HETEROGENEITY OF CEREBRAL CHROMATIN

M. KUROKAWA, T. TASHIRO, F. MIZOBE and N. SETANI

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

Received 28 May 1973

1. Introduction

Neuronal nuclei have been shown to synthesize significantly more RNA than oligodendroglial and liver nuclei [1]. It has recently been reported that, in addition to neuronal nuclei, astroglial nuclei are also capable of RNA synthesis at a high rate [2]. In the course of our studies carried out for the purpose of explaining the enhanced RNA formation, evidence is obtained indicating that in neuronal chromatin a part of the steric restrictions imposed on RNA synthesis appears to be intrinsically weakened.

2. Materials and methods

Nuclei were isolated from the cerebral cortex and liver of the adult guinea pig as described previously [1, 3], and stored frozen at −80°C until use. Cerebral nuclei were also fractionated in a similar way as described by Austoker et al. [2]. In case of guinea pigs, the nuclear subfraction collected at interphase between 2.3 M and 2.4 M sucrose appeared to be more enriched with neuronal nuclei than the equivalent fraction obtained from rats by Austoker et al. [2]. The cerebral nuclear sample in which the population of neuronal nuclei averaged approx. 50% is expressed as P_{50}. The remaining half of the nuclei was of glial origin, with a dominance of astroglial over oligodendroglial nuclei. In the P_3 nuclear preparation, approx. 90% was of oligodendroglial origin, and the remainder of astroglial and, to a lesser extent, of neuronal origin.

Chromatin was prepared from purified nuclei essentially as described by Shaw and Huang [4] and stored at 0–4°C, and used for assay of RNA polymerase, and also for recording of circular dichroism (CD) spectra within 10 hr.

RNA polymerases A and B were solubilized from nuclei by sonication in 0.3 M $(NH_4)_2SO_4$ and 25% (v/v) glycerol, and purified by $(NH_4)_2SO_4$ precipitation, followed by passage through a DEAE-cellulose column (25 cm × 1.2 cm) eluted with linear $(NH_4)_2SO_4$ gradient ranging from 0.04 M – 0.40 M, essentially as described by Mandel and Chambon [5], and stored frozen at −80°C.

In nuclei and chromatin, the RNA polymerase reaction was initiated in the medium (0.35 ml) with or without trypsin (10 μg/tube; 2 × crystallized, Worthington Biochem. Corp., Freehold, N.J., USA). After incubation for 10 min at 37°C, 0.15 ml of $(NH_4)_2SO_4$ solutions of appropriate molarities, adjusted to pH 8 with aq. NH_3, were added to give prescribed final concentrations, and the incubation continued for further 30 min at 37°C. The medium (final volume, 0.5 ml) contained (final concentrations): Tris–HCl buffer, pH 8.0 (100 mM); $MnCl_2$ (2 mM); β-mercaptoethanol (10 mM); $(NH_4)_2SO_4$ (0–0.9 M as indicated); ATP, CTP and GTP (0.5 mM each); UTP (0.01 mM) and 1.5 μCi of [5–^{3}H] UTP (26.9 Ci/mmol; New England Nuclear, Boston, Mass., USA); and appropriate amounts of nuclei or chromatin (30–60 μg as DNA). In case of purified RNA polymerases, native or heat-denatured calf thymus DNA (10 μg; type I, Sigma Chemical Co., St. Louis, Mo., USA) was incorporated in the assay medium as a template, and incubation carried out at 37°C for 10 min. The reaction was terminated by the addition of 1.0 ml of ice-cold 10% (w/v) trichloroacetic acid (TCA) in 40 mM-sodium pyrophosphate. The precipitate was collected by centrifugation, together with 1 mg of bovine serum albu-

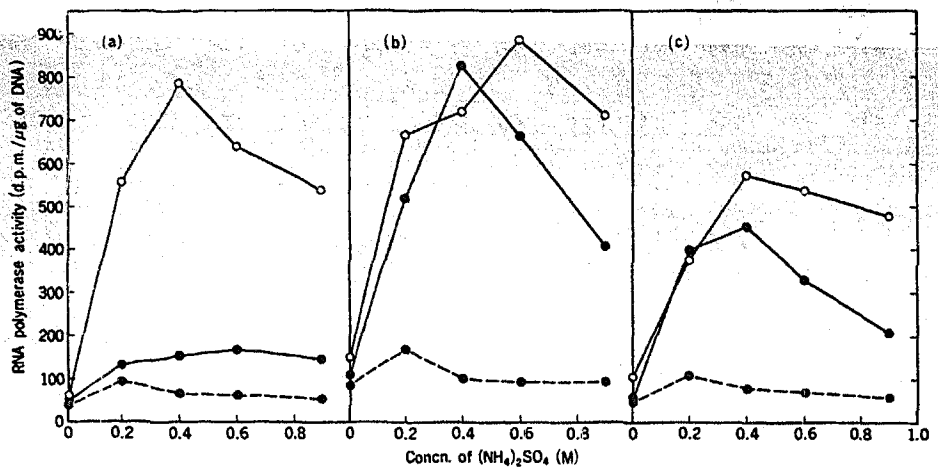

Fig. 1. RNA polymerase activities in cerebral P_{50}, P_3 and liver nuclei: (a) Liver nuclei; (b) P_{50}, enriched with neuronal nuclei; (c) P_3, enriched with oligodendroglial nuclei. Reaction was initiated in medium (0.35 ml) with (o—o) or without (•—•) 10 μg of trypsin, and after 10 min at 37°C appropriate $(NH_4)_2SO_4$ solutions (0.15 ml) added so as to give final concentrations indicated. Reaction was terminated 30 min thereafter. (•—•) α-Amanitin (1 μg/ml) present, in the absence of trypsin.

mim when appropriate, washed three times, each with 8 ml of ice-cold 5% (w/v) TCA in 20 mM-pyrophosphate, transferred into a counting vial with a minimal amount of water, dried, and solubilized in 0.5 ml of Soluene-100 (Packard Instrument Co., Downers Grove, Ill., USA). Radioactivity was measured in a Packard Model 3380 liquid scintillation spectrometer, with 10 ml of scintillation fluid, containing, in 1 l of toluene, 4 g of 2,5-diphenyloxazole and 0.25 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene.

CD spectra were recorded with a JASCO Model J-20 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan). The spectrum was first taken at 25°C with 1.5 ml of native chromatin in water, adjusted to pH 8 by aq. NH_3, which gave an A_{260} of approx. 1.3 in a 1.0 cm light-path. Spectra were subsequently recorded each time after the addition of 15 μl of 1 M Tris–HCl (pH 8.0), 20 μl of trypsin (1 μg/μl), and 80 μl of 10% (w/v) sodium dodecyl sulphate in 10 mM Tris–HCl (pH 8.0), respectively. Mean residue ellipticity, (θ), is expressed in deg · cm^2 · $dmol^{-2}$ on the basis of DNA residue concentration.

DNA was determined according to Kissane and Robins [6] with calf thymus DNA as standard. In case of chromatin, DNA was determined also by ultraviolet absorption at 260 nm in 0.25% sodium dodecyl sulphate (cf. [7]), using an ϵ_p of 6800. Protein was determined according to Lowry et al. [8] with bovine serum albumin as standard.

3. Results and discussion

In the absence of $(NH_4)_2SO_4$ in the reaction medium, [^{3}H] UTP incorporation in cerebral P_{50} nuclei is only twice as high as in liver nuclei (fig. 1). RNA synthesis in P_{50} nuclei is, however, increased more prominently than in liver nuclei by raising the ionic strength of the medium. Thus the stimulation by $(NH_4)_2SO_4$ is 8~9-fold at 0.4 M in P_{50}, while only about 3-fold at 0.6 M in liver nuclei. α-Amanitin when incorporated in the medium to a concentration of 1 μg/ml, completely counteracts the effect of increasing ionic strength (fig. 1).

RNA polymerase B was eluted from the DEAE-column with $(NH_4)_2SO_4$ at around 0.25 M. On the basis of DNA in the starting materials, P_{50} nuclei tended to yield 3~4-fold as much enzyme protein in comparison with liver nuclei (data not shown). However, it is not clear whether the apparent higher yield represents the relative ease with which the P_{50} enzyme is solubilized from the nuclear structure, or an in-

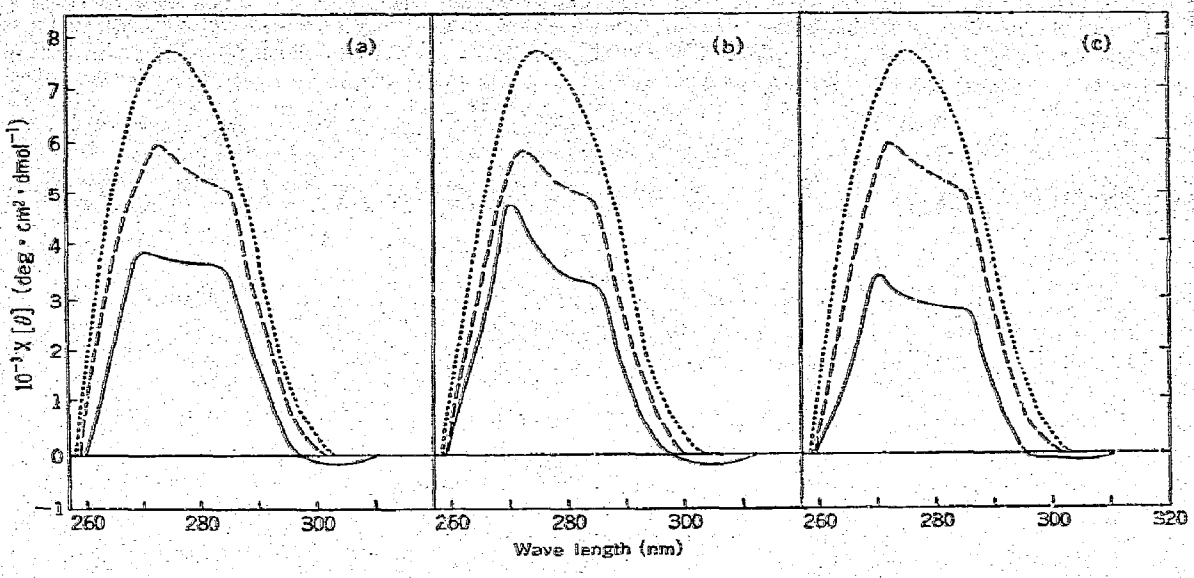

Fig. 2. Circular dichroism spectra of cerebral P_{50}, P_3 and liver chromatins: (a) Liver chromatin; (b) P_{50} chromatin; (c) P_3 chromatin. Spectrum was first taken at 25°C in native chromatin in water, pH 8 (——), and subsequently in the presence of 10 mM Tris–HCl, pH 8.0 (not depicted), of trypsin (13 μg/ml, – –), and finally of 0.5% sodium dodecyl sulphate (– – –), respectively.

creased number of enzyme molecules per se. Chambon et al. [9] have recently presented evidence indicating that the actual number of RNA polymerase B molecules per haploid DNA complement does not drastically differ amongst brain, liver and other tissues in rat.

Studies with the purified polymerase B failed to furnish any positive evidence to indicate that enzymes from P_{50} and liver nuclei differed from each other in respect to elution pattern, specific activity (4–5 nmol of UMP incorporated/mg of protein/10 min at 37°C), optimum concentration of $(NH_4)_2SO_4$ required for activation (60–80 mM), preference for denatured DNA as template, cation requirement (Mn^{2+} in preference to Mg^{2+}) and α-amanitin sensitivity.

When RNA synthesis in liver nuclei is initiated in a low ionic strength and in the presence of trypsin (3–10 μg/tube), the polymerase B reaction later disclosed by the addition of $(NH_4)_2SO_4$ is found greatly enhanced (fig. 1a). Trypsin alone activates RNA formation to only a very limited extent. It is also noted that in trypsinized liver nuclei, the concentration of $(NH_4)_2SO_4$ required for maximal activation of polymerase B reaction is lowered to 0.4 M, in contrast to 0.6 M in non-trypsinized samples. Equivalent results were obtained also with unsheared liver chromatin (data not presented).

Activation of RNA polymerase B reaction by trypsin is demonstrated also in P_3 nuclei in the range of 0.4 M – 0.9 M $(NH_4)_2SO_4$ (fig. 1c), which is ascribable to the property of oligodendroglial nuclei. Results with P_{50} nuclei given in fig. 1b indicate that the neuronal (and possibly also astroglial) nuclei barely respond, if at all, to trypsin by an enhancement of RNA polymerase B activity, in contrast to liver and oligodendroglial nuclei. Elevation of RNA synthesis by trypsin observed in P_{50} nuclei in the concentration range of $(NH_4)_2SO_4$ between 0.6 M and 0.9 M may be attributable to oligodendroglial nuclei present in this preparation.

In CD spectra, the mean residue ellipticity, (θ), exhibited by native P_{50} chromatin in wave length range between 260 nm and 300 nm is found significantly higher as compared with P_3 and liver chromatins (fig. 2). In addition, a steeper change in ellipticity in the range of 270 nm – 290 nm appears to characterize P_{50} chromatin. Digestion of chromatin with trypsin leads to reversion of the spectrum toward that of free DNA, which can be observed with chromatin in the presence of 0.5% sodium dodecyl sulphate ([7], fig. 2). It is noteworthy that, in contrast to native

chromatin, there exists practically no difference in spectra among three species of trypsinized chromatins (fig. 2). Trypsin has been shown to exert limited proteolysis of chromatin, presumably affecting histone bridging between adjacent DNA segments [10], which is thought to stabilize supercoiled conformation in chromatin. It seems likely that in liver and oligodendroglial chromatins, prior destabilization of the supercoiled structure by trypsin is required, in addition to increased ionic strength, to ensure sufficient interaction between RNA polymerase B molecules and DNA in chromatin, while neuronal chromatin has fewer such structural restrictions. This is rather in line with the view of Johns [11, 12] that linear parts of chromatin can be transcribed, whether or not they have histones, and that the supercoiled parts are repressed. Another possible explanation might be that the DNA in neuronal chromatin is enriched with the B form relative to C form (cf. [13]).

The present experiment clearly demonstrates an aspect of the heterogeneous nature of cerebral chromatin arising from the difference in cell species. However, more satisfactory separation between neuronal and astroglial chromatins remains to be made.

Acknowledgements

We thank Professor Th. Wieland for his generous gift of α-amanitin. Thanks are also due to Professor K. Imahori for provision of spectropolarimeter and for his helpful discussions, and Professor K. Iso for his valuable suggestions.

References

[1] Kato, T. and Kurokawa, M. (1970) Biochem. J. 116, 599–609.
[2] Austoker, J. Cox, D. and Mathias, A.P. (1972) Biochem. J. 129, 1139–1155.
[3] Kato, T. and Kurokawa, M. (1967) J. Cell Biol. 32, 649–662.
[4] Shaw, L.M.J. and Huang, R.C.C. (1970) Biochemistry 9, 4530–4542.
[5] Mandel, J.L. and Chambon, P. (1971) FEBS Letters 15, 175–180.
[6] Kissane, J.M. and Robins, E. (1958) J. Biol. Chem. 233, 184–188.
[7] Shih, T.Y. and Lake, R.S. (1972) Biochemistry 11, 4811–4817.
[8] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[9] Chambon, P., Gissinger, F., Kedinger, C., Mandel, J.L., Meilhac, M. and Nuret, P. (1972) Acta Endocrinol., Suppl. 168, 222–242.
[10] Simpson, R.T. (1972) Biochemistry 11, 2003–2008.
[11] Johns, E.W. (1969) in: Homeostatic Regulators. Ciba Foundation Symp. (Wolstenholme, G.E.W. and Knight, J., eds.), pp. 128–140, Churchill, London.
[12] Johns, E.W. (1972) Nature New Biol. 237, 87–88.
[13] Hanlon, S., Johnson, R.S., Wolf, B. and Chan, A. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3263–3267.