CHARACTERISTICS OF CEREBRAL NON-HISTONE CHROMATIN PROTEINS AS REVEALED BY POLYACRYLAMIDE GEL ELECTROPHORESIS

Tomoko TASHIRO, Fumio MIZOBE and Masanori KUROKAWA

Biochemistry Department, Institute of Brain Research, Tokyo University Faculty of Medicine, Tokyo, Japan

Received 6 November 1973

1. Introduction

A rapid method has been developed for the polyacrylamide gel electrophoretic anàlysis of the whole chromatin proteins in small amounts, and comparison of non-histone proteins has been made among chromatins prepared from neurone-rich, oligodendroglial as well as liver nuclei. Neuronal chromatin is characterized by its unusual wealth of high molecular weight non-histone proteins. Neuronal chromatin is also provided with a group of non-histone proteins of higher molecular weights, which is apparently absent in oligodendroglial and liver chromatins.

2. Materials and methods

2.1. *Isolation of nuclei*

Nuclei were isolated at 0–4°C from the cerebral cortex and liver of the adult guinea pig. Brain was briefly perfused in situ as described previously [1]. All the sucrose solutions used for the preparation of nuclei contained $CaCl_2$ to the final concentration of 1.5 mM. Cerebral cortices collected from 30 animals (approximately 50 g) were homogenized in 5 volumes of 0.32 M sucrose, filtered through eight layers of gauze, and finally made up to about 5% by adding appropriate volumes of 0.32 M sucrose. Two 500 ml portions of the homogenate were centrifuged at 1000 *g* for 10 min, pellets were ground in 3.5 ml of 0.32 M sucrose, and made to 2 M with respect to sucrose by mixing with appropriate volumes of 2.4 M sucrose. Three 60 ml portions of the suspension were centrifuged at 75 000 *g* for 60 min, pellets were ground in 5 ml of 0.32 M sucrose, and made to 2.3 M with respect to sucrose by mixing with appropriate volumes of 2.5 M sucrose. A 9 ml portion of this suspension was layered on the top of a discontinuous density gradient consisting of 2.5 ml of 2.5 M and 2.5 ml of 2.4 M sucrose in an 18 ml tube fitting the Hitachi RPS 25-3A rotor, and on the top of the suspension was layered 1.5 ml of 2.0 M sucrose. Six equivalent tubes were centrifuged at 78 000 *g* for 60 min. A white band at the interphase between the 2.3 M and 2.4 M sucrose layers was collected by a bent-tip Pasteur pipette, 6 equivalent bands were combined and diluted to about 0.5 M with 0.32 M sucrose, and centrifuged at 1000 *g* for 10 min. This produced the neurone-rich nuclear preparation (cf. [1]). Oligodendroglial nuclei were recovered as the pellet at the bottom of the 18 ml tube. Liver nuclei were isolated as described previously [2]. All nuclear preparations were suspended in 0.32 M sucrose without $CaCl_2$, and stored at −80°C until use. They were further washed with 0.5% Triton X-100–0.32 M sucrose–1.5 mM $CaCl_2$ immediately before preparing the chromatin.

2.2. *Preparation of chromatin*

Unsheared chromatin was prepared from Triton X-100-washed nuclei according to Shaw and Huang [3], and immediately used for dissociation of chromatin proteins in Method A, or freeze-dried in Method B, as described below. Approximately 70% of DNA in nuclei was recovered in chromatin.

Abbreviations: SDS, sodium dodecyl sulphate; DTT, dithiothreitol; Bis, *N, N'*-methylenebisacrylamide; TEMED, *N, N, N', N'*-tetramethylethylenediamine.

2.3. *Treatments of chromatin*

(a) *Method A.* One-fourth volume of 10% (w/v) SDS–5% (w/v) β-mercaptoethanol solution was added to freshly prepared chromatin in water (pH 8). After homogenization, the chromatin solution was kept at room temperature for 30 min, and then centrifuged at 85 000 *g* for 24 hr to sediment DNA. Supernatant which contained dissociated proteins with no detectable amount of DNA was dialyzed against water to remove excessive SDS, and then freeze-dried. The freeze-dried protein was dissolved in appropriate volumes of 10% (w/v) sucrose – 10 mM Tris – 1 mM EDTA – 40 mM DTT (pH 8.0) to give the final concentration of about 1 mg of protein/ml, incubated at 40°C for 1.5–2 hr, and a 30–100 μl portion was subjected to polyacrylamide gel electrophoresis, with Pyronin G as a tracking dye. Concentration of SDS in the sample was estimated to be 1–1.5%, on the basis of the weight of SDS remaining in the freeze-dried material.

(b) *Method B.* The freeze-dried chromatin was suspended in appropriate volumes of 1% SDS – 10% (w/v) sucrose – 10 mM Tris – 1 mM EDTA – 40 mM DTT (pH 8.0) to give the concentration of about 1 mg of protein/ml. After incubation at 37°C overnight with occasional mixing, the suspension turned into an almost transparent and viscous solution. A 30–100 μl portion of the solution was subjected to electrophoresis, with Pyronin G as a tracking dye.

2.4. *Analytical polyacrylamide gel electrophoresis*

This was carried out as described by Fairbanks et al. [4], but with minor modifications. The gel (pH 7.4) finally contained 7.5% acrylamide, 0.2% Bis, 1% SDS, 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, 0.15% ammonium persulphate and 0.025% TEMED. Each column (6 mm × 85 mm) was overlaid gently with a solution of 0.1% SDS – 0.15% ammonium persulphate – 0.05% TEMED, and after 1 hr, top of gel was rinsed and overlaid with 0.5 ml of electrophoresis buffer (1% SDS – 40 mM Tris – 20 mM sodium acetate – 2 mM EDTA, pH 7.4). The gels were then left to stand overnight at room temperature. Electrophoresis was carried out in the anode direction with the current at 8 mA/tube until the tracking dye migrated 8 cm from the origin (about 2 hr). The gels were stained with 0.05% Coomassie Brilliant Blue R-250 in 25% (v/v) isopropanol – 10% (v/v) acetic acid, destained as

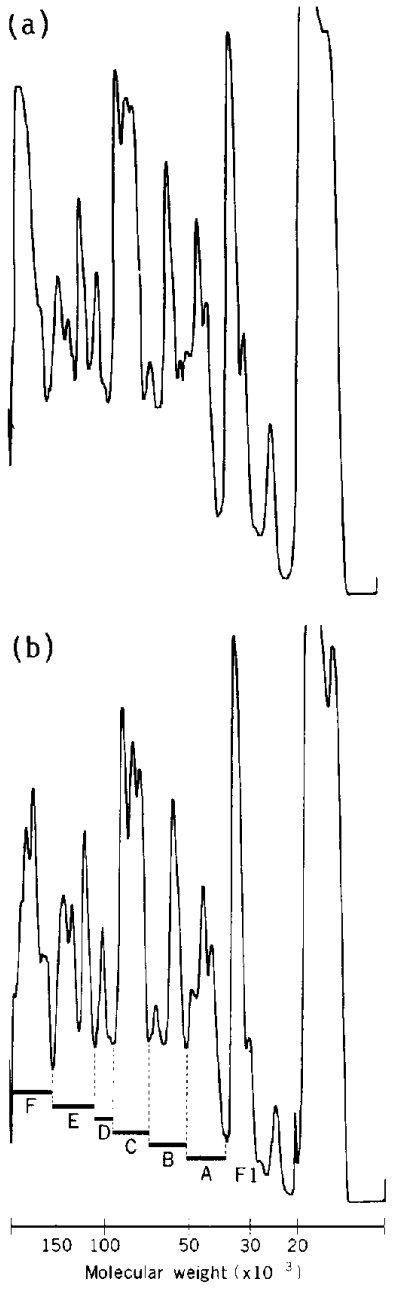

Fig. 1. Gel electrophoretic analysis of chromatin proteins prepared from neurone-rich nuclei. (a) Chromatin was treated by the Method A (see Materials and methods), and proteins devoid of DNA were subjected to electrophoresis. (b) Chromatin was treated by the Method B, and proteins were applied to gel with the concomitant presence of DNA.

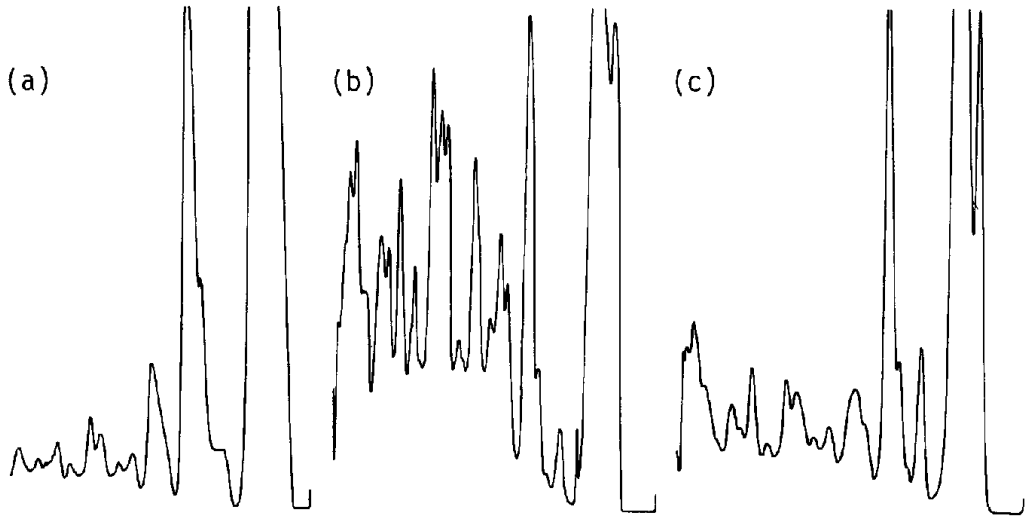

Fig. 2. Comparison of the non-histone proteins of liver (a) neurone-rich (b) and oligodendroglial (c) chromatins: respective chromatins were treated by the Method B (see Materials and methods).

described by Fairbanks et al. [4], and densitometric tracings were taken at 550 nm in a Joyce Loebl Chromoscan. Molecular weight markers for SDS gel calibration were cytochrome *c* (mol. wt. 12 400), trypsin (23 300), glyceraldehyde 3-phosphate dehydrogenase (36 000), ovalbumin (43 000), glutamate dehydrogenase (53 000) and bovine serum albumin (68 000 and 136 000).

2.5. *Assays*

DNA was determined according to Kissane and Robins [5] with calf thymus DNA as standard, and protein according to Lowry et al. [6] with bovine serum albumin as standard.

3. Results and discussion

In the present system, proteins whose molecular weights range from 10 000 to 200 000 can be resolved on the basis of their molecular size. The procedure is highly reproducible. Methods A and B give essentially the same electrophoretic banding patterns in any of the three types of chromatins examined (for neurone-rich chromatin, see fig. 1), indicating that the prior removal of DNA from chromatin is not critical for satisfactory resolution. Thus in Method B, rather lengthy procedures to remove DNA are obviated. Also Method B has advantages over Method A in that there is no possibility of losing proteins during the treatment of chromatin, and that the procedure can be carried out with much less amount of chromatin as starting material. The best electrophoretic resolution of non-histone proteins is obtained with 30–100 μg of the total protein per gel; larger amounts of samples may cause streaking and entrapment of material at the origin.

Histones migrate farthest in the anode direction. In the present system, some minor components of non-histone proteins which have been reported to have similar molecular size to histones [7, 8] are not well differentiated. Non-histone proteins which migrate at slower rates than F1 histone are tentatively grouped in 6 classes; A, 40 000–50 000 in molecular weight; B, 50 000–70 000; C, 70 000–90 000; D, 100 000; E, 100 000–150 000; and F, higher than 150 000 (fig. 1).

The ratio of total histones extracted by 0.4 N H_2SO_4 to DNA is comparable among three types of chromatin examined (approximately 1:1). In contrast, the amount of non-histone proteins on a histone (or DNA) basis is much larger in neurone-rich chromatin as compared with oligodendroglial and liver chromatins (figs. 2 and 3).

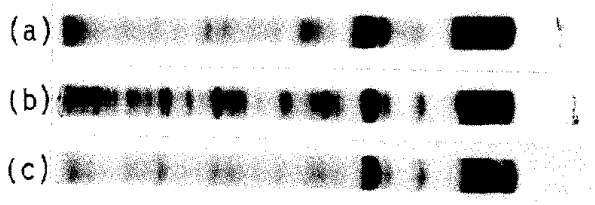

Fig. 3. Electrophorograms of liver (a) neurone-rich (b) and oligodendroglial (c) chromatins. Amount of chromatin (as DNA) loaded was: (a) 33 μg; (b) 16 μg; (c) 17 μg.

In liver chromatin, group A is the major component, and group F is absent (fig. 2). Neuronal chromatin is characterized by its relative wealth of groups B, C and D, and also by a unique presence of group F. In oligodendroglial chromatin, all the components present in neurone-rich chromatin are detected, but in exceedingly smaller amounts. This may be due to a small population of neuronal nuclei (less than 5%) that are present in the oligodendroglial nuclear preparation (cf [9]). Since in neurone-rich nuclear preparation of neuronal nuclei amounts to only about 50%, the actual difference in the amount of non-histone proteins between neuronal and oligodendroglial chromatins must be even greater. However, contribution of astroglial chromatin present in the neurone-rich nuclear preparation to the electrophoretic pattern remains to be known (cf [9]).

Paucity of high molecular weight non-histone proteins in liver chromatin will raise the question of a possible loss of some of these proteins during the preparation of chromatin. To eliminate this possibility, extracts at respective steps of chromatin preparation were freeze-dried and similarly analyzed by gel electrophoresis. None of the extracts of liver nuclei were found to contain any significant amount of such proteins as seen in neurone-rich chromatin.

Recently, relative abundance in the high molecular weight non-histone proteins in cerebral chromatin has been reported by two groups of workers [10, 11]. In view of our present observation, this can evidently be attributable to the characteristic of neuronal chromatin, although the population of neuronal nuclei in cerebral nuclear preparations used by these workers are not well defined.

Neuronal nuclei have been shown to synthesize significantly more RNA as compared with oligodendroglial and liver nuclei [2], and evidence is presented indicating that in neuronal chromatin a part of steric restrictions imposed on RNA synthesis appears to be intrinsically weakened [9]. The relative wealth of high molecular weight non-histone proteins and the unique presence of a group of high molecular weight non-histone proteins in neuronal chromatin may provide a molecular basis for maintaining such a chromatin conformation.

Acknowledgement

We thank Miss N. Setani for her help with certain phases of this work.

References

[1] Kato, T. and Kurokawa, M. (1967) J. Cell Biol. 32, 649–662.
[2] Kato, T. and Kurokawa, M. (1970) Biochem. J. 116, 599–609.
[3] Shaw, L.M.J. and Huang, R.C.C. (1970) Biochemistry 9, 4530–4542.
[4] Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617.
[5] Kissane, J.M. and Robins, E. (1958) J. Biol. Chem. 233 184–188.
[6] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[7] Elgin, S.C.R. and Bonner, J. (1970) Biochemistry 9, 4440–4447.
[8] Elgin, S.C.R. and Bonner, J. (1972) Biochemistry 11, 772–781.
[9] Kurokawa, M., Tashiro, T., Mizobe, F. and Setani, N. (1973) FEBS Letters 34, 165–168.
[10] MacGillivray, A.J., Cameron, A., Krauze, R.J. Rickwood, D. and Paul, J. (1972) Biochim. Biophys. Acta 277, 384–402.
[11] Wu, F.C., Elgin, S.C.R. and Hood, L.E. (1973) Biochemistry 12, 2792–2797.