A COMPARATIVE STUDY OF SEVERAL MEMBRANE PROTEINS FROM THE NERVOUS SYSTEM*

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Summary: A comparative study is carried out on the amino acid composition and average hydrophobicity of several membrane proteins from the central and peripheral nervous system. For this purpose, the amino acids are grouped into different sets whose ratios are defined in terms of the proportion of hydrophilic and hydrophobic residues. The analysis results in the classification of proteolipid proteins and the cholinergic receptor protein of Electrophorus as integral proteins, and acetylcholinesterase and basic proteins as peripheral.

Introduction

The characterization of membrane proteins is still limited to the determination of the three-dimensional structure of a few of them and the amino acid sequence of a few more; so far we do not have at our disposal the necessary objective criteria for a universal definition of membrane proteins as such. Along these lines Singer and Nicolson recently proposed a classification of membrane proteins according to some of their physico-chemical characteristics. The two categories proposed are those of "integral" (formerly called core, intrinsic or structural) and"peripheral" (elsewhere extrinsic) proteins (1). The latter require mild treatments for detachment from the membrane, they dissociate free of lipids, and once dissociated from

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the membrane they are relatively soluble in neutral aqueous buffers. Integral proteins, on the other hand, require more drastic procedures for their isolation and often remain associated with lipids. In their delipidated state they are usually insoluble in neutral aqueous buffers and found in aggregates (1).

Myelin is one of the biomembranes which has been more extensively studied. The most important protein components of this membrane are the basic protein, the Folch-Pi-Lees proteolipid and the Wolfgram protein. The former can be extracted with weak acids and is soluble in water (2), and the amino acid sequence of the bovine (3) and human (4) basic proteins has been determined. The Folch-Pi proteolipid protein occurs in a great variety of biomembranes (5), and in the case of myelin accounts for 50% of the total protein content. This type of protein is soluble in the organic solvents used for its isolation, it tends to aggregate in more polar media (5), and it shows a capacity to bind phospholipids after being delipidised (6,5). It is clear then that proteolipid proteins fulfill most of the requirements for inclusion in the integral category mentioned above, whereas the basic proteins seem to belong to the peripheral type. However, recent studies on highly delipidised proteolipids, showing their aqueous solubilisation, question the validity of rigid definitions, and reinforce the need for establishing additional criteria for defining membrane proteins (7).

In this paper the comparative analysis of several proteins from the nervous system, based on their amino acid composition and average hydrophobicities, leads to the classification of two distinct groups of membrane proteins, in coincidence with the criteria of Singer and Nicolson (1).

Methods and Materials.

Amino acid analysis of Protein fraction I. Total lipid extracts from cerebral

cortex were prepared as described in the first paper of this series (5). A slight modification in technique improved the resolution of the separative procedure, resulting in the obtention of several proteolipid protein fractions. The first fraction eluted from the Sephadex LH 20 column with N,N-dimethylformamide-0.1 N HCl (9:1 v/v), was practically devoid of lipids. Complete delipidisation was achieved by dialysis against chloroform-methanol (2:1 v/v). After 72 hr dialyses only fatty acids remained associated with the proteolipid protein (Protein fraction I). After evaporation of the chloroform-methanol about 300 µg of the protein were hydrolysed for periods of 18 to 72 hr in 100 µl of 6 N HCl containing 0.1% phenol. The HCl was removed from the sealed tubes which were repeatedly washed and evaporated. Finally, duplicates of the protein were analysed with a Technicon automatic amino acid analyser.

Comparative study. The amino acid composition of Protein fraction I together with those of other membrane proteins obtained from the references listed were submitted to a comparative analysis, for which purpose sets of amino acids, ratios of sets and average hydrophobicities were defined as follows: BASIC: histidine + lysine + arginine; ACID: aspartic acid + asparagine + glutamic acid + glutamine; TOTAL CHARGED (TCHARG): basic + acid; HYDROPHILIC (PHY): total charged + threonine + serine; HYDROPHOBIC (FO): valine + methionine + isoleucine + leucine + tyrosine + phenylalanine; APOLAR: (hydrophobic) -tyrosine; RATIO 1 (R 1): hydrophilic/hydrophobic; RATIO 2 (R 2): hydrophilic/apolar; RATIO 3 (R 3): total charged/hydrophobic; RATIO 4 (R 4): total charged/apolar; Average hydrophobicity or hydrophobicity index was calculated by multiplying the residue percentage of each amino acid by its side chain hydrophobicity factor (8,9,10), adding up all the totals, and then dividing by the total number of residues.

RESULTS AND DISCUSSION

Table I lists the amino acid composition of several membrane proteins of nervous origin. Protein fraction I, the proteolipid protein from cerebral cortex, is relatively rich in proline and

TABLE I

AMINO ACID COMPOSITION OF SEVERAL MEMBRANE PROTEINS

	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
Proteolipid protein peripheral myelin (16)	4.20	4.60	7.80	5.50	2.10	9.00	7.10	1.60	12.00	1.80	6.80	15.90	4.40	6.90	2.30	1.80	3.60
Proteolipid protein, heart (17)	6.09	7.16	6.71	4.77	5.51	9.23	10.11	.18	5.40	5.17	7.03	14.58	3.35	7.47	2.78	2.31	2.13
Proteolipid protein, central w. matter (19)	4.20	8.50	5.40	6.00	2.90	10.30	12.50	4.20	6.90	1.70	4.90	11.10	4.70	7.90	4.30	1.90	2.60
Protein fraction 1, (this study)	4.16	7.69	6.46	6.25	6.13	10.59	10.14	2.84	6.51	4.46	4.29	10.92	3.86	7.01	2.12	2.67	3.90
Proteolipid protein, central myelin (18)	3.91	8.15	5.25	5.82	3.35	10.44	11.66	2.71	7.19	1.44	4.86	11.21	4.83	7.98	4.59	2.34	2.83
Protein N-2,central myelin (20)	4.79	8.41	6.32	7.23	2.83	11.17	12.03	4.17	6.55	1.59	4.38	11.67	4.89	8.00	4.35	2.34	1.80
Acetylcholine receptor, Electrophorus (21)	11.44	5.61	6.16	10.20	5.73	5.86	5.83	1.97	8.60	1.95	6.38	10.53	4.02	5.74	4.57	2.21	6.18
Acetylcholinesterase, Electrophorus (22)	9.60	3.80	6.10	8.30	7.20	6.90	4.60	0.10	6.20	2.70	3.30	8.00	3.40	4.80	3.90	2.00	4.80
Protein "K" erythrocyte (23)	12.90	6.70	6.50	6.70	3.80	10.90	10.00	1.30	10.70	2.40	6.10	6.00	1.70	4.20	7.80	2.50	3.80
Wolfgram protein, central myelin (2)	9.90	5.16	5.84	12.95	4.64	8.00	8.45	1.01	5.84	2.13	4.27	9.63	2.87	4.22	6.95	2.29	5.83
Basic protein (P2) peripheral m, (24)	9.09	6.06	6.06	9.09	6.06	10.10	8.08	0.00	6.06	2.02	4.04	8.08	2.02	4.04	10.10	3.03	6.06
Basic protein,central myelin (24)	6.71	3.71	9.46	7.63	7.24	15.49	8.90	0.00	1.38	1.28	1.57	6.02	2.79	5.36	7.79	5.04	9.47
Basic protein, central w. matter(25)	6.99	4.15	9.71	6.37	7.44	15.08	8.81	0.00	1.54	1.16	1.76	6.35	2.49	4.78	7.56	5.67	10.14

methionine, its overall composition being similar to that of other proteolipid proteins.

A comparison of amino acid sets, ratios of these, and average hydrophobicities is given in Table II, in which proteins are listed according to increasing ratio 3 (R 3). Basic and acid sets are relatively scarce in proteolipids, both sets (i.e. total charged) amounting jointly to less than 20% of the total. These sets are quite high in the basic proteins and other peripheral proteins, supporting the recent X-ray diffraction evidence suggesting their peripheral location in the membrane (11). The hydrophilic set also shows the difference between integral and peripheral proteins, accounting for about 30% in the former and 50% in the latter. The proportion of small amino acids (Gly, Ser and Ala) not listed here, is also a special feature of proteolipid proteins. But even more conspicuous is the high proportion of hydrophobic (or apolar) amino acids in these proteins; the unusually high percentage of these residues results in the fact that proteolipid proteins have the highest hydrophobicity indices, even though the Trp contribution is not computed, since many papers in the literature do not report on it. In spite of this exclusion, the average hydrophobicity of proteolipids is significantly higher than the great majority of "soluble" proteins (unpublished results) and than those of the peripheral proteins listed here. The acetylcholine receptor extracted from the electroplax (20) is also found in this group of proteins, whereas acetylcholinesterase (22), the membrane-bound enzyme from the same source, has values ranging between those of peripheral proteins.

The difference between the two categories of Singer and Nicolson (1) is more clear when ratios of groups of amino acids are compared. As seen in Table II, the mean value of ratio 3 (R 3) for proteolipid

TABLE II

AMINOACID SETS, RATIOS, AND AVERAGE HYDROPHOBICITIES

	BASIC	ACID	TCHARG	PHY	FO	APOLAR	R 1	R 2	R 3	R 4	AVERAGE HYDROPHOBICITY
Proteolipid protein, peripheral myelin(16)	7.50	9.70	17.20	29.60	47.80	43.40	0.619	0.682	0.360	0.396	1291.499
Proteolipid protein, heart (17)	7.22	10.86	18.08	31.95	43.00	39.65	0.743	0.806	0.420	0.456	1281.914
Proteolipid protein, central w. matter (19)	8.80	10.20	19.00	32.90	37.20	32.50	0.884	1.012	0.511	0.585	1180.999
Protein fraction I, (this study)	8.69	10.41	19.10	33.25	37.05	33.19	0.897	1.002	0.516	0.575	1166.239
Proteolipid protein, central myelin (18)	9.76	9.73	19.49	32.89	37.51	32.68	0.877	1.006	0.520	0.596	1185.719
Protein N-2,central myelin (20)	8.49	12.02	20.51	35.24	37.08	32.19	0.950	1.095	0.553	0.637	1171.529
Acetylcholine receptor,Electrophorus(21)	10.96	21.64	32.60	41.37	37.16	33.14	1.113	1.248	0.877	0.984	1177.154
Acetylcholinesterase, Electrophorus (22)	10.70	17.90	28.60	38.50	28.40	25.00	1.356	1.540	1.007	1.144	956.749
Protein "K" erythrocyte (23)	14.10	19.60	33.70	46.90	31.10	29.40	1.508	1.595	1.084	1.146	1017.699
Wolfgram protein, Central myelin (2)	15.07	22.85	37.92	48.92	28.96	26.09	1.689	1.875	1.309	1.453	1002.279
Basic protein (P2) peripheral m, (24)	19.19	18.18	37.37	49.49	26.26	24.24	1.885	2.042	1.423	1.542	1003.939
Basic protein, central myelin (24)	22.30	14.34	36.64	49.81	18.40	15.61	2.707	3.191	1.991	2.347	866.904
Basic protein, central w.matter(25)	23.37	13.36	36.73	50.59	18.08	15.59	2.798	3.245	2.032	2.356	861.759

proteins is about 1/4 of that for basic proteins, the latter being even higher than the mean values established by Hatch and Bruce (13) for membranous lipoproteins.

The combination of the solubility properties and the study of the amino acid composition of membrane proteins facilitates the classification of several proteins into two distinct groups. The solubility criteria justify the inclusion of proteolipid proteins into the integral category and that of basic proteins into the peripheral one. Other proteins isolated with organic solvents, such as the N-2 (26), or with detergents, such as the acetylcholine receptor (20) show similar characteristics to those of proteolipid proteins, suggesting that the high degree of hydrophobicity is a common feature of all integral proteins. The hydrophobic portion of some proteins is known to be maintained over long evolutionary periods, as shown by Perutz, Kendrew and Watson (14). This constancy also seems to be present in cytochrome c, a typical peripheral protein, though its total amino acid composition has changed by about 50% throughout its evolution (15). Although the hydrophobic domains of hemoglobin and myoglobin (14) result from special amino acid sequences rather than from a high proportion of hydrophobic residues, it is highly likely that such hydrophobic domains also exist in integral proteins, thought to be in contact with the membrane interior. Since no amino acid sequence of these proteins is yet available, and hence no homologies between the types of proteins depicted in this study can be drawn, the objective criteria here suggested provide a tentative means towards defining membrane proteins.

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