HETEROGENEITY OF CHROMATIN SUBUNITS

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1. Introduction

Recent investigations suggest that chromatin is organized in linearly arranged, compact subunits (nucleosomes) connected by relatively short DNA threads [1–7]. It is not clear, however, whether all nucleosomes are similar in structure. Neither is it clear whether they undergo structural changes related to chromatin functioning in the cell-cycle.

Mild nuclease treatment of the nuclei or chromatin cuts chromatin into fragments consisting of monomers (mononucleosomes), dimers etc. [8]. It was shown that mononucleosomes can be separated into two electrophoretic fractions, only one of which containing H1 histone [9]. In the present study it has been found that mononucleosomes include several fractions (up to 4) varying in their protein moieties and sizes of DNA segments. Additionally, the distribution of rapidly-labelled RNA and newly synthesized DNA among electrophoretic chromatin fragments has been investigated.

2. Materials and methods

Nuclei and nucleosomes were obtained from rat thymus by the method described in ref. [8]. Electrophoresis of nucleosomes was done in a 5% polyacrylamide slab-gel.

To analyze the distribution of rapidly-labelled RNA in nucleosome fractions the nuclei isolated from hepatoma or L-cells labelled for 15–30 min with [^{14}C]uridine (10 μCi/ml, Czechoslovakia) were digested with DNAase II (Worthington) in the presence of the protease inhibitor, phenylmethanesulfonylfluoride. A single extraction of free RNP-particles was made [10] prior to DNAase II digestion. DNA was pulse-labelled (5 min) with [^{3}H]thymidine (50 μCi/ml, Czechoslovakia). In the pulse-chase-experiment (3 h chase) the radioactive medium was replaced with a fresh medium containing 20 μg/ml of non-labelled thymidine. Micrococcal nuclease was used for digestion. The resulting radioactive chromatin fragments were subjected to electrophoresis in 5% polyacrylamide gel. Gel was sliced into 4–5 mm fractions and the slices hydrolyzed with 1 ml of 30% hydrogen peroxide at 60°C. Radioactivity of the samples was counted in a toluene scintillator containing Triton X-100 in a Mark II scintillation spectrometer (Nuclear Chicago).

3. Results and discussion

The sedimentation coefficient of mononucleosomes ranged from 10–11 S (average 10.55 S) which is in agreement with the results of other authors [2,4]. Low ionic strength electrophoresis allowed us to separate the mononucleosomes into 4 fractions (fig.1). It was previously found that nucleosomes obtained from isolated chromatin of the Ehrlich ascites cells contained two fractions [9].

We found also that mononucleosomes obtained from nuclei contained an additional non-histone protein as a minor component (fig.2). Its content varies but none the less it occurred in all nucleosome preparations tested. This additional protein did not prove to be a large fragment of histone H1 because, in contrast to histone H1, one could not extract it with either 5% $HClO_4$ or 0.6 M NaCl. Its extractability and SDS–electrophoretic mobility were very similar to those of 'histone-like' non-histone protein [11]. According to two-dimensional electrophoresis the additional protein,

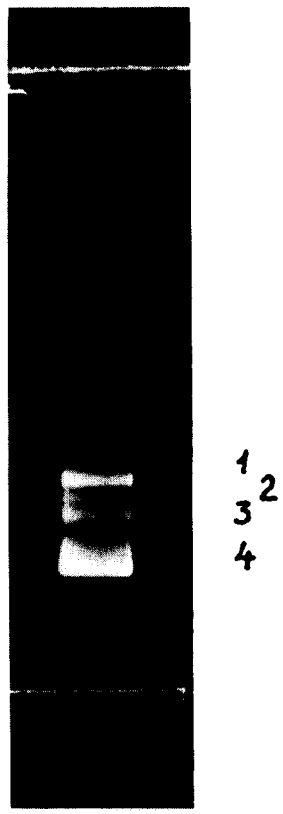


Fig.1. Electrophoretic separation of mononucleosomes. The figures designate mononucleosome fractions according to their increasing electrophoretic mobility. Electrophoresis was done in a 5% polyacrylamide slab-gel 1.5 mm thick (ratio, acrylamide/bisacrylamide 30:1) Tris–borate buffer, 0.01M, with 0.001 M EDTA, pH 8.3, was used as electrophoretic buffer. Gel was stained with ethidium bromide and visualized under ultraviolet light.

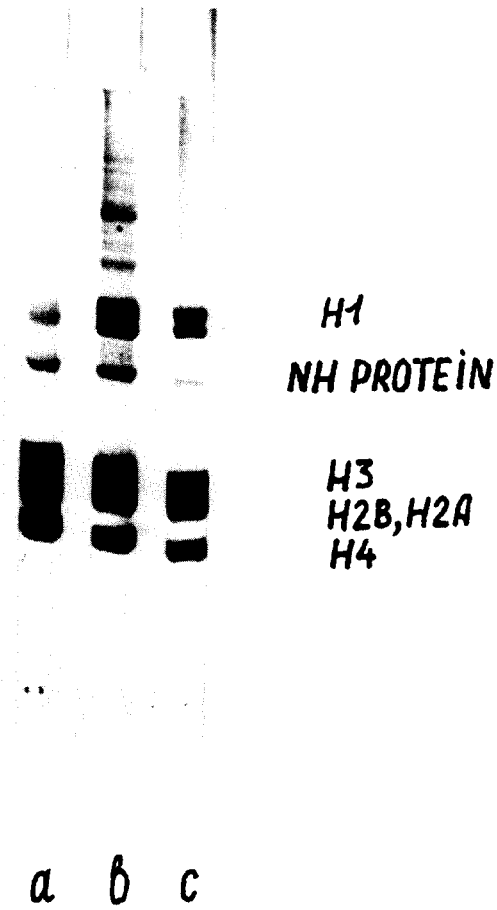


Fig.2. Electrophoresis of mononucleosome proteins. 'a' and 'c'. Mononucleosome proteins, 'b', Acid-extracted protein from nuclei. Nucleosomes and acid-extracted protein were dissolved in electrophoretic buffer containing 1% β-mercaptoethanol, 1% SDS and 20% sucrose, then heated on a boiling water bath. Electrophoresis was according to Laemmli [13]. Proteins were stained in 0.1% Coomassie R250 solution (Serva).

like histone H1 is absent from some mononucleosome fractions (fig.3). Mononucleosome fractions were designated 1, 2, 3 and 4 according to their increasing electrophoretic mobility (fig.1). One can see that the fastest of these, fraction 4, lacks both histone H1 and the additional protein. Fraction 5 shown in fig.3 occurs when the gel is greatly overloaded and is likely to be subnucleosomal material containing all four histone fractions with the exception of H1.

What is the nature of the electrophoretic heterogeneity of mononucleosomes? The presence or absence of histone H1 in nucleosomes determines the most essential differences in electrophoretic mobility (fractions 1 and 4, figs.1 and 3). As shown recently nucleosomes from Ehrlich ascites cell lacking histone H1 do not contain a short DNA fragment of about 30 nucleotides which can be removed together with histone H1 [9]. Two-dimensional DNA electrophoresis of nucleosome fractions supports this conclusion. Moreover nucleosome fractions of intermediate mobility are found to contain a DNA segment of a size intermediate between that of the DNA fragments of fractions 1 and 4 (fig.4). Comparison of the results

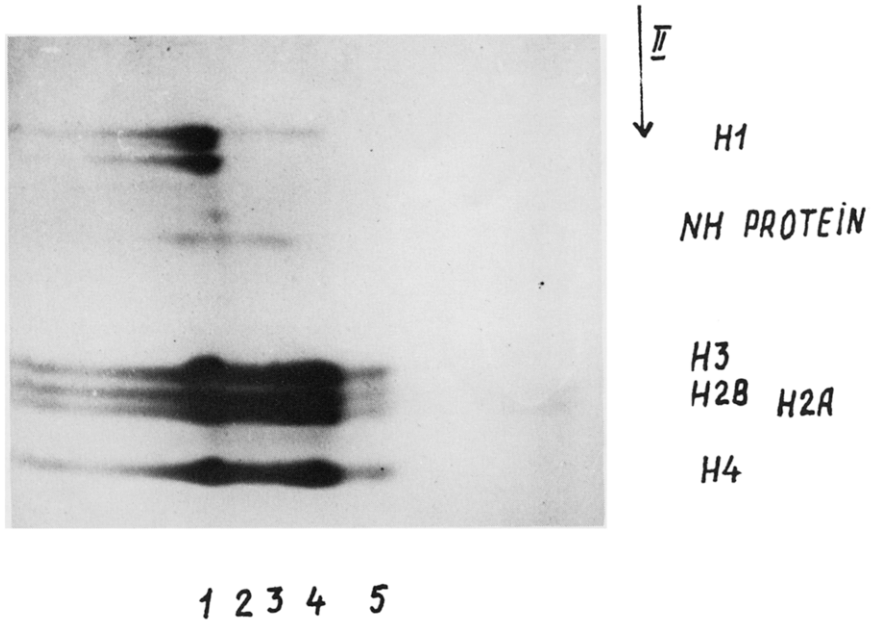
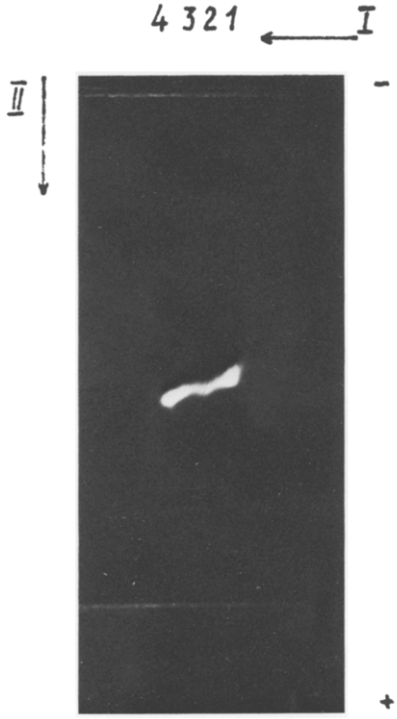


Fig.3. Two-dimensional electrophoresis of proteins of mononucleosomal fractions. I, Low ionic strength electrophoresis of nucleosomes in 5% polyacrylamide gel (see Materials and methods). II, SDS-electrophoresis of nucleosomal proteins in 15% polyacrylamide gel according to Laemmli [13]. Arrows indicate the direction of electrophoresis. Figures are the same as in fig.1. Fraction 5 is a subnucleosomal material.



given in figs.3 and 4 allows the following suggestions to be made: first, on nuclease digestion of nucleosomes a small DNA fragment is lost, together with histone H1, and this is a prerequisite to the formation of nucleosomes 2 and 3, which are intermediate as regards their electrophoretic mobilities. Second another small DNA fragment is lost which is accompanied by removal of additional protein and this produces nucleosome 4.

It is of interest to elucidate if the discrete pattern of mononucleosome fractions is related to their function during chromatin transcription and replication processes. We studied localization of rapidly-labelled RNA bound to chromatin fragments after nuclease digestion. For these experiments we used another nuclease, DNAase II, since micrococcal nuclease also shows RNAase activity [12]. Special experiments

Fig.4. Two-dimensional electrophoresis of DNA fragments of mononucleosomal fractions. I, as in fig.3. II, SDS-electrophoresis of nucleosomal DNA in 7.5% polyacrylamide gel.

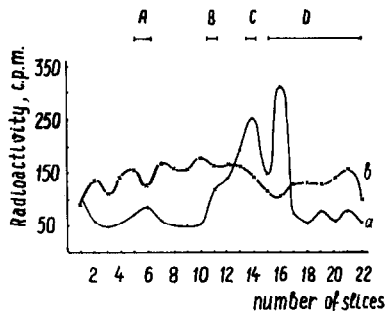


Fig.5. Electrophoresis of [^{14}C]uridine labelled (a) nucleosome fractions and (b) RNP-particles extracted from nuclei. (A) Dinucleosomes. (B) Mononucleosomes with histone H1 (fraction 1, fig.1). (C) Mononucleosomes lacking histone H1 (fraction 4, fig.1). (D) Subnucleosomal region.

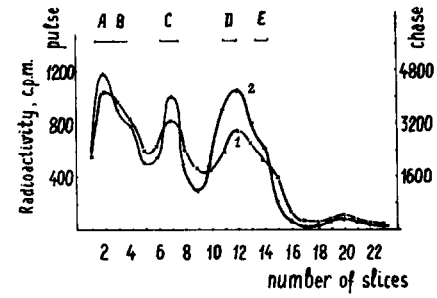


Fig.6. Distribution of radioactive [^{3}H]thymidine in nucleosome fractions. (1) Pulse-label (5 min). (2) Pulse-label (5 min) followed by chase (3 h) in the presence of 20 μg/ml of non-labelled thymidine. (A–B) Trinucleosome and tetranucleosomes. (C) Dinucleosomes. (D) Mononucleosomes with histone H1. (E) Mononucleosomes lacking histone H1.

indicate that electrophoretic characteristics of nucleosomes produced by these two nucleases practically coincide. DNAase II - fragmented chromatin was analyzed by electrophoresis on 5% polyacrylamide gel and the radioactivity of RNA bound to different chromatin fragments was counted (fig.5).

As can be seen from fig.5(a), rapidly-labelled RNA is distributed among different electrophoretic chromatin fractions. Radioactive RNA was found in subnucleosomal particles, in mononucleosomes and dinucleosomes, as well as in large chromatin fragments which fail to enter the gel. Of interest is the fact that among different mononucleosomes rapidly-labelled RNA is found predominantly in mononucleosomes lacking histone H1.

Electrophoresis of the RNP-particles obtained [10] has shown a rather diffuse distribution of the radioactivity along the gel (fig.5(B)). This result excludes the possibility of RNP- and DNP-particles co-migrating. We studied also the co-precipitation of unlabelled nucleosomes and labelled RNP-particles in the presence of Mg^{2+}. The results of this control experiment also indicate that the presence of radioactivity in nucleosomes is not due to the absorption of RNP-particles on to nucleosomes. Incorporation of [^{14}C]uridine into nucleosomal DNA in our experiments is negligible. Thus, on the basis of data obtained it can be assumed that RNA synthesis occurs in chromatin regions with typical nucleosomal structure.

In other series of experiments distribution of radioactivity on electrophoregrams was examined after a pulse of [^{3}H]thymidine incorporation (5 min) and a 3 h chase. In this instance the localization of radioactivity coincides with that of electrophoretic fractions of mononucleosomes, dinucleosomes, trinucleosomes etc. both after short-labelling and chase-experiments (fig.6). Consequently nascent chromatin has a subunit structure typical of the whole chromatin even after 5 min incorporation of [^{3}H]thymidine when only short DNA fragments (Okazaki fragments) are synthesized [14].

Thus, replication and transcription of chromatin is not associated with any significant and prolonged transformation of its subunit structure as revealed by electrophoresis. Apparently, these processes involve chromatin unfolding, which is presumably associated with the changes in localization of histone H1 on nucleosomes.

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