PHOSPHORYLATION OF NONHISTONE CHROMATIN PROTEINS FROM NEURONAL AND GLIAL NUCLEI-ENRICHED FRACTIONS OF RAT BRAIN

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1. Introduction

One of the metabolic differences between mature neuronal and glial nuclei is the considerably higher synthesis of RNA in neurones [1–4]. This agrees with the less pronounced steric restrictions of neuronal chromatin template in respect to RNA synthesis than that of the oligodendroglial chromatin template [5].

The extent of phosphorylation of tissue-specific [6,7] nonhistone chromatin (NHC) phosphoproteins has been correlated to changes in rates of in vitro RNA synthesis [8,9].

The present communication compares the in vitro phosphorylation of NHC phosphoproteins from a rat brain fraction enriched in 'neuronal' nuclei and from another fraction containing up to 95% small nuclei of oligodendro- and microglial origin.

The 'neuronal' fraction is characterized by a higher ratio of NHC proteins to DNA as well as to histone content and exhibits higher endogenous RNA polymerase activities than the 'glial' fraction.

We could indeed demonstrate that most of the 'neuronal' NHC fractions resolved in polyacrylamide gels show a considerably higher phosphorylation than NHC fractions of the 'glial' population.

2. Methods

2.1. *Nuclear fractions*

Female Wistar rats of 150–170 g were starved for 12 hr and killed by decapitation between 7 and 8 a.m.

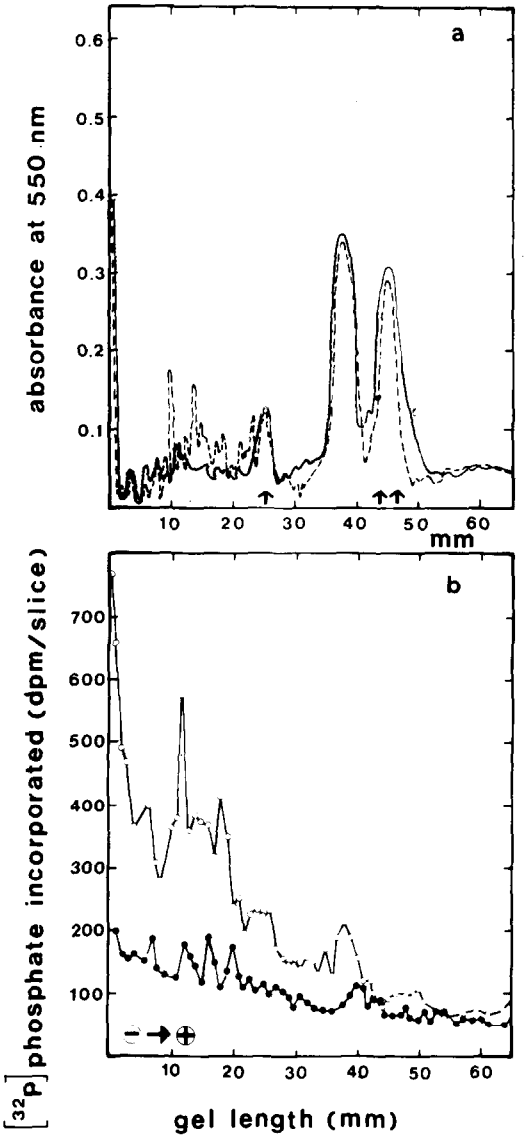
Brain cell nuclei were isolated and two different populations were fractionated as described in ref. [10]. However treatment with Triton X-100 was omitted.

From the morphological criteria [11–13] and chemical data ([2,4,14]; table 1) for differentiating brain nuclei we conclude that the large, mostly pale nuclei collected at the layer between 2.4 M and 2.5 M sucrose represent neurones and astrocytes with a dominance of the former. This fraction will be referred to as 'neuronal' nuclei. The nuclear fraction collected at the bottom of the gradient tube (2.6 M sucrose) contains small dark nuclei of oligodendro – or microglial origin – 'glial' nuclei.

Table 1
Protein/DNA ratio in chromatin from 'neuronal' and 'glial' material

	DNA	Histone	Nonhistone	Total proteins
'Neuronal' chromatin	1	0.914 ± 0.048	0.949 ± 0.036	1.863 ± 0.017
'Glial' chromatin	1	0.862 ± 0.087	0.619 ± 0.102	1.481 ± 0.035

Histones were extracted from chromatin twice with ice-cold 0.25 N HCl. The values for the nonhistone proteins were obtained by subtracting the histone values from the data for total chromatin protein. Values are arithmetic means ± S.D. from 3 assays, using 30–40 pooled rat brains per experiment.



Optical scans (a) and distribution of ^{32}P-radioactivity (b) associated with chromatin proteins from 'neuronal' (o-o-o) and 'glial' (•-•-•) fractions, which were prepared from 80 pooled rat brains. Chromatin proteins were labeled by incubating 3 mg DNA as chromatin with 3 μmol γ-labeled ^{32}P-ATP (spec. act. 250 nCi/nmol) and isolated as described in 'Methods'. 122 μg 'neuronal' chromatin proteins were electrophorized in triplicate at 4.5 mA per gel for 18 hr. (↑) f-1 (KAP), (↑↑) f2a1 (GRK) identified by coelectrophoresis of calf thymus histone fractions.

2.2. *Phosphorylation*

In vitro phosphorylation of NHC proteins was carried out as described elsewhere [15]: the standard reaction mixture consists of 20 mM $MgCl_2$, 100 mM NaCl, 80 mM Tris–HCl (pH 7.5), ^{32}P-ATP,γ-labeled (spec.act. 250 nCi/nmole) and DNA as chromatin in the amounts given in the legend of fig. 1.

The mixture was incubated at 37°C for 5 min. The incubation was stopped by adding ice-cold 10% trichloracetic acid (TCA) containing 50 mM pyrophosphate. The TCA-insoluble material was extracted twice with ice-cold 0.25 N HCl, treated for 10 min with 15% TCA at 90°C, washed four times with 5% TCA, then dissolved in a toluene based scintillation mixture [16] with 0.2 ml NCS-tissue solubilizer (Amersham-Searle, Ill., USA) and counted in a Packard model 3388 scintillation spectrometer. Complete incubation mixtures (treated with ice-cold 10% TCA before adding the ^{32}P-ATP) served as blanks and were subtracted from each value given.

When NHC proteins were phosphorylated for gel electrophoretic fractionation, the incubation was stopped by adding an excess of 1.5 mM sodium chloride with 0.15 mM sodium citrate containing a tenfold excess of unlabeled ATP.

After centrifugation the ^{32}P-labeled chromatin proteins were solubilized in 50 mM Tris–HCl, pH 8.0, containing 8 M urea, 1% sodiumdodecylsulfate (SDS) and 1% mercaptoethanol, then dissociated from DNA by centrifugation at 70 000 *g* for 36 hr [17], dialyzed against deionized water for 48 hr and lyophilized.

2.3. *Gel electrophoresis*

Lyophilized chromatin proteins were fractionated in 10% polyacrylamide gels with 0.1% SDS [18] using an apparatus developed by us, which allows in one run the electrophoresis of a gel block consisting of 25 cylindrical gels of 5 × 10 mm. The gels were stained with Coomassie Brilliant Blue G 250 (Serva, Heidelberg), destained in 7% acetic acid containing 35% ethanol, scanned in a Gilford spectrophotometer with a scanning attachment and sliced in 1 mm fractions using the slicer and technique described elsewhere [19]. The gel slices were placed in scintillation vials, dried and counted in a scintillation mixture [16].

Chromatin was prepared as described in refs. [18] and [20], DNA was determined according to ref. [21].

In the case of chromatin quantitation by ultraviolet absorption at 260 nm was also performed, using calf thymus as a standard. Protein was assayed by the method of Lowry et al. [22].

3. Results and Discussion

In order to study phosphorylation of NHC proteins we optimized the conditions for incubating 'neuronal' and 'glial' chromatin fractions with ^{32}P-ATP. The uptake of ^{32}P-phosphate into NHC proteins increases linearly in both the 'neuronal' and the 'glial' fraction up to the sixth min of incubation reaching plateau values between 8 and 10 min. Maximal ^{32}P-phosphate incorporation into 'neuronal' as well into 'glial' NHC proteins occurred at 20 mM Mg^{2+}. This was found to be optimal also for chromatin associated kinase activities in rat liver [23,24] and rat kidney [15].

The ^{32}P-phosphate incorporation into NHC proteins of 'neuronal' and 'glial' fractions increases linearly with ATP-concentrations up to 100 μM and 50 μM ATP respectively. At higher concentrations the rate of phosphorylation remains constant. The phosphorylation of 'neuronal' NHC proteins was considerably higher than that of 'glial' ones under all conditions tested.

Incubation of chromatin under optimal conditions and fractionation of phosphorylated chromatin proteins in polyacrylamide gels, containing 0.1% SDS, revealed that most of the NHC protein fractions of 'neuronal' origin contained considerably higher amounts of ^{32}P-phosphate than the fractions prepared from 'glial' material (fig. 1b).

The densitometric tracings of the chromatin proteins point out the great preponderance of histones. However, histones are clearly less phosphorylated than the NHC proteins.

Fig. 1a further shows a higher content of NHC proteins in chromatin of 'neuronal' origin to that in 'glial' material – if related to the respective amounts of histones or DNA (table 1). A recent study reports a higher nonhistone/DNA ratio of 'neuronal' than of 'glial' chromatin from guinea pig brain [4].

Though these findings and the optical scans of NHC proteins (fig. 1a) indicate qualitative differences between 'neurone' enriched and 'glia' enriched fractions, this remains to be confirmed using different methods for the dissociation of cell specific nuclear types as well as for preparation and resolution of NHC proteins.

The presence of high molecular weight NHC proteins has been claimed to be characteristic for brain [26,27]. This is true for 'neuronal' rather than for 'glial' fractions ([4]; present study). Most of NHC proteins of higher molecular weight are highly phosphorylated. Since phosphorylated NHC proteins have been shown to be tissue specific [6,7] and their phosphorylation has been correlated with transcriptional activity [8,9], we suggest a connection between the considerably higher, in comparison to the 'glial' nuclei, phosphorylation rate of NHC proteins from 'neuronal' fractions and the likewise significantly higher RNA synthesis of the latter.

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