NUCLEAR PARTICLES FROM RAT BRAIN: COMPLEXITY OF THE MAJOR PROTEINS AND THEIR PHOSPHORYLATION *IN VIVO*

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1. Introduction

The protein composition of the nuclear particles containing DNA-like RNA has been studied by several investigators. In rat liver particles, Krichevskaya and Georgiev [1] found a unique protein (mol. wt. 40 000–45 000) whereas many species (mol. wt. 32 000–130 000) were found by Niessing and Sekeris [2, 3]. In rat brain we described two major proteins in the mol. wt. range 30 000–42 000 and minor species of higher molecular weight [4]. In spite of the lack of agreement concerning the existence of proteins of molecular weight higher than 42 000 in the particles, there is general agreement as to the presence of relatively large quantities of the 30 000–42 000 mol. wt. species. These will be designated here as 'major' proteins. Using polyacrylamide gel electrophoresis and DEAE-cellulose chromayography, we show that this fraction is complex, and in addition that some of its proteins are phosphorylated *in vivo*.

2. Methods

0.5 mCi per rat of [^{32}P]phosphate (CEA, Saclay, France) were injected intracisternally into 12 adult rats. After 15 hr, a nuclear extract (NE) containing particles and soluble proteins was prepared from purified brain nuclei as previously described [4, 5].

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NE was centrifuged on a 10–25 p. 100 sucrose gradient for 4 hr at 69 500 *g* [4]. Fractions containing the bulk of the particles (or particles of known sedimentation coefficient) and the gradient supernatant (NS) containing the soluble proteins were pooled.

The particles (NP) were collected by centrifugation (2 hr at 100 000 *g* in a R40 rotor) and suspended in 10 mM Tris–HCl, pH 8. RNA was hydrolyzed by a 1 hr incubation at 0°C with 2 μg/ml of pancreatic ribonuclease (RNAase). Samples from the NE and from the NS were incubated with RNAase in the same conditions. They were then dialyzed against 1 mM Tris–HCl pH 7.3, 0.1 mM dithiothreitol (DTT) to remove small molecular weight material such as the DOC used to lyse the nuclei.

The 3 fractions (NE, NP, NS) were dialyzed against 10 mM Tris–HCl, 6 M urea, 0.1 mM DTT, the final pH being adjusted to 7.6 (TUD buffer). The fractions were left in the presence of TUD at least overnight to insure complete dissociation of proteins.

The proteins of the particles were further fractionated by chromatography on DEAE-cellulose (Whatman 23) in the presence of TUD. The effluent (F_o) was ollected and the column was eluted with 2 M NaCl (F_{2M}). F_o and F_{2M} were concentrated as follows: the pooled fractions were dialyzed against 1 mM Tris–HCl, pH 7.4, 0.1 mM DTT to remove urea and lyophilized. The dry residue was dissolved in a small volume of TUD.

Samples from the various fractions were analyzed by polyacrylamide gel electrophoresis (12 p. 100 acrylamide) in the presence of SDS and DTT [4]. Zones

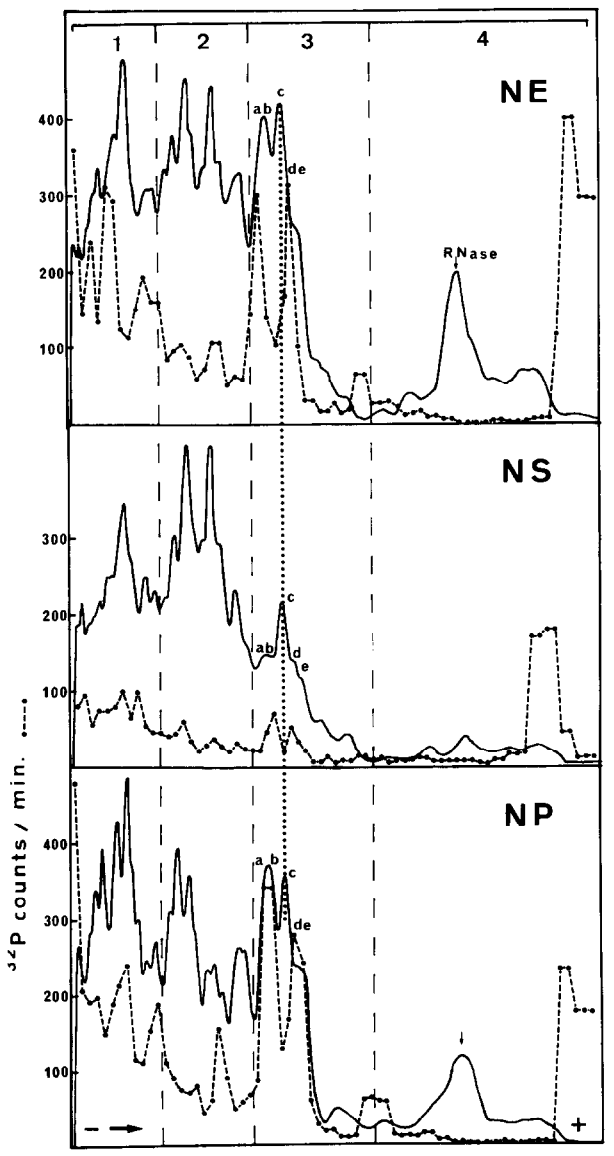

Fig. 1. Polyacrylamide gel electrophoresis of proteins from nuclear extract (NE) fractionated into soluble proteins (NS) and 60–190 S particles (NP). The fractions were prepared and analyzed as described under Methods. Molecular weights were determined by comparison with known markers and myoglobin (mol. wt. 17 800) was used as internal reference. Zones were defined as previously [4]. Zone 2 corresponds to proteins from (mol. wt. 42 000–70 000), zone 3 to proteins from mol. wt. 23 000–42 000. Mol. wt.: proteins 3a–b, 37 000; 3c, 34 000; 3d–e, 30 000. The difference in molecular weights between 3d and 3e were about 1000–2000 and 2000–3000 between 3a and 3b. Full lines: absorbance; dotted lines: ^{32}P cpm.

of molecular weight were defined as previously [4] and are indicated in the figures. Zone 3 contained the 'major' proteins. After recording, 1 mm slices were cut from frozen gels and counted for ^{32}P radioactivity.

3. Results and discussion

3.1. *Comparison of the soluble proteins and the proteins from particles*

A ^{32}P-labelled nuclear extract (NE) was fractionated on sucrose gradient. 60–190 S particles (NP) and soluble proteins (< 30 S) (NS) were collected.

As already pointed out [4], the electrophoretic profile of NE was complex in zones 1 and 2 (fig. 1). In zone 3 we found three main peaks and the presence of shoulders in some experiments suggested that there were at least five species 3a, 3b, 3c, 3d, 3e. Two prominent peaks of radioactivity were found at the level of 3a–b and 3d–e. The species 3c was not labelled or only slightly.

The relative propertion of the proteins of zone 3 decreased considerably in NS while it increased in the particles (NP). The same species 3a–b and 3d–e were labelled in NS and NP as in NE.

The comparison of the profiles NS and NP suggested that the proteins of zones 1 and 2 of the particles might represent a contamination by aggregated soluble proteins. It has been shown [6] with amino acid-labelled proteins that such aggregates indeed exist but sediment only with particles of low sedimentation coefficients (up to 50–60 S). Therefore we only analyzed particles larger than 60 S. When particles of different sedimentation coefficients (60–80 S, 120–140 S, 155–175 S) were analyzed, the same electrophoretic profiles were found and the same proteins were labeled.

Our comments will primarily concern the 'major' proteins of zone 3. At least 2 of these proteins were regularly labelled with ^{32}P and can be considered as phosphoproteins. Indeed the radioactivity was totally removed by pronase digestion and approximately 70 p. 100 of the total ^{32}P radioactivity from the RNAase treated NE, NS or NP were insoluble in 10 p. 100 TCA at 90°C. ^{32}P material was also found in zones 1 and 2; it is likely that some of the many proteins in these zones are also phosphorylated.

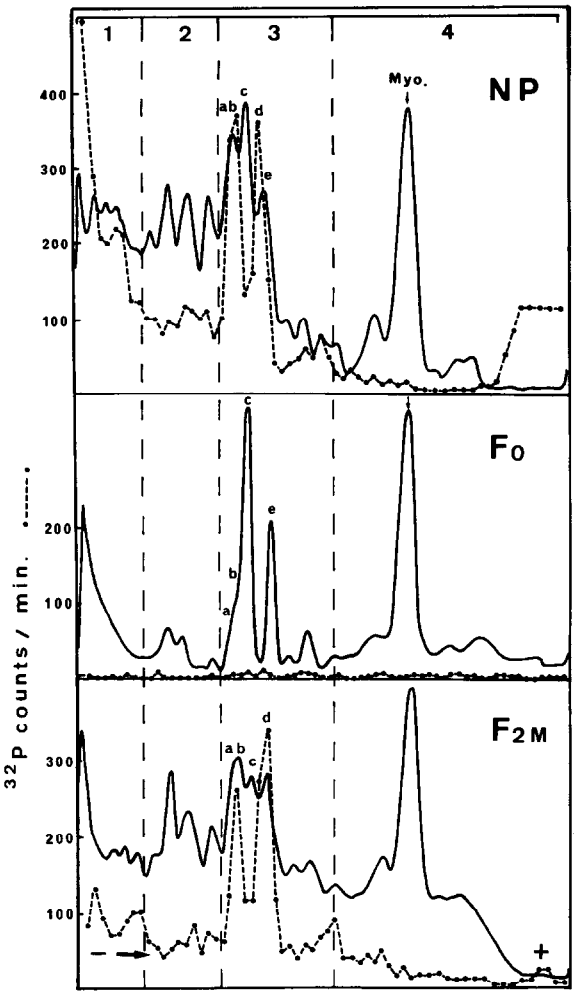

Fig. 2. Polyacrylamide gel electrophoresis of proteins from 60–190 S particles (NP) fractionated on DEAE-cellulose. F_o represents the effluent, F_{2M}, the proteins attached to the column and eluted with 2 M NaCl. Zones and symbols as in fig. 1.

3.2. *Fractionation of the proteins from the nuclear particles by chromatography on DEAE-cellulose*

More informations about the composition of the major proteins of zone 3 were obtained by chromatography on a DEAE-cellulose column (at pH 7.6, in the presence of 6 M urea). A small portion of the proteins (about 10 p. 100) was not attached to the column (fig. 2). This fraction (F_o) contained only a small amount of the proteins of zone 1 and 2 but considerably enriched in protein 3c and 3e. The amount of 3e was variable according to the experiments. 3d was regularly absent as shown by the empty interval between 3c and 3e. There were also small amounts of 3a and 3b seen in fig. 1 as shoulders or in other experiments as small peaks in the ascending branch of 3c. None of these proteins were labelled with ^{32}P.

The proteins remaining on the colum were eluted with 2 M NaCl. Most of the proteins of zones 1 and 2 were found in this fraction (F_{2M}). Some of them were labelled with ^{32}P. 2 M NaCl also eluted proteins of zone 3; 3a and 3b probably in similar amounts were not resolved. All the ^{32}P radioactivity from the 3a–b region of the particles (NP) was found in F_{2M}. It is likely, although not proven yet, that both 3a and 3b were labelled with ^{32}P. The amount of the species 3c in F_{2M} was usually small and the peak was absent in several experiments. Moreover it had apparently not incorporated ^{32}P. All the radioactivity from the 3d–e region from the particles (NP) was found in F_{2M}. The presence of relatively large amounts of 3e in F_o suggests that this radioactivity was associated to 3d.

The results are compatible with the following interpretation: among the 5 main proteins from zone 3, two (3c and 3e) are not phosphorylated *in vivo* and behave as basic in the conditions of the experiment (pH 7.6). 3a and 3b are partly phosphorylated; the fraction found in F_o is not phosphorylated and this suggests that 3a and 3b from F_o are basic at pH 7.6 like 3c. On the other hand 3d is either entirely phosphorylated or if only partly, behaves as acidic at pH 7.6.

Although the proteins 3a, 3b, 3c from F_o and F_{2M} had the same mobility, as checked in split-gels, our results do not exclude the possibility that they are different proteins. Only a further purification and a study of the primary structure of the proteins would permit to clarify this problem.

Acknowledgements

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