AMINO ACID ANALYSIS AND N–TERMINAL SEQUENCE DETERMINATION OF P7 PROTEOLIPID APOPROTEIN FROM HUMAN MYELIN

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1. Introduction

There is a general agreement that myelin from central nervous system has a relatively simple protein composition, which makes it an excellent material for protein–lipid interaction studies in a membrane. Three main protein types have been described: basic proteins, proteolipid proteins and acidic Wolfgram proteolipid proteins (see ref. [1] for review). The distribution ratio between these proteins is variable depending on the procedures used [2–5] and animal species examined [2,6,7].

In human myelin, most work has been devoted to the unique basic protein [8–10], and a sequence has been published [11,12]. To our knowledge no comparable information is available for the other proteins; so, during the course of the sequence study of the major rat brain myelin proteolipid P7 protein [13], we also decided to elucidate the primary structure of the corresponding human protein. This was done in order to provide information of possible homology in protein sequence of the proteolipid apoprotein during evolution. In the present paper, we present results concerning the amino acid composition and the sequence of the first 20 amino acids of the N-terminal moiety.

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2. Material and methods

Myelin was isolated from human white matter by a modification of the method of Kurihara et al. [14] using an initial 5% homogenate. Human material was obtained from a body without any neuropathological signs within 3 hr after death. Up to 8.5 g of dry weight myelin was prepared starting with 195 g of white matter from one adult brain.

The procedures for preparation of the myelin proteolipids were those described in detail in previous works [5,13]. The preparative SDS-gel electrophoresis of P7 apoprotein was done in an apparatus similar to that described by Waehneldt [15], entirely automated [13]. The recuperation of the native apoprotein from fractions containing pure P7 was carried out by acidification with acetic acid extraction with diethyl ether as mentioned earlier [13]. The protein precipitate was collected, dissolved in 98% formic acid and dialyzed during 48 hr against acetic acid solutions of decreasing molarity. Finally the protein was obtained by lyophilisation.

The other analytical methods used, for amino acid determination, oxidation by performic acid, automated Edman degradation with a Socosi sequencer and digestion by carboxypeptidase A, were identical to those indicated in a foregoing work [13].

3. Results

The purity of the myelin was checked by electron microscopy. Fig. 1 shows the electrophoretic pattern of rat and human myelin proteins after electrophoresis on polyacrylamide gels in the presence of SDS. Proteolipids P7 and P8M in both species have identical migration rates, indicating very close molecular weights.

Amino acid analyses have been performed on purified human P7 preparations using three hydrolysis times (table 1); the nearest integer obtained was

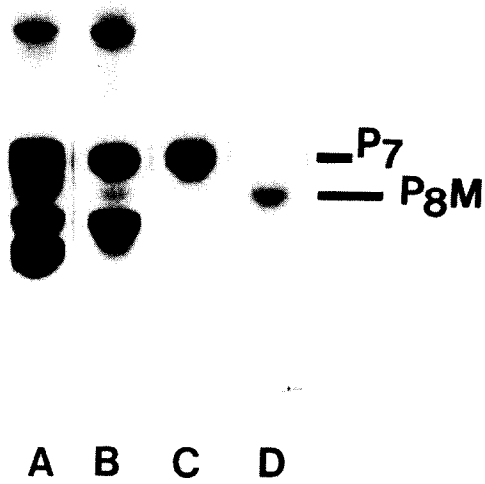

Fig. 1. SDS-polyacrylamide gel electrophoresis of the individual human myelin proteolipids P7 (gel C) and P8M (gel D) isolated by a preparative scale method in comparison to total myelin proteins from human (gel B) and rat (gel A) brain. The homogeneity of each isolated proteolipid is indicated by the same relative mobilities on SDS gels as those observed in the original myelin preparation.

Table 1
Amino acid analysis of the major human brain myelin proteolipid P7 apoprotein: residues/mole of mol. wt. 23 500. Comparison with the rat brain myelin proteolipid P7

Amino acid	Human brain				Rat brain nearest integer [13]
	Time of hydrolysis			Nearest integer	
	18 hr*	48 hr	72 hr		
Asp	10.2	9.1	9.0	11	11
Thr	16.1	15.1	14.4	17	17
Ser	12.4	10.5	10.0	12–13	12
Glu	13.0	12.9	13.1	13	13
Pro	6.2	5.8	5.4	6	6
Gly	21.4	21.7	22.0	22	22
Ala	24.0	24.0	24.0	24	24
Val	14.3	15.0	15.9	16–17	17
(Cys-)	8.3	8.0	8.0	8–9	8
Met	3.0	2.7	2.9	3	3
Ile	10.5	10.9	11.4	12	12
Leu	22.3	22.9	23.1	23–24	24
Tyr	9.7	9.5	10.0	10	10
Phe	16.9	16.1	16.3	17	17
Trp**				2	2
Lys	8.9	9.6	9.7	10	10
His	5.7	5.2	5.2	5–6	5
Arg	5.4	5.9	5.4	6	6
Total				217–222	219

*Residues per mole calculated on the basis of 24 residues of Ala.
**Characterized following the procedure of Spies and Chambers [20].

Table 2
N-terminal sequence of the major human brain myelin proteolipid P7 apoprotein determined with a Sequencer (For experimental details, see ref. [13])

I	Gly–	Leu–	Leu–	Glu–	Cys–	Cys–	Ala–	Arg–	Cys–	Leu–
II	Val–	Gly–	Ala–	Pro–	Phe–	Ala–	X–	Leu–	Val–	Ala–
										20

X, unidentified amino acid.

compared with that of rat brain. No significant differences can be observed.

The proposed N-terminal sequence of P7 apoprotein, after performic oxidation, is given in table 2; it corroborates the sequence found by studying the P7 of rat brain [13]. Until now, there exist some difficulties in identifying the amino acid in position 17. Treatment of the oxidized apoprotein with carboxypeptidase A released only phenylalanine, identified by using the amino acid analyzer.

4. Discussion

The methodology developed in the course of a previous study [13] led us to examine the amino acid composition and a part of the N-terminal sequence of human myelin P7 proteolipid apoprotein obtained in pure state as checked by gel electrophoresis (fig. 1). Gagnon et al. [16] have also claimed the isolation of a highly purified human myelin protein with similar properties to that of proteolipids. Glycine was the N-terminal amino acid reported, but the authors concluded that it was a glycoprotein since some carbohydrates are present in appreciable amounts. This conclusion is not in accordance with other workers using classical proteolipids [17], so that we feel that Gagnon's preparation might be rather an aggregation of denaturated proteolipid with some other minor proteins of myelinic origin. Other studies on unpurified proteolipid protein preparations from myelin of human and calf brain have also shown that glycine was the most abundant N-terminal amino acid [18]. Finally, Whikehart and Lees [19] have found that the major N-terminal residue of white matter and myelin proteolipid was glycine with glutamic acid (or glutamine) as the minor N-terminal group. These authors also suspected phenylalanine to be the C-terminal amino acid corresponding to the N-terminal glycine. Our results confirm their assessment.

The present results on human P7 proteolipid apoprotein do not allow to detect any differences with that originating from rat brain; this would most probably indicate that the structure of this protein has been stable during evolution as did myelin basic proteins where only 11 amino acids substitutions were observed between the human and the bovine proteins [11]. Nevertheless, definite conclusions can only be drawn when the entire sequence is known.

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