils by heparin. The two-stage character of the change in viscosities of the DNP suspension (Fig. 2a) may reflect differences in the kinetics of loosening of the structure of different fibrils.

At the same time, it can be postulated that changes in the compactness of DNA fibrils under the influence of heparin, manifested as an increase in the sedimentation resistance of DNP systems (the appearance of slowly sedimenting DNA), an increase in viscosity, and a decrease in the turbidity of the suspension, are induced by dissociation of protein. To discriminate dissociated protein from protein which can bind with slowly sedimenting DNA, the same mixtures of DNP–heparin were centrifuged for a longer time (12 h) under the same conditions. Under these circumstances DNA was not fixed in the supernatant, but protein was found. Clearly this protein had dissociated from DNA.

The attempt to determine the protein composition of the supernatants by electrophoresis was unsuccessful. Protein bands could not be distinguished in the polyacrylamide gel. Accordingly the residues, in which there must have been only a little heparin, were subjected to electrophoresis. Densitograms of the gels are illustrated in Fig. 3. The decrease in intensity of the histone bands becomes noticeable with high heparin/DNA ratios (12.5) in chromatin–heparin mixtures. The first bands to be reduced in intensity are histones H1, H2A, and H2B. No predominant dissociation among them could be detected. When the ratio of heparin to DNA was 60.5, the bands of these histones were reduced in intensity still more. Meanwhile the amplitude of the inflection corresponding on the densitogram to histone H3 was reduced. However, this could be attributed to a decrease in the contribution of the overlapping band of histone H2B to the band of histone H3. Evidence against dissociation of histone H3 is given by the constancy of the amplitude of the histone H4 band and the fact that histone H3 has higher affinity for DNA than all other histones in a medium of physiological ionic strength [11].

The only definite conclusion that can be drawn is thus that the presence of heparin in the chromatin suspension in a medium of physiological ionic strength causes dissociation of histones H1, H2B, and H2A. Failure of attempts to demonstrate this phenomenon in previous studies using both heparin [10] and other polyanions [1] can evidently be attributed to the use of insufficient concentration of the polyanions in the experiment.

The question arises: Has dissociation of chromatin histones by the polyanionic components of the cell any biological meaning if the phenomenon requires polyanion/DNA ratios that

are so high that they are never found in the cell? However, it may be supposed that high heparin concentrations were necessary in the present experiment simply to establish biochemically the fact that histones are dissociated. The possibility cannot be ruled out that dissociation takes place in the presence of substantially lower heparin/DNA ratios, but that aggregation of heparin–protein complexes in a medium of physiological ionic strength leads to their cosedimentation along with DNA. To make this possibility less likely, we centrifuged a DNP–heparin mixture with a low DNP concentration (C_{DNA} =40 μg/ml). However, the presence of cosedimentation cannot be ruled out. The results of the present investigation show that quite low concentrations of the polyanion ($C_{heparin}/C_{DNA} \geq 0.01$) can induce loosening of the compact structure of chromosome fibrils in a medium of physiological ionic strength and with neutral pH.

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