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COMPARISON OF NONHISTONE CHROMOSOMAL PROTEINS FROM NEURONAL AND GLIAL CHROMATIN BY ISOELECTRIC FOCUSSING AND MICRODISC ELECTROPHORESIS

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Summary: The present study describes optimal conditions for preparative fractionation of nonhistone chromosomal proteins from neuronal and glial nuclei.
We have detected about 1200 nonhistone chromosomal proteins. Microgels of glial origin contained 20% more protein bands than those of neuronal origin. However, neuronal bands prevailed clearly in the acidic respectively in the high-molecular range. Amino acid analysis confirmed the observed heterogeneity.

1. Introduction

Significant differences exist between neuronal and glial brain cells in regard to nuclear processes such as amino acid incorporation (1), RNA polymerase (2-4) and phosphorylation of nonhistone proteins (5). As these processes may be involved in the regulation of gene expression, their study in the various brain cell types could help in elucidating that complex problem. In this report we have focussed our attention on the NHCP[+] and have proceeded to characterize them by a two-step electrophoretic system. The first step is isoelectric focussing by means of a Valmet apparatus on a preparative scale (6-8); the second is microdisc electrophoresis on SDS-acrylamide gels, which proved to be a much superior analytical tool than the currently in use electrophoretic procedures.

2. Methods

2.1. Isolation of cell nuclei: Cerebral nuclei from 250 male Wistar rats (140-160 g) were isolated by means of hypertonic sucrose and Triton X-100 (1,9). Neuronal- and glial-enriched populations were fractionated and characterized by discontinuous sucrose density

[+] Abbreviations used: NHCP (nonhistone chromosomal proteins); SDS (sodium dodecylsulphate); N (neuronal); G (glial).

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gradient centrifugation as described previously (1,5).All buffers for nuclei isolation and chromatin preparation contained 1-5 mM of the protease inhibitor sodium hydrogensulphite. All preparations were carried out at 4°C.

2.2. Preparation and dissociation of chromatin: After extraction of the informofers (RNP particles) by the method of Samarina et al. (10), chromatin essentially was prepared according to Graziano and Huang (11). Chromatin proteins were separated from DNA essentially as described by Levy et al. (12).
Briefly, chromatin was dissociated at a final concentration of about 1 mg/ml DNA in 6 M urea (ultra pure), 0,35 M guanidine hydrochloride, 1.5 M $NaHSO_3$, 2 mM EDTA, 2 mM dithiothreitol and 0.1 M Na_3Po_4 (pH 7.0). The partially dissociated chromatin DNA precipitated at 285 000 x g, 48 h (1st dissociation stage, Fig. 1). The pellet (I) was sheared in a small quantity of 6 M urea, 3 M NaCl, 1.5 M $NaHSO_3$, 2 mM EDTA, 2 mM dithiothreitol and 0.1 M Na_3Po_4,pH 7.0(Ultra Turrax, 60 V, 3 min). Centrifugation of the suspension (285 000 x g, 60 h) sedimented DNA and tightly bound proteins (2nd dissociation stage, Fig. 1).
Both supernatants (I,II) contain histones as well as the NHCP. The latter were separated from the strong basic chromosomal proteins (histone fraction) by ion-exchange chromatography on Bio Rex 70(12). The non-absorbed NHCP precipitated by dialyzing against 4 M ammoniumsulphate, 2 mM mercaptoethanol and reduction with 50 mM dithiothreitol for 3 h at 37° C (13).

2.3. Preparative isoelectrofocussing and microgel electrophoresis: NHCP were preparatively fractionated in a zone convection isoelectrofocussing apparatus developed by Valmet (6). The employed apparatus was constructed in our workshop. (For details see Ref. 8). Its 31 chambers, holding about 50 ml, contained the NHCP (0.5-1.5 mg/ml) in 7 M urea, 2 mM dithiothreitol and 4%-ampholine (LKB, pH 3.5-10). 50 mM sodium bicarbonate (pH 10) with 12%-sucrose served as catolyte and 1%-acetic acid (pH 2.8) with 12%-sucrose as anolyte. Focussing was carried out at 4°C (3W, 60 h).
The NHCP were fractionated according to their isoelectric points in 50 fractions totally, (for each nuclear population 15 fractions after the 1st, respectively 10 fractions after the 2nd dissociation). Then the NHCP were dialyzed against 5 mM mercaptoethanol and lyophilized. The lyophilized protein fractions were dissolved (1mg/ml) in 0.035 tris-sulphite buffer (pH 8.6) containing 1% SDS, 1% dithiothreitol and 10% glycerol, incubated at 100°C for 2 min (14) and analysed by polyacrylamide microgel electrophoresis (1-40% gradient) (15). Protein samples of 2 µg were applied on top of a continuous acrylamide gradient in 10 µl capillaries and electrophoresed in a continuous buffer system (0.05 M tris-glycine,pH 8.4; 0.1% SDS and 0.1% thioglycolic acid (v/v) for about 3 h (16)). The gels were stained with Coomassie Brilliant Blue R 250 (0.18% w/v solution, 50°C, 20 min), destained in 7% acetic acid and scanned in a Gilford spectrophotometer, with a scanning attachment for microgels.

2.4. Analytical methods: Proteins were determined by a modification of Lowry's method (17,18) DNA by diphenylanine and RNA by orcinol (20). The amino acid analysis was carried out in Durrum autoanalyser after hydrolyzing usually 50 to 100 µg in 100 µl of 6 N HCl (110°C, 24 h).

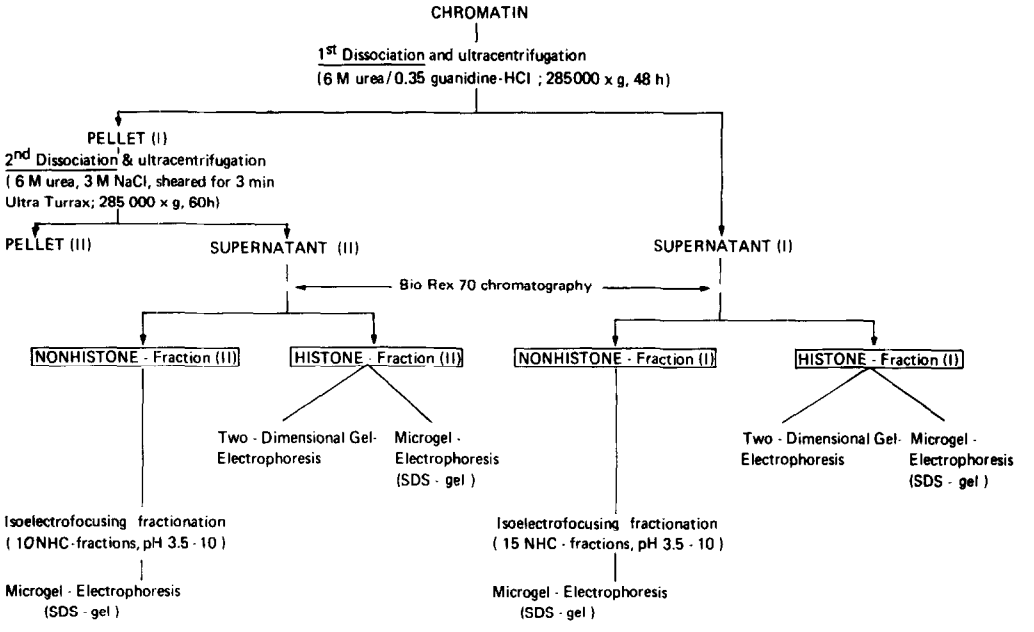

Fig. 1 PREPARATION SCHEME OF THE CHROMOSOMAL PROTEINS

3. Results and discussion

The present study describes optimal conditions for fractionation of NHCP from neuronal (N) and glial (G) nuclei,purified of all detectable cytoplasmatic contaminations (1). Chromosomal proteins were dissociated from chromatin by a high urea-salt buffer (Fig. 1), as previously applied by various workers (12,13,21-24). Up to 95% of the chromatin proteins were recovered, the remainder being still tightly bound to DNA (Tab. 1; Ref. 25).Most NHCP were then separated from the histones by Bio Rex 70 chromatography(12, 23); the non-absorbed NHCP represented nearly 70% of total chromatin proteins both for N and G.(This is in contrast to Ref. 26 but is similar to Ref. 27). Some strong basic NHCP were retained with the histones on the ion-exchanger. (The retained fraction will be considered elsewhere).

The NHCP were then submitted to isoelectric focussing on the Valmet apparatus which combines high analytical resolution with high capacity, thus enabling us to differentiate 50 IP-fractions. As far we are aware of, no similarly rewarding method exists for preparative fractionation of NHCP from N and G, (compare Ref. 27-29). NHCP fractions were subsequently analysed by the highly sen-

Table 1: Fractionation of chromosomal proteins (in % of total)

Dissociation	1st	2nd	3rd *
N-fraction	65.0 ± 0.2	29.4 ± 0.2	5.6
G-fraction	61.1 ± 0.3	31.1 ± 0.1	7.8

Values are arithmetic means ± S.D. from 3 determinations.
(* in preparation)

sitive microelectrophoretic technique, permitting the resolution of small amounts (1-2 µg) of protein, a prerequisit for any engagement in work dealing with scarce and valuable material like the glial and neuronal fractions.

By means of photographic enlargements and optical scanning of the microgels we have detected about 1200 NHCP components (Fig 3). The obtained gel patterns were highly reproducible. In most fractions gels of glial origin contained 20% more protein bands than gels of neuronal origin. This is in contrast with Refs. 28,29.
After the 1st urea dissociation we have counted 330 N-and 405 G-bands in the released proteins. Only in the acidic pH-range more N-bands than G-bands were observed. (Tab. 2) Similarly, the quantitative distribution of NHCP in the various pH-ranges shows more acidic N-than G-proteins. (Fig. 2) The N-bands are enriched in polypeptides with M.W. over 92 500 which includes the main peak of neuronal NHCP. This is due to the mentioned predominance of acidic proteins in the N-fractions. (Fig. 3).
40% of the total NHCP are found after the 2nd urea dissociation in the supernatant . There were more G- than N-bands with an exception in the high-molecular range, as observed after the 1st urea dissociation.

The observed heterogeneity between N and G was confirmed by amino acid analysis. The ratio of acidic to basic amino acids in the fractions obtained after the 1st urea dissociation was 5.5 - 1.8 for the N- and 3.3 - 1.2 for the G-proteins; similar values were found in the proteins obtained after the 2nd urea dissociation. Detailed results - as well as for various brain regions-will

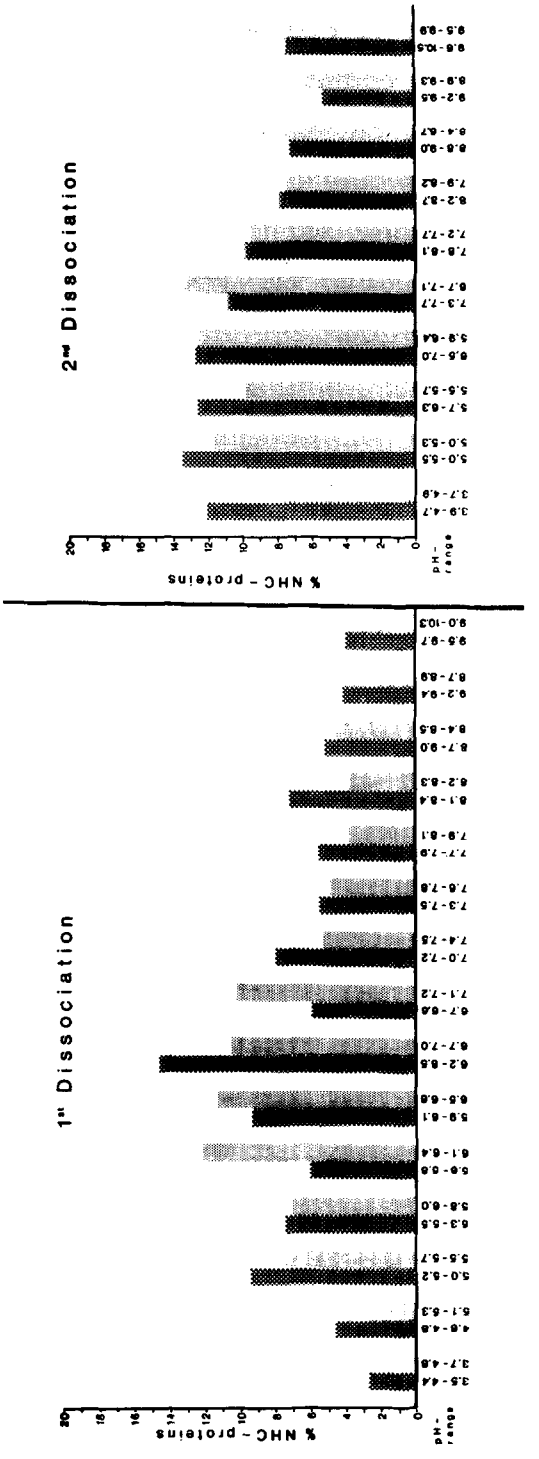

Fig. 2: pH-range and percentage - related to the total of dissociated NHCP from neuronal ( ) and glial ( ) fractions.

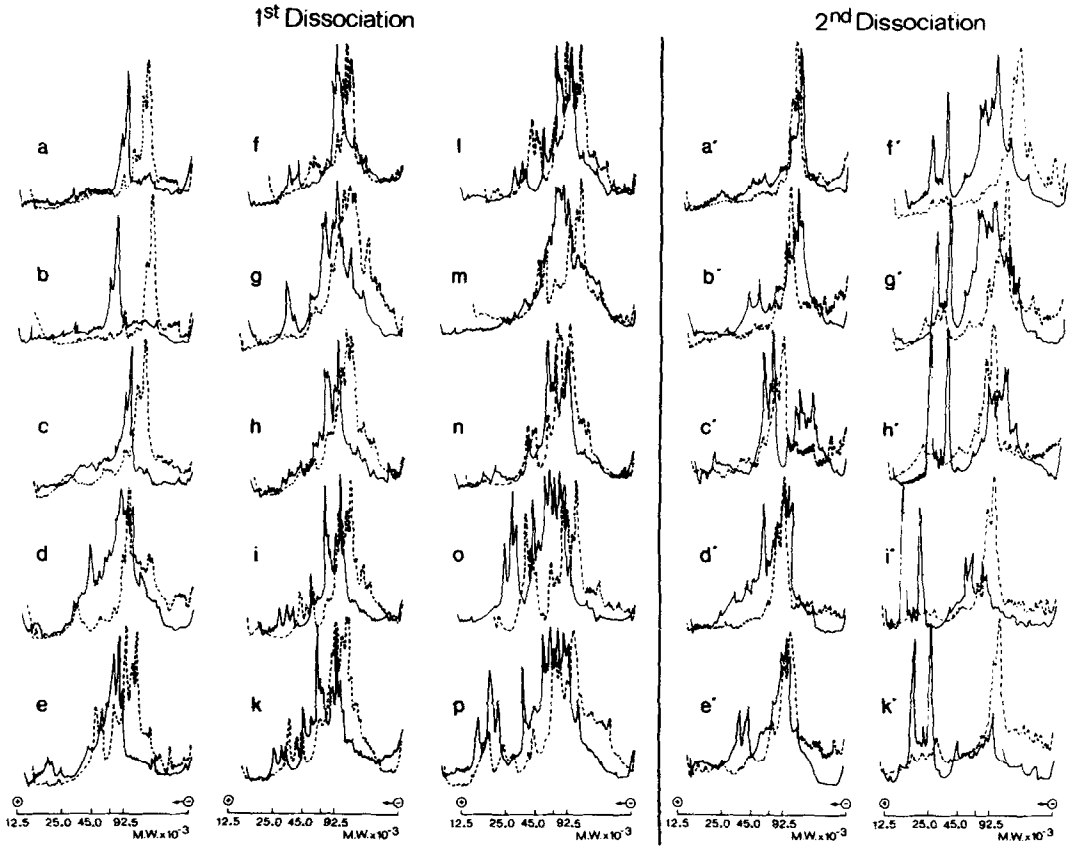

Fig. 3: Absorbance profiles (600 nm) of NHCP-fractions from neuronal (- - -) and glial (———) populations analysed by microgel-electrophoresis. Lettering stands for pH-ranges(adapt to values of Fig. 2, acidic pH - range beginning at a and a').

Tab. 2: Absolute numbers and distribution of NHCP components, classified by pH and molecular weight.

	1st Dissociation								2nd Dissociation							
	N	G	N	G	N	G	N	G	N	G	N	G	N	G	N	G
pH - range until:	6.1	6.0	7.5		> 7.5		total pH		6.3	6.4	7.7		>7.7		total pH	
% of bands M.W. >92 500 (I)	57.6	37.9	50.5	40.1	50.5	38.9	53.3	39.0	60.6	47.4	56.3	47.3	56.9	47.5	57.9	47.4
- 45 000 (II)	25.0	25.0	24.2	25.8	25.2	28.7	24.9	26.7	19.7	25.3	18.7	23.7	18.9	22.9	19.1	23.9
- 25 000 (III)	12.1	19.0	14.3	17.4	15.9	17.8	13.9	18.0	15.2	15.8	10.4	15.8	11.6	13.5	12.5	14.7
< 25 000 (IV)	5.3	18.1	11.0	16.7	8.4	14.6	7.9	16.3	4.5	11.5	14.6	13.2	12.6	16.1	10.5	14.0
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
total numbers:	132	116	91	132	107	157	330	405	66	95	48	38	95	118	209	251
ratios: I : IV	10.9	2.1	4.6	2.4	6.0	2.7	6.8	2.4	13.3	4.1	3.9	3.6	4.5	2.9	5.5	3.4
I/II : III/IV	4.7	1.7	3.0	1.7	3.1	2.1	3.6	1.9	4.1	2.7	3.0	2.5	3.1	2.4	3.3	2.5
I : III/IV	3.3	1.0	2.0	1.2	2.1	1.2	2.4	1.1	3.1	1.7	2.3	1.6	2.3	1.6	2.5	1.7

be given in a future publication.

Our present findings clearly demonstrate the tissue-specific heterogeneity of NHCP, in contrast to results of others (30; comp. Ref. 13). The possibility of isolation of defined NHCP fractions in sufficient quantities by use of the described electrofocussing system will certainly be of help for the isolation of specific nuclear proteins and for evaluating their role in nuclear metabolism.

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