# REORGANIZATION OF CHROMATIN IN MEDIUM OF PHYSIOLOGICAL IONIC STRENGTH IN THE PRESENCE OF EXTRA TOTAL HISTONE

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Investigation of the ability of chromatin to undergo structural reorganization under the influence of natural polyions *in vitro*, in the writers' opinion, is a promising approach to the understanding of the structural and functional dynamics of chromatin in the living cell. In such investigations it is desirable to use chromatin preparations obtained by widely different methods, for each method preserves something but at the same time modifies something in the spectrum of properties of the cell chromatin. Preparations of chromatin obtained from eukaryote cells in medium of physiological ionic strength (henceforward they will be called physiological deoxyribonucleoproteins — $DNP_p$), we consider, are the closest to native. This view is supported by the fact that all other chromatin preparations are obtained by subjecting the cells to additional procedures besides homogenization in physiological saline. The writers described previously the results of an analysis of structural changes in $DNP_p$ under the influence of polyanions [2, 8, 13-15]. In those experiments a situation was created when the polyanionic components of the mixture (acid chromatin proteins, DNA, added polyanions) were present in excess relative to histones, and, consequently, they could compete for histones and cause their redistribution. Recently [2] the writers showed for the first time that three histone fractions — H1, H2A, and H2B — are redistributed from $DNP_p$ to the natural polyanion heparin, added in a great excess to medium of physiological strength.

The object of the present investigation was to analyze the reorganization of chromatin by natural polyions with a polycationic character. For this purpose total histone of chromatin was used. The aim was to discover whether histones of $DNP_p$ can be redistributed if an excess, not of polyanions but of all five histone fractions, is created in the system. Redistribution could take place if the histones added to a chromatin suspension in physiological medium could compete for DNA with intrinsic proteins of chromatin. To assess the character of possible reorganization of chromatin in the presence of added total histone, accessibility of chromatin DNA to DNase II was analyzed.

## EXPERIMENTAL METHOD

Chromatin ($DNP_p$) was obtained from calf thymus by homogenizing 20 g of the gland for 1 min in 200 ml of standard solution: 0.024 M EDTA—$Na_2$ + 0.075 M NaCl, pH 8.0. The residue obtained after centrifugation (2500 g, 10 min) was homogenized for 10 sec and again centrifuged, the operation being repeated six times. The resulting $DNP_p$ was dispersed in standard solution and diluted to $C_{DNA}$ = 400 μg/ml. To the DNP suspension an equal volume of a solution of calf thymus total histone was added so that the ratios of chromatin DNA to added histone were 1:5 and 1:20 in two different samples. Histones were obtained by acid extraction of chromatin in 0.4 N HCl. The mixtures and original chromatin were incubated overnight at 4°C in the presence of 1 mM phenylmethylsulfonylfluoride. The mixtures were then centrifuged to separate the free histones. The residue was used for electrophoretic analysis of the protein composition of the nucleoprotein complexes [6] after washing twice in standard solution. In parallel tests the washed residue was resuspended in a solution of 10 mM tris-HCl, pH 7.2, and DNase II was added (ratio DNase II/DNA = 1 by weight). Incubation with the enzyme was carried out for 1 h at 37°C. Some of the material was then used for electrophoretic analysis of DNA degrada-

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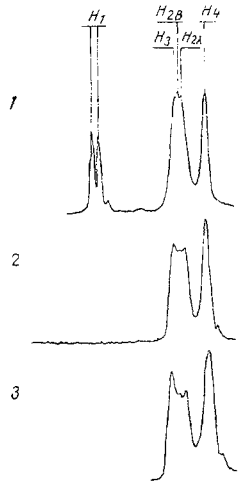


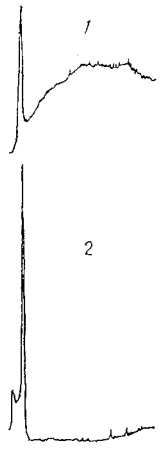


Fig. 1 Fig. 2

Fig. 1. Densitograms of polyacrylamide gels after electrophoresis of proteins of chromatin and nucleoprotein complexes formed in chromatin–total histone mixtures. 1) Electrophoresis of proteins of original chromatin, 2) of residues of chromatin–total histone mixture; ratio of added histone/chromatin DNA 5:1, 3) electrophoresis of residues of chromatin–total histone mixture; ratio of added histone/chromatin DNA 20:1.

Fig. 2. Densitograms of gels after electrophoresis of DNA fragments obtained by treating original chromatin and chromatin reorganized by total histone with DNase II. 1) Fragments of DNA of original chromatin, 2) fragments of DNA complex formed in chromatin–total histone mixture; ratio of added histone/chromatin DNA 5:1. Direction of DNA migration from left to right.

tion products [7], the rest for determination of the amount of acid-soluble DNA appearing in the preparations after the action of DNase II. Acid-soluble DNA was extracted in 0.5 N $HClO_4$. The DNA concentration was determined by Spirin's method [3].

## EXPERIMENTAL RESULTS

Densitograms of the gels after electrophoresis of proteins of chromatin and nucleoprotein complexes formed in a chromatin–total histone mixture in medium of physiological ionic strength (0.075 M NaCl + 0.024 M EDTA–$Na_2$, pH 8.0) with ratios of chromatin DNA to added histones of 1:5 and 1:20, respectively, are shown in Fig. 1. Clearly a fivefold excess of total histone led to complete removal of histone H1 from chromatin. Some of the H2B and H2A histones also were removed. This can be deduced from the fact that the peaks corresponding to these histones became smaller than the histone H4 peak, whereas these peaks in the densitogram of proteins of the original chromatin were about equal. The peak corresponding to histone H3 in the chromatin preparation treated with total histone also was reduced somewhat. Previously, by means of competitive analysis of histone binding with DNA the writers showed [11] that histones H3 and H4 are more firmly bound with DNA than the other fractions and form a complex with DNA in equimolar proportions. Accordingly, it can be postulated that the decrease in size of the histone H3 peak mentioned above takes place on account of a decrease in the contribution of the histone H2B peak, which overlaps it.

In a 20-fold excess total histone caused some additional decrease in the content of histones H2A and H2B in chromatin compared with a fivefold excess. However, this decrease was not very large, indicating great differences in the strength of binding of histone H1 and the group of histones H2A and H2B with DNA in $DNP_p$. The writers showed previously that if DNA and

total chromatin histone are mixed in medium of physiological ionic strength (0.15 M NaCl + 0.7 mM Na-phosphate buffer, pH 7.0) with a ratio of histone/DNA equal to 20:1, only histones H3 and H4 form a complex with DNA. It now appeared that a 20-fold excess of histones removes only a small quantity of histones H2A and H2B from chromatin. The reason for this may be either some difference in the media used in the two cases, or the effect of nonhistone chromatin proteins on the strength of bonds between histones and DNA in the experiments described above, or some difference in the relations of histones with DNA when mixed in physiological saline and in chromatin formed directly in the cell (in $DNP_p$).

It is clear that the changes found in the composition of $DNP_p$ in the presence of extra total histone are due to competition between histone fractions for DNA and they reflect the dynamic character of DNA–histone relations in the chromatin preparations used.

To analyze the organization of histones on DNA in chromatin when reorganized by extra total histone, original and reorganized chromatin were treated with DNase II. It was shown previously that addition of histones to chromatin in medium of low ionic strength weakens degradation of chromatin DNA by DNase I [10] and by staphylococcal nuclease [5]. This was explained by binding of the extra histone to free phosphate groups of the chromatin DNA. This hypothesis was confirmed by the discovery of a decrease in the concentration of $Mn^{++}$ ions bound with phosphate groups of chromatin DNA after addition of histones [5]. However, we know [12] that the number of free phosphate groups of DNA in chromatin remains practically unchanged after its treatment with an excess of total histone in medium of physiological ionic strength. This fact could be connected with differences in the organization of chromatin in media of low and physiological ionic strength.

The present experiments with DNase II showed that under conditions when acid-soluble fragments of DNA extracted with 0.5 N $HClO_4$ from original chromatin amounted to 50%, only about 9 and 6% of DNA of chromatin treated with fivefold and 20-fold excesses of total histone, respectively, changed into the acid-soluble state.

Densitograms of gels obtained by electrophoresis of DNA after treatment of original chromatin and chromatin treated with a fivefold excess of total histone with DNase II are shown in Fig. 2. In the latter case nearly all the DNA was held up at the starting line, and only a small quantity of the degradation products of lowest molecular weight migrated into the gel. Polymer fragments formed after treatment of original chromatin with DNase II were completely absent in enzymic digestion products of chromatin treated with total histone.

Chromatin in a medium of physiological ionic strength is thus modified by the addition of an excess of total histone so that it loses histone H1 and some of its histones H2A and H2B, and becomes highly resistant to the action of DNase II. The decrease in accessibility of the DNA of chromatin treated with total histone to DNase II may be connected with increased ability of chromatin to undergo aggregation, despite removal of histone H1 from it.

Previous investigations showed that natural polyanions cause structural modification of chromatin, leading to redistribution of histones [2] and increasing the accessibility of the chromatin DNA to the action of DNases [14]. It now becomes clear that polycationic components of the cell nucleus such as histones can also reorganize chromatin, by inducing redistribution of histone fractions on DNA, thereby excluding from chromatin the more weakly bound H1 fraction and some of the H2A and H2B fractions, and reducing the accessibility of the chromatin DNA to DNase II. The data as a whole suggest that the appearance of any polyionic components in the nucleus must affect the structural (and functional) state of chromatin in the cell. That is why to preserve the state of chromatin the content of polyions of the cell nucleus must be strictly determined by certain factors not only quantitatively, but also qualitatively.

There are indications that some cells may contain 1000-fold excesses of histones relative to DNA [4, 9]. The consequences of such a high histone content for the organization of the chromatin of such cells still awaits study, with allowance for the data now obtained. However, it may be that certain factors preventing competition between histones for DNA and, consequently, protecting chromatin against the reorganization which we observed *in vitro*, may exist in such cells, and that these factors are absent when histones are present in an excess over DNA.

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# EFFECT OF SELENIUM-CONTAINING COMPOUNDS ON ELECTRICAL ACTIVITY OF THE ISOLATED GUINEA PIG RETINA DURING INDUCED LIPID PEROXIDATION

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Data in the literature [5] and our own experimental observations [3] indicate that accumulation of endogenous products of lipid peroxidation (LPO) inhibits electrical activity of the isolated retina of cold-blooded animals. At the same time, LPO in the retina is known to be inhibited by various selenium compounds, among which organic compounds have proved to be most effective [4].

In connection with the facts described above it was interesting to study the possibility of regulation of electrical activity of the isolated retina of warm-blooded animals by means of selenium-containing compounds under conditions of induced lipid peroxidation.

## EXPERIMENTAL METHOD

Experiments were carried out on 120 male guinea pigs weighing 300-350 g, kept under standard vivarium conditions. The retina of dark-adapted animals (for not less than 2 h) was removed by a special surgical method from the eye in weak red light. The preparation thus obtained was placed on filter paper with the receptors facing downward and laid in a special continuous-flow chamber with two recording electrodes. The retina was perfused with Ringer's solution for warm-blooded animals, regularly saturated with oxygen. The electroretinogram (ERG) was recorded by means of the UBP 1-02 biopotentials amplifier and photographed from the screen of an S1-18 oscilloscope. Photic stimulation was applied by means of a xenon flash tube, giving flashes from 0.005 to 0.19 J in intensity. The light was focused on the light guide of the chamber by an optical system. The flash duration was 150 μsec.

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