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A SIMPLE AND REPRODUCIBLE METHOD FOR ANALYSIS OF CHROMATIN CONDENSATION

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A method for examination of chromatin condensation with the help of gel electrophoresis in low-density agarose gels was suggested. This method provides a way for the degree of chromatin condensation to be estimated at different ionic conditions of the medium. It led to results which are in close agreement with the results of other traditional methods. Thus it was inferred that this method offers an alternative to the method of density-gradient ultracentrifugation for chromatin condensation study.

Higher eukaryotes' chromosomes are constituted by chromatin fibers with a diameter of about 30 nm. This filaments unwind at low ionic strength of the medium and the absence of bivalent cations, resulting in chromatin filaments of about 10 nm in diameter (review [1]). The main structural unit of the filament is nucleosomal core particle that is made up of histones H2A, H2B, H3, H4, and DNA fragment of 146 bp in length. More complex particle, the chromatosome, consists of octamer of histones H2A, H2B, H3, H4, a molecule of lysine-rich histone H1 (or H5), and 165-170 bp DNA fragment. According to present views, the histone H1 is involved in 10 nm filament

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condensation and has no significant effect on nucleosomal structure as such.

There are traditional methods of chromatin condensation study: electron microscopy [1-3], ultracentrifugation in density gradient of sucrose [2-4], metrizamid [5, 6], $CsCl_2$ (see review [7]), and electric dichroism measurements [8-10], as well as neutron scattering [11, 12]. The undoubted advantages of these methods are accompanied by a number of disadvantages. For instance, the complicated and cumbersome equipment, elaborate procedures of forming and analysing the gradients, long persistence of the experiment all are limitations of the ultracentrifugation. In the present work we propose a simple and reproducible method of chromatin condensation analysis with the help of electrophoresis in agarose gels of low concentration.

Materials and Methods

Rat liver chromatin was isolated as described by Keinz et al. [2]. All procedures were conducted at 0°C. The liver was gomogenized in buffer A (50 mM Tris HCl pH 8.0, 0.25 M sucrose, 5 mM $MgCl_2$, 5 mM Na-butyrate, 5 mM 2-mercaptoethanol, 0.1 mM PMSF). Then the equal volume of the same buffer with 2M sucrose and 1% NP40 was added. After the incubation for 10 min, the nuclei were pelleted by centrifugation at 5,000g for 20 min. The pellet was suspended in buffer A that contained 1 M sucrose an 0.25% NP40 and centrifugated at 5,000g for 10 min. This procedure was repeated twice. Then the nuclei were treated in the same manner with buffer A containing 50 mM KCl and suspended in buffer A with 100 mM KCl , 1 mM $CaCl_2$, up to final concentration of $2.5x10^8$ nuclei/ml. The nuclei were then treated with micrococcal nuclease (2u/10^6 nuclei) at 0°C for 5 minutes. The reaction was terminated by adding EDTA up to 10 mM and the reaction mix then was dialysed overnight against 1 mM EDTA. The remains of nuclear membranes were pelleted at 5,000g for 20 min, supernatant contained 50-80% DNA in the state of nucleosomal filament fragments. Isolated chromatin was used in the following procedures directly as well as after additional purification by gel-filtration on a column TOYOPEARL HW75F (1x14 in) equilibrated with buffer A containing 25 mM NaCl and 2 mM EDTA. Chromatin fragments of 15 nucleosomal links were used.

For removal of H1 histone, chromatin was transferred by dialysis into the buffer containing 50 mM Na-phosphate pH 7.0, 0.2 mM EDTA, 100 mM NaCl. Then it was mixed with 1/4 volume of an ionite AG50W-X2 equilibrated with the same buffer and stirred for 2 hours at 0°C. Thereafter the ionite was pelleted by centrifugation at 3,000g for 10 min. Supernatant contained the chromatin without H1 histone [1].

For isolating the chromatin in the different states of condensation, the chromatin portions were dialysed for 20 hours against solution containing 5 mM triethanolamine pH 8.0, 0.25 mM EDTA, and NaCl in different concentrations (from 0 to 120 mM). Then the chromatin was treated with 0.1% glutaraldehyde [1] overnight at 4°C temperature. Fixed chromatin obtained in this manner, afterwards was dialysed overnight against 1 mM EDTA at

4°C, and analyzed on 0.25-0.4% agarose gel with Tris-acetate buffer [13]. Histone were extracted from the chromatin by 0.2 M H_2SO_4 and, after precipitation by 4 volumes of ethanol, were analyzed in 10% polyacrylamide gel with 50 mM Na-phosphate (pH 7.0) and 0.25% SDS [14].

Densitometric scans were obtained with the help of a 300A Fast Scan computing densitometer, Molecular Dynamics.

Results and Discussion

Intact chromatin in the solution of low ionic strength contains all histone and forms 10 mn nucleosomal filament with nucleosomes which are easily observable by electron microscopy [1]. The filament packs up into more compact fiber with the diameter of 25-30 nm in the presence of bivalent cations [15] or with the increase of ionic strength of the medium [1, 2]. In the present view [1, 16-18], this fiber has solenoidal structure with 3-6 nucleosomes per turn depending on NaCl concentration. These processes of chromatin condensation were studied in our work using electrophoresis in the agarose gels of low concentration. Chromatin nucleosomal fiber fragments (average size of 12-15 nucleosomes) were obtained by the soft treatment of rat liver nuclei with micrococcal nuclease. Fresh chromatin solution then was portioned and dialysed against buffers containing 5 mM triethanolamine, 0.25 mM EDTA , and NaCl at different concentrations. Afterwards the chromatin was fixed by 0.1% glutaraldehyde at 4°C for 15 hours.

The results of the chromatin electrophoresis in 0.3% agarose gel are shown in Fig. 1. As is obvious from Fig. 1 the chromatin fixed at higher NaCl concentration has a higher electrophoretic mobility. It reflects the process of salt-induced nucleosomal solenoid compactization and correlates well with the results obtained previously by ultracentrifugation in density gradient of sucrose [1, 2], and metrizamid [6].

Histone H1-depleted chromatin is incapable of forming the regular solenoidal structure and has structure of a random tangle, which parameters depend only weakly on the ionic strength of the medium. In our work we removed the H1 histone by treatment of the chromatin with an ionite AG50W-X2 [1] at moderate ionic strength (100 mM NaCl, 50 mM Na-phosphate pH 7.0). In these conditions H1 histone may be completely removed without considerable nucleosomes dissociation (Fig. 2). As expected, electrophoretic mobility of the chromatin stripped of H1 histone is essentially independent of the NaCl concentration

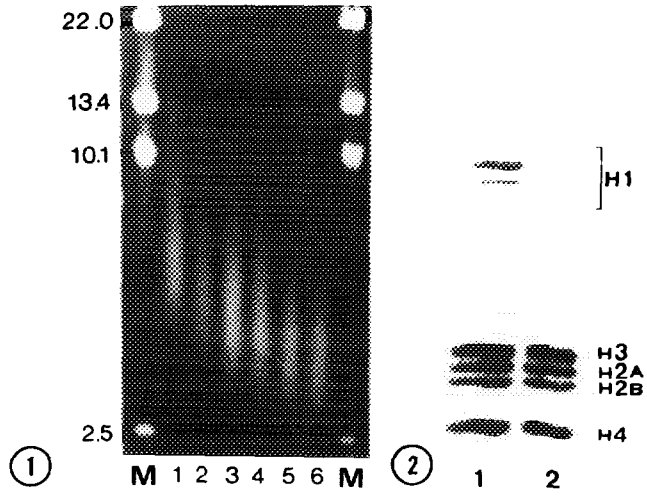


Figure 1. Electrophoretic analysis of chromatin samples in different states of condensation. Chromatin was fixed by glutaraldehyde at 4° C in the solution containing 5 mM triethanolamine, 0.25 mM EDTA, and NaCl in the concentrations: 1 - 0 mM, 2 - 10 mM, 3 - 20 mM, 4 - 30 mM, 5 - 60 mM, 6 - 120 mM. M - marker DNA (Lambda digested by restriction endonuclease Bgl II), fragment sizes are given in kb.

Figure 2. Intact chromatin histone (1) and histone of the chromatin treated with an ionite AG50W-X2 (2).

during the chromatin fixation (Fig.3). Figure 4 gives densitometric tracing of the gel patterns shown in Figures 2,3.

The dependence of chromatin relative electrophoretic

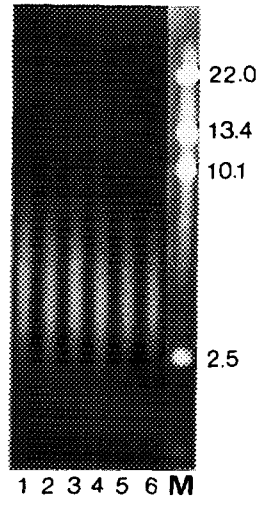
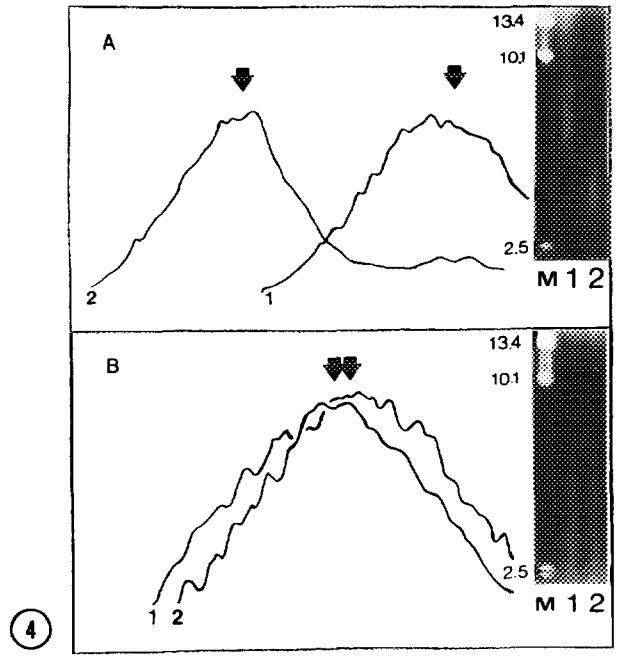


Figure 3. Electrophoretic analysis of H1-depleted chromatin samples in different states of condensation. Chromatin treated with ionite was fixed by glutaraldehyde in the solution containing 5 mM triethanolamine, 0.25 mM EDTA, and NaCl in the concentrations: 1 - 0 mM, 2 - 10 mM, 3 - 20 mM, 4 - 30 mM, 5 - 60 mM, 6 - 120 mM. M - marker DNA (Lambda digested by restriction endonuclease Bgl II), fragment sizes are given in kb.



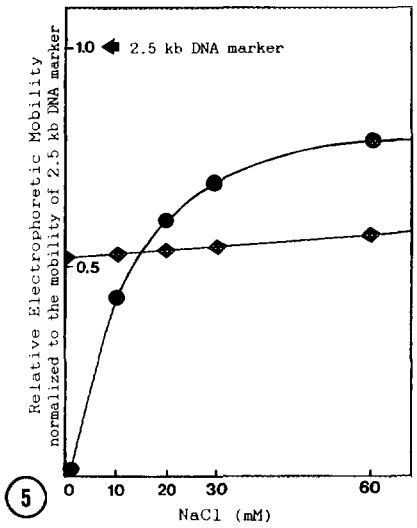


Figure 4. Densitometric tracing for the gel patterns of chromatin electrophoresis in 0.3% agarose. A - intact chromatin, B - H1-depleted chromatin. The chromatin was fixed by glutaraldehyde in the buffer containing 5 mM triethanolamine, 0.25 mM EDTA, and NaCl in concentration of 5 mM (curve 1) and 60 mM (curve 2). M - Lambda DNA digested by restriction endonuclease Bgl II; fragment sizes are given in kb. The arrows pinpoint the centers of chromatin electrophoretic distribution.

Figure 5. Relative electrophoretic mobility of the chromatin samples in 0.3% agarose gel depending on ionic strength during the fixation by glutaraldehyde. The electrophoretic mobility is normalized to the mobility of 2.5 bp DNA marker.
● - chromatin fixed at 4°C; ◆ - H1-depleted chromatin.

mobility on NaCl concentration is plotted in Fig. 5. The mobility slightly decreases as NaCl concentration decreases from 60 to 30 mM, markedly decreases with decreasing of NaCl concentration from 30 mM to 10 mM, and drastically falls as NaCl ranges from 10 mM to about 0 mM. This effect, apparently, results from condensation-decondensation processes involving H1 histone. It is confirmed by the results of electrophoresis of H1-depleted chromatin, whose electrophoretic mobility depends only weakly on NaCl concentration ranging from about 0 to 60 mM. Thus our results lead to the conclusion that the dramatical decrease of chromatin electrophoretic mobility with NCl concentration reflects nucleosomal fiber "unfolding" because of change in H1 histone interactions.

Our results obtained by proposed method agree well with the data on chromatin ultracentrifugation [1, 2, 6]. This

enables us to suggest a simple and reproducible method of electrophoresis in low-concentration agarose gels for the study of chromatin condensation as alternative to high-speed density-gradient ultracentrifugation .

Acknowledgment

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