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AN ELECTROPHORETIC ANALYSIS OF PROTEOLIPIDS FROM DIFFERENT RAT BRAIN SUBCELLULAR FRACTIONS

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Proteolipid proteins were extracted from adult rat brain subcellular fractions and purified by chromatography on Sephadex LH-60. Polyacrylamide gel electrophoresis of the delipidized proteins, in the presence or absence of 8 M urea, was carried out with all fractions. The distribution of the various types of proteolipid proteins was studied and their molecular weight calculated by the Ferguson relationship. Several bands of proteolipid proteins were found in the five membrane fractions analyzed. Some of them, such as the 17.5 K and 37 K components were very prominent in mitochondria and synaptosomes. The 30 K component was found in myelin-derived membranes and in microsomes, while the 20 K and 25 K proteolipid proteins were present in all subcellular fractions. The 30 K component (proteolipid protein (PLP)), typical of the purified myelin membranes, showed a similar distribution to that of 2′,3′-cyclic-nucleotide 3′-phosphohydrolase (EC 3.1.4.37) activity, while the other major proteolipid protein present in all subcellular fractions (25 K) did not show such parallelism, indicating that it might not be an exclusive component of myelin. The electrophoretic pattern of microsomal proteolipid proteins did not show the high molecular weight components (aggregates of PLP) which are found in myelin. Furthermore, the 30 K component showed a smaller Y_0 value than that of the 30 K found in myelin. Thus the presence of 30 K proteolipid protein in microsomes should not be considered as being due to myelin contamination.

Introduction

The lipoprotein complexes defined as 'proteolipids' contain a special type of proteins, soluble in organic solvents, which can be transferred to water by different procedures [1–3]. They were first described by Folch and Lees [4] in bovine brain white matter but have also been found in other tissues in lesser quantities [5]. They are the main protein constituents of central nervous system myelin [6] and their subcellular distribution has been studied in rat cerebral cortex by Lapetina et al. [7] and in mouse brain by Nussbaum and Mandel [8]. The latter investigators also described the subcellular distribution of several species of proteolipid proteins separated by SDS-polyacrylamide gel electrophoresis. They concluded that mitochondria, myelin and synaptosomes were the only fractions containing proteolipid proteins and indicated that the main myelin proteolipid protein (P_7) was a specific constituent of this membrane since it was absent from the electrophoretic pattern of total brain proteolipids from myelin-deficient mutants.

In the present paper we present the results of our study by SDS-polyacrylamide gel electro-

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phoresis and SDS-urea-polyacrylamide gel electrophoresis of the proteolipid proteins of purified myelin, and other subcellular fractions obtained from adult rat brain, calculating their molecular weights by the Ferguson relationship [9]. We correlate the presence of the main myelin proteolipid protein with the activity of 2′,3′-cyclic-nucleotide 3′-phosphohydrolase (EC 3.1.4.37) in purified myelin and in other subcellular fractions and discuss the possibility that the proteolipid protein PLP present in the microsomal membranes could act as a precursor of that found in myelin.

Materials and Methods

Analytical reagents and redistilled solvents were used throughout. Adult Wistar rats of either sex were used in all our experiments. They were killed by decapitation and the forebrain rapidly removed and placed in the cold. Subcellular fractions were obtained by the method of De Robertis et al. [10] except that the crude mitochondria were fractionated on a discontinuous density gradient of 0.8 M, 1.0 M and 1.25 M sucrose. The material floating on top of 0.8 M sucrose was myelin (fraction A); the 0.8 M–1.0 M layer, fraction B; the 1.0 M–1.25 M interface, purified synaptosomes (fraction C) and the pellet, mitochondria. Myelin was further purified according to Norton and Poduslo [11]. The microsomal fraction was obtained by centrifugation of the post-mitochondrial supernatant at $100000 \times g$ for 60 min while purified nuclei were isolated from the initial total homogenate as described by Krawiec et al. [12].

Isolation and purification of proteolipid proteins

Lipids and proteolipids were extracted from an aliquot of the original total homogenate and from all subcellular fractions by the method of Folch et al. [13]. The total lipid extracts from all subcellular fractions were treated with a solution of KCl according to González Sastre [14] and the lower phases or total lipid extracts *, were concentrated under vacuum to a suitable volume and applied to a column of Sephadex LH-60 (1.8×40 cm) equilibrated with chloroform/methanol (1:1, v/v) containing 5% 0.1 M HCl. The column was eluted with the same solvent mixture, at room temperature and at a flow rate of 0.3 ml/min. 3-ml fractions were collected, aliquots of which were used for protein determination. This chromatographic procedure, which has been recently developed in our laboratories [15] allows us to obtain in a very short time (2 h) the proteolipid proteins from the different subcellular fractions, free of adventitious lipids and as a single peak that elutes with the void volume of the column with a recovery of 95% or better.

Preparation of the samples for polyacrylamide gel electrophoresis

The tubes containing protein were pooled, concentrated under vacuum, placed in cellulose dialysis tubing and dialyzed against a solution containing 0.1 M Tris-phosphate buffer (pH 7.5), 1% SDS, 0.1% 2-mercaptoethanol for six days, which is the time necessary to form a single phase. This solution was changed at least twice a day. At the end of the dialysis, the contents of the bag were limpid. In order to bring the solvent composition of the sample similar to that of the gel [16], the contents of the bag were now dialyzed against 0.1 M Tris-phosphate buffer containing 0.1% SDS, 0.1% 2-mercaptoethanol. When the samples were prepared for electrophorpesis in gels containing urea the dialysis was done against the above solution containing 8 M urea. The recovery of protein in the bag, at the end of the procedure, determined in this case by the method of Schaffner and Weissman [17] with minor modifications, ranged in all cases between 90 and 108%.

Polyacrylamide gel electrophoresis

Two systems were employed:

(a) SDS system: Gels at 8, 10, 12 and 14% of total acrylamide concentration, pH 7.5 were used.

(b) SDS-urea system: Gels at 6, 8, 10, 12% of total acrylamide concentration, pH 7.5 containing 8 M urea were used. In both cases electrophoresis was carried out in 6×120 mm glass tubes and the running buffer was 0.1 M Tris-phosphate (pH 7.5) containing 0.1% SDS.

* We define proteolipid proteins as those proteins which are soluble in the organic phase after treatment of the lipid extract with KCl.

Electrophoretic procedure

Electrophoresis was carried out in a similar way for both systems. Gels were pre-runned for 2 h to eliminate the ammonium persulphate and 50–100 μl (20–50 μg of protein) of sample containing 10% glycerol and 5 μl of 0.05% Bromophenol blue solution were applied onto the gel. After electrophoresis, the gels were removed, measured and the dye front marked with a piece of wire. Gels were fixed for two days in 10% trichloroacetic acid which was changed several times.

Staining and destaining

The fixed gels were stained overnight with 0.25% Coomassie blue R-150 (Sigma Chemical Co.) in methanol/acetic acid/water (45:7:48, v/v), destained by transverse electrophoresis (Bickle and Traut [18]) and the relative mobility of the band (R_m) was calculated (Weber and Osborne [16]).

The gels were scanned by densitometry at 595 nm on a Chromoscan Spectrophotometer fitted with a linear transport accesory and an automatic integrator. In order to estimate the proportion of each band, the integrated area under each peak was related to the total integrated area.

Determination of proteins and of 2',3'-cyclic-nucleotide 3'-phosphohydrolase activity

Assay of 2',3'-cyclic-nucleotide 3'-phosphohydrolase activity was carried out by the method of Prohaska et al. [19]. 2',3'-Cyclic AMP (sodium salt) was obtained from Sigma Chemical Co. Total proteins were determined according to Lowry et al. [20] and proteolipid proteins according to the method described by Hess and Lewin [21].

Results

(A) Subcellular distribution of proteolipid proteins

Table I shows that myelin was the fraction containing the highest amount of proteolipid proteins. Added to the amount present in synaptosomes and mitochondria it represents 75–80% of all the proteolipid proteins that can be extracted from the different subcellular fractions. Fraction B, constituted by myelin fragments, small synaptosomes and curved membranes also contained significant quantities of these proteins. Mitochondria and synaptosomes contained quite similar amounts of proteolipid proteins.

TABLE I

DISTRIBUTION OF PROTEOLIPID PROTEINS IN ADULT RAT BRAIN SUBCELLULAR FRACTIONS

Subcellular fractions were obtained by the method of De Robertis et al. [10] (with minor modifications) and purified nuclei from a separate aliquot of the total homogenate [12]. Percent values were calculated from the sum of proteolipid proteins recovered in the seven fractions.

Subcellular fraction	% of total proteolipid proteins		
	Expt. 1	Expt. 2	Expt. 3
Purified nuclei	1.4	1.6	1.5
Myelin	50.1	56.2	50.5
Fraction 'B'	14.5	12.8	14.0
Synaptosomes	11.9	14.4	7.0
Mitochondria	12.8	8.9	16.0
Microsomes	9.1	6.1	11.0
Cytosol	n.d.	n.d.	n.d.

n.d., non detectable.

At variance with results presented by other investigators [8] the microsomal fraction contained small but measurable amounts of proteolipid proteins, which represent approx. 2% of the total protein in this fraction (see Table IV) and 8–10% of total rat brain proteolipid proteins (Table I). Purified nuclei contained only traces of proteolipid proteins while they were absent in the soluble fraction.

(B) Electrophoretic analysis

Logarithm of the free mobility (Y_0) of the SDS-protein complex is obtained by extrapolation to 0% of total acrylamide concentration (0% T) of the straight line in the plot: $\log R_m$ vs. % T. Since Chan and Lees [22] demonstrated that the $\log Y_0$ of proteolipid proteins from bovine white matter were higher than those of the standard proteins used for the calibation curves, we decided to use the Ferguson procedure, to estimate the molecular weights. Ferguson plots and calibration curves for standard proteins in both electrophoretic system used, are shown in Fig. 1.

SDS electrophoresis

The electrophoretic patterns of proteolipid pro-

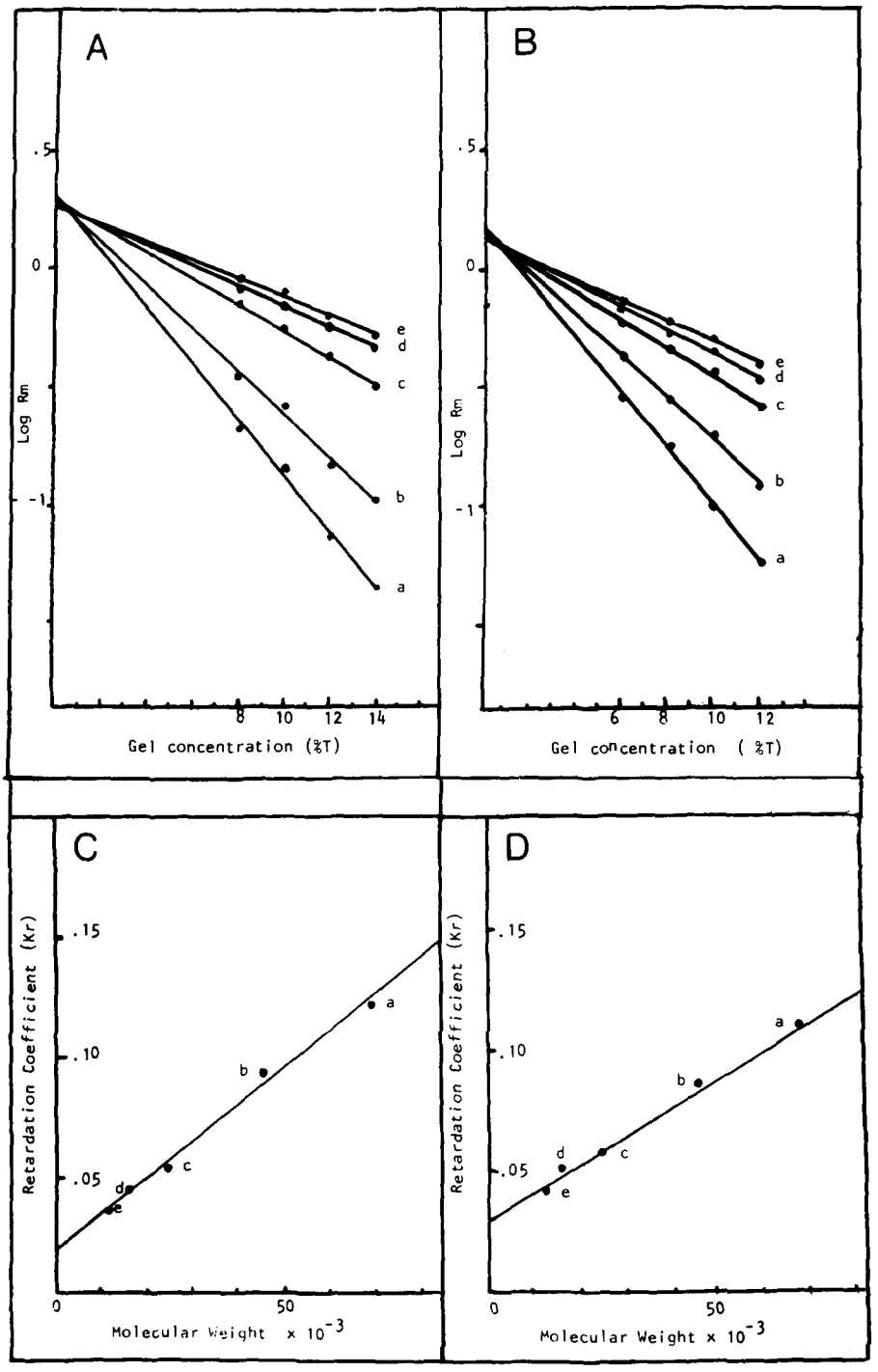

Fig. 1. Ferguson plots and calibration curves of standard proteins after SDS-polyacrylamide gel electrophoresis (A and C) and SDS-polyacrylamide gel electrophoresis containing urea (B and D). Standard proteins used for the preparation of calibration curves were: bovine serum albumin (M_r 67000)=a; egg albumin (M_r 43000)=b; trypsin (M_r 23000)=c; hemoglobin (M_r 16700)=d and cytochrome *c* (M_r 11700)=e. All of them were obtained from Sigma Chemical Co.

TABLE II

MOLECULAR WEIGHT OF PROTEOLIPID PROTEINS FROM ADULT RAT BRAIN SUBCELLULAR FRACTIONS

The molecular weight was determined by the SDS-polyacrylamide gel electrophorpesis using the following relationships. F (Ferguson method): K_r vs. M_r [9]. W (Weber and Osborne method): log R_m vs. M_r [15]. Figures in parenthesis represent % of each band, determined by densitometric scanning of the gels [a]. Molecular weight values represent the mean of three independent experiments and are given $\times 10^{-3}$. K_r, retardation coefficient; R_m, relative mobility.

Band No.	Whole brain		Myelin		Fraction B		Synaptosomes		Mitochondria		Microsomes	
	F	W	F	W	F	W	F	W	F	W	F	W
1	65.1	56.6 (6)	–	–	–	–	64.1	55.1 (8)	63.0	55.6 (9)	–	–
2	55.2	47.7 (20)	54.9	47.9 (20)	55.0	47.5 (14)	53.5	46.5 (12)	53.2	45.7 (6)	–	–
3	–	–	–	–	–	–	–	–	44.1	37.0 (5)	–	–
4	37.7	31.5 (5)	–	–	38.2	32.1 (6)	37.8	30.6 (13)	37.0	30.1 (40)	–	–
5	–	–	34.7	27.5 (9)	34.0	27.0 (10)	35.1	28.0 (6)	–	–	–	–
6	29.8 [b]	24.2 (33)	30.7 [b]	25.5 (41)	30.5 [b]	24.9 (34)	31.0 [b]	24.5 (7)	–	–	29.8 [b]	27.4 (29)
7	26.0 [c]	20.3 (18)	25.5 [c]	19.3 (19)	24.3 [c]	19.0 (22)	25.5 [c]	19.9 (29)	26.5 [c]	20.8 (9)	24.4 [c]	22.0 (24)
8	20.8	16.3 (4)	19.8	15.3 (11)	20.1	15.5 (4)	20.4	15.1 (7)	21.7	16.0 (5)	20.4	16.5 (9)
9	18.5	15.0 (8)	–	–	17.6	14.5 (10)	17.5	13.9 (18)	18.0	13.7 (12)	17.2	14.0 (38)
10	14.8	11.8 (6)	–	–	–	–	–	–	15.2	12.0 (10)	–	–
11	–	–	–	–	–	–	–	–	11.0	8.5 (4)	–	–

[a] Some aggregated material does not enter the gel. Since the proportion of each band in many different experiments and under the same experimental conditions was absolutely reproducible, we think that giving the results as percentage is valid.

[b] Reported in the text and in Table IV as 30 K proteolipid protein.

[c] Reported in the text and in Table IV as 25 K proteolipid protein.

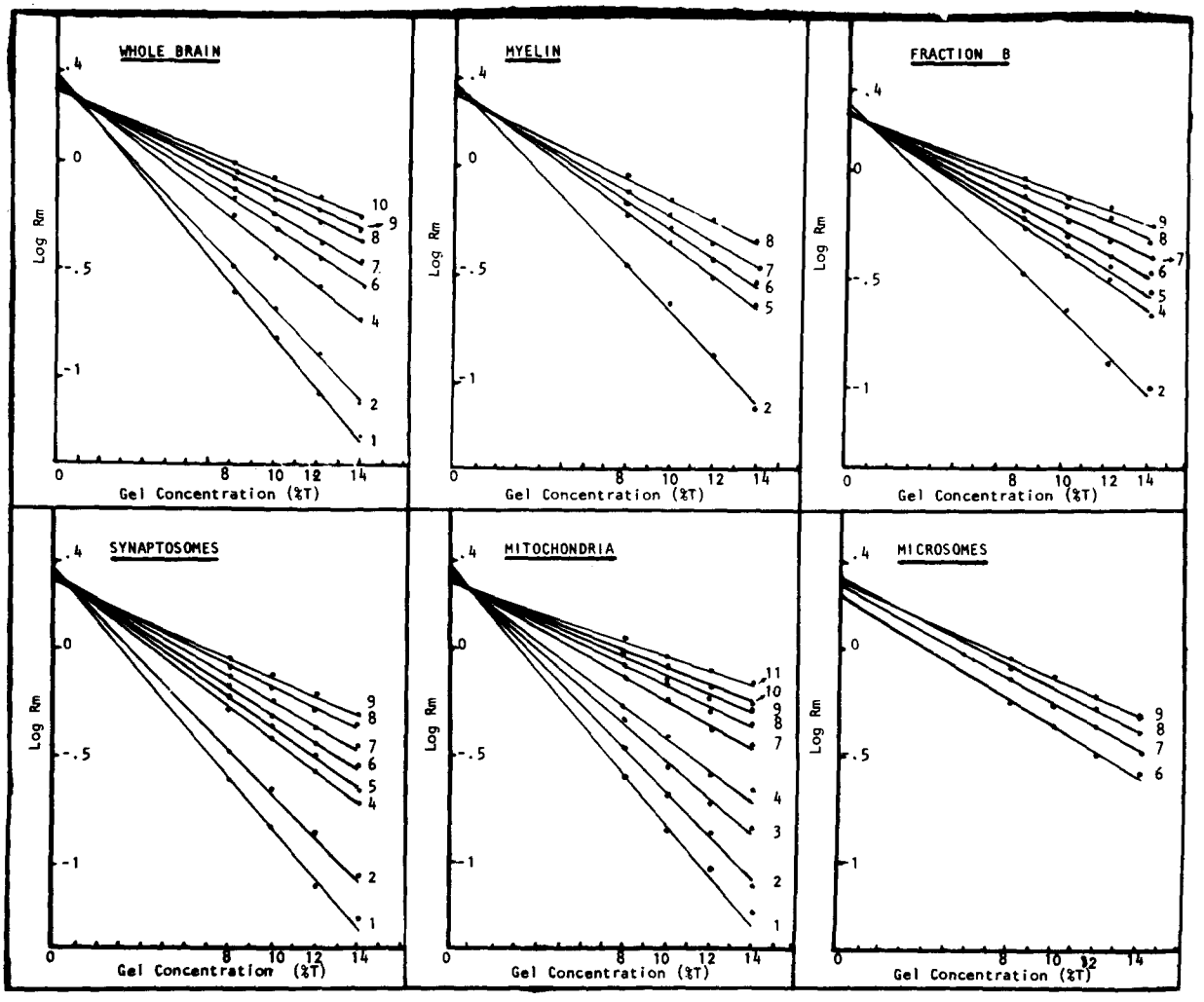

Fig. 2. Ferguson plots of bands observed after SDS-polyacrylamide gel electrophoresis (notation as in Table II).

teins of the various subcellular fractions in SDS-polyacrylamide gels are shown in Fig. 4a. The molecular weights as well as the Ferguson plots are shown in Table II and Fig. 2.

The electrophoretic analysis of proteolipid proteins obtained from whole brain revealed eight bands, three of which, with molecular weights 55.2 K, 29,8 K and 26 K were the most prominent and represented approx. 70% of the proteolipid proteins present in whole brain. These three components were also present in myelin, showing molecular weights of 30.7 K and 25.5 K for the bands corresponding to proteolipid protein and DM_{20} described by other investigators [22] and 54.9 K for the third component, which probably represents aggregates of the former proteins (unpublished results). Two other components (34.7 K and 19.8 K) were also present in small amounts. When the gels were overload or re-stained, two minor additional bands with molecular weights 15.2 K and 11.0 K were observed in the latter fraction. The high molecular weight band, as has been previously described for bovine white matter proteolipid proteins by Chan and Lees [22], was diffuse. The free mobilities (Y_0) of myelin proteolipid proteins were found to be higher than the corresponding standard proteins used for the calibration curves (Figs. 1 and 2), and no protein bands

with Ferguson plots similar to those of basic protein were detected. Presence of myelin membranes in fraction B was reflected in a pattern which was quite similar to that of myelin, except that two additional bands (M_r 38.2 K and 17.6 K) that were also observed in mitochondria and synaptosomes, were detected. This might be due to the presence in this fraction of curved membranes of synaptic origin.

Proteolipid proteins from synaptosomes showed a different pattern than myelin; there was a remarkable decrease in the proportion of the proteolipid protein PLP or 30 K component and an increase in bands of M_r 17.5 K and 37.8 K. Synaptosomes and mitochondria were the only subcellular fractions presenting a minor high molecular weight component of 64.1 K. The latter fraction showed an electrophoretic pattern where the classical proteolipid protein was absent. There were nine bands present, five of which were the most prominent. Two major bands had M_r 37 K and 18 K and three minor ones, characteristic of this fraction had M_r 44.1, 15.2 and 11.0 K.

The microsomal fraction was enriched in a band which was also present in synaptosomes (17.2 K). Two other major components (M_r 29.8 K and 24.4 K) and a minor one (20.1 K) were found in this fraction. The 29.8 K component of the microsomal

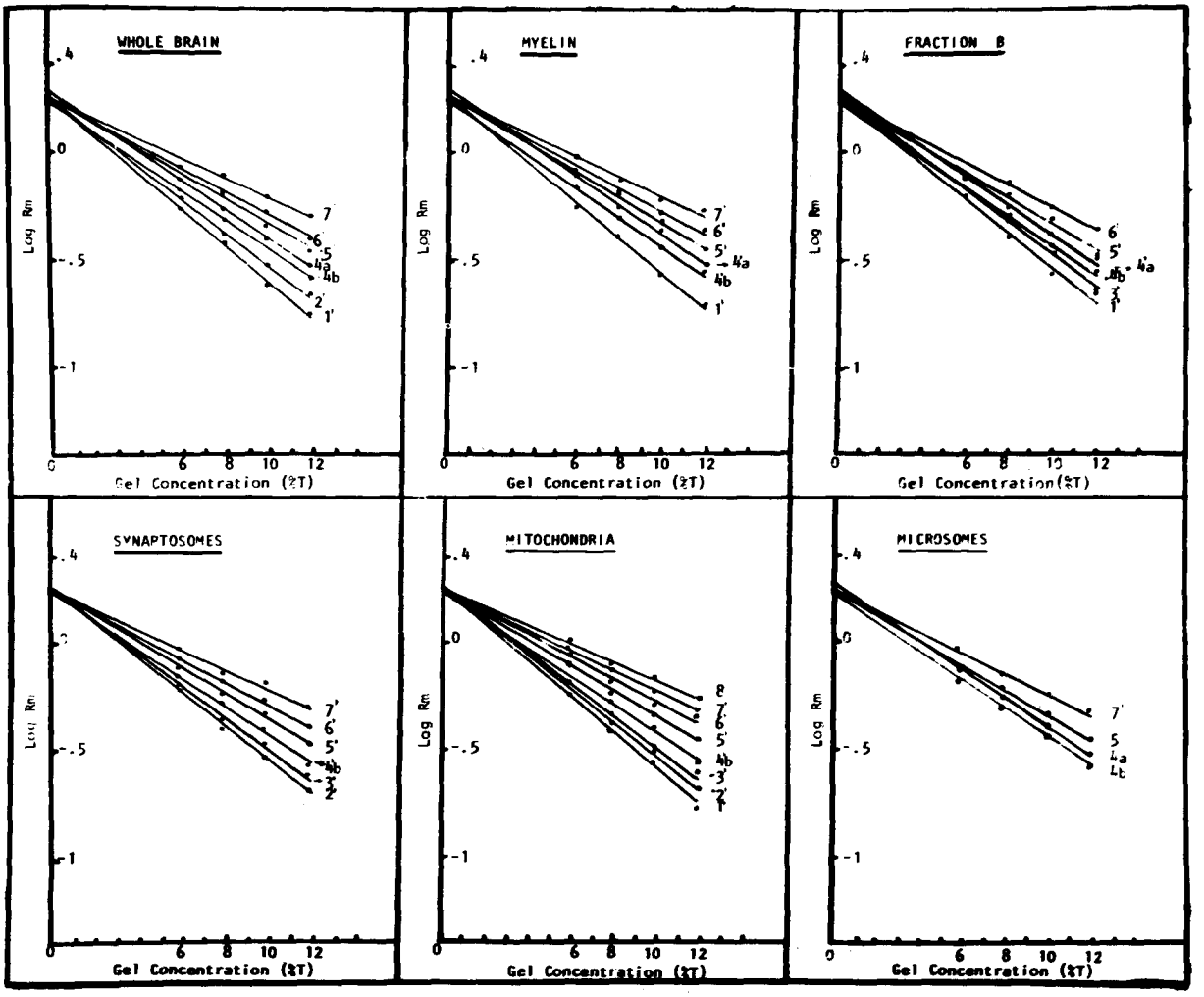

Fig. 3. Ferguson plots of bands observed after SDS-polyacrylamide gel electrophoresis containing urea (notation as in Table III).

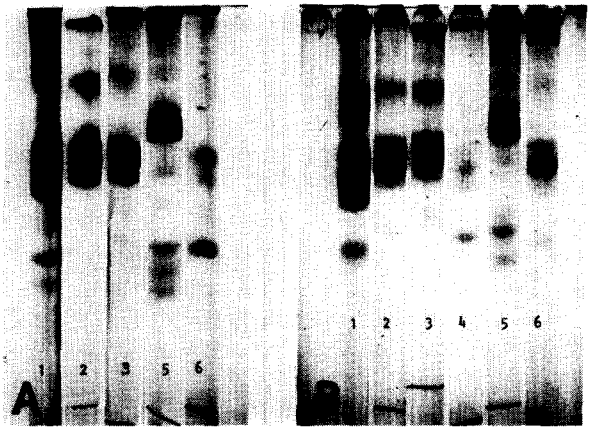

Fig. 4. Proteolipid proteins from adult rat brain subcellular fractions separated by polyacrylamide gel electrophoresis. (A) SDS system; (B) SDS-urea system. For both systems the proteolipid proteins were reduced with 2-mercaptoethanol; 1, whole brain; 2, myelin; 3, fraction 'B'; 4, synaptosomes; 5, mitochondria; 6, microsomes.

fraction which appears to correspond to myelin proteolipid protein PLP (30 K), was the only one showing an Y_0 similar to that of the standard proteins.

SDS-urea electrophoresis

Ferguson plots and electrophoretic patterns of proteolipid proteins of the various subcellular fractions in SDS-urea are shown in Figs. 3 and 4B. In total brain, seven bands were present. Two of them were isofactors (same retardation coefficient but different free mobility (Y_0)) and represented 60% of the total proteolipid proteins in the gel. Myelin showed a pattern very similar to that found in the absence of urea, except that the band corresponding to M_r 34.7 K was absent. The isofactor complex or doublet, probably represents proteolipid protein and the band of M_r 24 K corresponds to the DM_{20} present in the SDS system without urea (Table III). The patterns found in the synaptosomal, mitochondrial and microsomal fractions were very similar to those found in the absence of urea.

Free mobility of detergent-protein complexes

The log Y_0 of the SDS-proteolipid protein complexes ranged in all cell fractions between 0.32 and 0.39 with the exception of the 30 K component of the microsomal fraction which showed a log Y_0 between 0.28 and 0.33. The standard proteins in the same electrophoretic system displayed a log Y_0 ranging between 0.25 and 0.29. In the presence of urea the protein standards had a log Y_0 ranging between 0.12 and 0.17 while the log Y_0 for the proteolipid protein of the various fractions varied between 0.22 and 0.28. For the isofactors, identical K_r values were obtained, with a log Y_0 of 0.23 and 0.28.

(C) Subcellular distribution of 2',3'-cyclic-nucleotide 3'-phosphohydrolase and of the main proteolipid protein of myelin

2',3'-Cyclic-nucleotide 3'-phosphohydrolase has been considered by several investigators as a marker enzyme of the myelin membrane [23]. Its presence in 'myelin related membranes' [24], in 'vesicular material' derived from the microsomal fraction [25] as well as its pattern of activity during brain development, suggests that it cannot be considered only as a marker of compact (mature) myelin, but as a marker of both precursor membranes of myelin and mature myelin. For these reasons, although its activity is high in myelin, fraction B and microsomes (Table IV), its presence in the latter fraction should not be considered as exclusively due to contamination with myelin.

Table IV also shows the subcellular distribution of the 30 K component (or PLP) and of the 25 K component. PLP was concentrated in purified myelin and was also found to a lesser extent, in fraction B and microsomes. Although the activity of 2',3'-cyclic-nucleotide 3'-phosphohydrolase followed a quite similar pattern of distribution it was not identical. Both PLP and 2',3'-cyclic-nucleotide 3'-phosphohydrolase activity were almost absent in synaptosomes as well as in mitochondria. The 25 K component, although concentrated in myelin, was also found in all the other subcellular fractions. Furthermore, no similarities between the activity of 2',3'-cyclic-nucleotide 3'-phosphohydrolase and the subcellular distribution of this component were found. If all the 25 K component present in fraction B and in microsomes would be derived from myelin contamination, the ratio PLP/25 K would be identical in the three fractions. However, this ratio was lower in fraction B and in microsomes when compared to myelin,

TABLE III

MOLECULAR WEIGHTS OF PROTEOLIPID PROTEINS FROM ADULT RAT BRAIN SUBCELLULAR FRACTIONS

The molecular weight was determined by 8 M urea-SDS-polyacrylamide gel electrophoresis using the following relationships. F: (Ferguson method): K_r vs. M_r [9] and W (Weber and Osborne method): log R_m vs. M_r [15]. Figures in parenthesis represent % of each band, determined by densitometric scanning of the gels [a]. Molecular weight values represent the mean of three independent experiments and are given $\times 10^{-3}$. K_r, retardation coefficient. R_m, relative mobility.

Band No.	Whole brain		Myelin		Fraction B		Synaptosomes		Mitochondria		Microsomes	
	F	W	F	W	F	W	F	W	F	W	F	W
1′	42.5	40.0 (9)	41.0	38.0 (10)	40.5	39.0 (12)	–	–	43.0	41.0 (4)	–	–
2′	38.0	33.4 (8)	–	–	–	–	38.9	35.1 (8)	39.0	35.0 (10)	–	–
3′	–	–	–	–	36.0	28.0 (2)	37.0	29.5 (6)	36.0	28.2 (65)	–	–
4′a	31.0	23.5 (30)	30.5	24.8 (28)	30.2	24.5 (30)	–	–	–	–	29.6	24.1 (22)
4′b	29.8	19.8 (34)	29.0	21.0 (43)	29.8	21.3 (37)	28.9	20.5 (9)	28.0	20.0 (3)	28.8	20.7 (46)
5′	23.5	17.8 (9)	24.0	18.0 (9)	25.1	18.7 (11)	24.5	17.2 (25)	23.1	17.0 (2)	23.6	17.7 (14)
6′	20.0	13.7 (2)	19.4	16.5 (3)	19.0	13.1 (8)	20.5	13.3 (19)	21.0	13.6 (5)	–	–
7′	14.0	10.9 (8)	14.9	12.5 (7)	–	–	14.1	11.5 (33)	15.1	11.0 (7)	16.0	11.5 (180)
8′	–	–	–	–	–	–	–	–	11.1	8.4 (4)	–	–

[a] See footnote in Table II.

TABLE IV

DISTRIBUTION OF TOTAL PROTEOLIPID PROTEINS; 25 K; 30 K AND 2',3'-CYCLIC-NUCLEOTIDE 3'-PHOSPHOHYDROLASE ACTIVITY IN RAT BRAIN SUBCELLULAR FRACTIONS.

CNP activity, 2',3'-cyclic-nucleotide 3'-phosphohydrolase activity. FT, fresh tissue. T.P.P., total proteolipid proteins. SC (specific concentration), mg of proteolipid proteins/mg total protein in the fraction. SA (specific activity), units/mg protein in the fraction (1 unit is the enzymatic activity producing 1 μmol of 2'-AMP from 2',3'-cAMP/min. *a*, CNP activity (U/g FT)/30 K (mg/g FT). *b*, 30 K (mg/g FT)/25 K (mg/g FT).

Subcellular fractions	T.P.P.		30 K component		25 K component		CNP activity		*a*	*b*
	mg/g FT	SC	mg/g FT	SC	mg/g FT	SC	U/g FT	SA		
Total homogenate	3.60	0.031	1.19	0.010	0.64	0.005	380	3.24	319	1.86
Myelin	1.71	0.37	0.72	0.156	0.31	0.067	115	25	160	2.32
Fraction 'B'	0.51	0.07	0.17	0.022	0.11	0.014	100	12.8	588	1.5
Synaptosomes	0.45	0.06	0.029	0.003	0.12	0.016	11	1.15	379	0.24
Mitochondria	0.50	0.04	–	–	0.04	0.003	9	0.78	–	–
Microsomes	0.32	0.016	0.093	0.005	0.077	0.004	36	1.86	387	1.2

indicating the possible existence of 25 K components in both microsomes and fraction B. Hence, the 25 K component present in the electrophoretic pattern of total brain proteolipid proteins should not be considered as exclusively derived from myelin, in agreement with results presented by Nussbaum and Mandel [8]. This does not imply that the 25 K component found in myelin and that found in the other fractions is the same protein species.

Discussion

The heterogeneous characteristic of proteolipid proteins was first described by Pasquini and Soto [26] and Einstein et al. [27] using polyacrylamide gel electrophoresis in phenol/acetic acid/water. In this system, the resolution of the various protein bands was generally poor and the molecular weight of the various proteins separated was not determined. Nussbaum and Mandel [8] analyzed proteolipids obtained from mouse brain by SDS-polyacrylamide gel electrophoresis and found eleven bands which they identified as $P_1, P_2 \ldots P_{11}$. Band P_7, which corresponds to PLP according to other nomenclatures [22,28], was described as the major proteolipid protein present in myelin but was almost absent in the other two fractions studied, while band P_8 was present in all of them. Although 25 K and 20 K proteolipid proteins have been related to myelin, their presence in microsomes and their clear electrophoretic characterization has not been accomplished in previous work.

As mentioned before and based on the findings of Chan and Lees [22] we have used the Ferguson method for our studies in order to avoid the errors in the determination of molecular weight produced by the use of the classical relationship proposed by Weber and Osborne [16] ($\log R_m$ vs. M_r). On the other hand we have carried out SDS-polyacrylamide gel electrophoresis in the presence of 8 M urea trying to avoid aggregation of the material [29]. Mitochondria and synaptosomes do not contain the classical PLP and their electrophoretic pattern in the two systems is quite similar. The electrophoretic pattern of mitochondrial proteolipid proteins agrees well with that found by Kadenbach and Harvey [30] for yeast mitochondrial proteolipids. The major protein band that we have found in both system (36 K) probably corresponds to the major proteolipid of the inner mitochondrial membrane [31].

In myelin there are three major protein bands PLP (30 K), DM_{20} (25 K), and (54.9 K) probably band P_3 as described by Nussbaum and Mandel [8] in mouse brain. In the presence of urea the electrophoretic profile did not change except that the PLP band appeared as a doublet constituted by two isofactors; this could be due to a different binding capacity of SDS or to an important effect

of the charge of the protein upon the total charge of the complex. The latter could probably be the most reasonable explanation of our results, since it is well known that in the presence of 8 M urea the binding capacity of proteins for SDS is reduced by 50% [32] and that the effect of the net protein charge upon the charge of the complex is increased. Chan and Lees [22] found a similar pattern in a preparation of proteolipid proteins obtained from bovine white matter and indicated, that reduced PLP in the presence of urea could appear as two isofactors as a result of a recombination at random of reduced S-S bridges, a process which has been previously described for ribonuclease by Haber and Anfinsen [33]. The microsomal fraction also contains a proteolipid protein similar to the PLP of myelin (M_r 30 K) which, however, shows a Y_0 similar to that of the standard proteins. This component represents an important proportion of the microsomal proteolipid proteins (30%) and two important pieces of evidence obtained from our data allow us to assume that its presence in the microsomal fraction is not due to myelin contamination: First, the difference in Y_0 between the PLP (30 K) of myelin and of the 30 K component of the microsomal fraction. It should be pointed out that proteolipid proteins are hydrophobic proteins containing covalently bound fatty acids [34]. Probably, differences in the degree of esterification of both proteins could produce differences in the hydrophobic binding capacity of SDS. Second: if the 30 K component found in the microsomal fraction would come from the contaminating myelin, one should expect to find in the electrophoretic profile of microsomal proteolipid proteins the high molecular weight band present in myelin, which is not the case. There is evidence [22,35] demonstrating that the high molecular weight proteolipid proteins from bovine myelin are hydrophobic aggregates of those of low molecular weight. Furthermore, recent observations [36] demonstrating the aggregation of proteolipid proteins in SDS solution induced by heating, were interpreted as due to intermolecular hydrophobic interactions of the fatty acid chains covalently bound to the protein. The present results agree with this interpretation, since if the microsomal PLP would have a lower amount of bound fatty acids, it would also have a lower binding capacity towards SDS (smaller Y_0) and there would be a smaller number of hydrophobic areas to form high molecular weight proteolipids.

The above mentioned data, as well as results previously reported [37,38] allow us to propose the possible presence of a microsomal pool of PLP, slightly different from that present in myelin. Its metabolic role as possible precursor of myelin PLP as well as the turnover of both components is being studied at present in our laboratories.

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