# STRUCTURAL DATA CONCERNING THE MAJOR RAT BRAIN MYELIN PROTEOLIPID P7 APOPROTEIN

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## 1. Introduction

In a previous paper, we described the isolation of the major rat brain myelin proteolipid P7 apoprotein by preparative sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis; the establishment of the N-terminal sequence up to the twentieth amino acid was thus possible [1]. Identical sequences were found by studying the P7 apoprotein of human myelin [2] and corresponding apoproteins of bovine white matter [3].

Proteolipid preparations have been described to be resistant to the action of endopeptidases [4]. However Lees et al. [5] concluded that proteolytic digestion was possible in the presence of non-ionic detergent. In the present paper, we report for the first time the isolation and the sequence of several tryptic peptides. The apoprotein N-terminal sequence could be lengthened and the C-terminal sequence precised. Furthermore, the previously reported arginine content of P7 apoprotein calculated as 6 residues/mol on a molecular weight basis of 23 500 [1] could be confirmed. The six arginine containing peptides were characterized.

## 2. Materials and methods

### 2.1. *Myelin isolation*

Isolation of myelin from brains of adult Wistar rats was carried out as previously reported [1]. The procedure for the preparation of myelin proteolipids was described in detail in earlier works [1,6]. A minor modification, precipitation of the basic proteins with 1.0 M KCl, was introduced in this technique. In order to separate the apoprotein moiety, the total diethyl ether proteolipid pellet was dissolved in a chloroform/methanol/0.1 N HCl (10 : 10 : 1, v/v/v) solvent and filtered on a Sephadex LH-20 column (120 × 2 cm) as indicated by Gagnon et al. [7]. In our conditions, the column excluded apoprotein fraction was found to be free from any detectable lipids on thin-layer chromatographic plates (0.5 mm thickness, silica gel H, Merck) using chloroform/methanol/25% ammonia (70:30:5, v/v/v) as solvent. Samples of the apoprotein moiety were also checked by SDS–polyacrylamide disc-gel electrophoresis [6] after appropriate solubilization of the material in an 8 M urea–1% SDS solution (Nussbaum, unpublished). The content of the tubes with the apoprotein was pooled and precipitated with 4 vol. of diethyl ether at −20°C. The precipitate, after centrifugation, was dissolved in 98% formic acid and subsequently lyophilised.

### 2.2. *Oxidation*

The oxidation of the apoprotein was achieved by the method of Hirs using performic acid during 150 min at 0°C [8].

### 2.3. *Tryptic digestion and separation of the fragments*

Oxidized proteolipid apoprotein (380 mg) was digested over 20 h with 6.5 mg trypsin (DCC treated, Sigma) at 37°C in 190 ml of 0.05 M *N*-ethylmorpholine–HCl buffer, at pH 8.0, containing 0.5% Triton X-100. The tryptic digest was extracted with 5 vol of a chloroform/methanol mixture (2 : 1, v/v). The



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peptides soluble in the resulting upper phase were lyophilised. After dissolution in 50% formic acid, the lyophilized material was filtered on a Sephadex G-25 column (180 X 1.8 cm) using 30% acetic acid as solvent. The long tryptic peptides contained in the first peak were further separated on a Sephadex G-50 column (200 X 1.8 cm) with 30% acetic acid as eluent. The shorter tryptic peptides were rechromatographed, in bulk, on a Dowex IX2 column (140 X 1.2 cm) equilibrated with a solution containing 1% pyridine and 1% collidine, the pH of which has been adjusted to 8.4 with acetic acid. Gradient elution was achieved as previously described [9]. The peptides were desalted on a Sephadex G-25 column (180 X 1.8 cm) with 30% acetic acid as eluent. When necessary, the peptides were finally purified by preparative paper electrophoresis, at pH 6.5 (pyridine/water/acetic acid; 100 : 900 : 4, v/v/v) or by preparative descending paper chromatography (I: *n*-butanol/pyridine/acetic acid/water; 15 : 10 : 3 : 12, v/v/v/v or II: *n*-butanol/formic acid/water; 75 : 15 : 10, v/v/v). The amino acid composition of the peptides was established with an autoanalyzer after total hydrolysis (6 M HCl, 18 h or 96 h, 110°C, under vacuum).

### 2.4. *Structure determination*

Some peptides were sequenced by the manual Edman-dansyl procedure. Automated Edman degradation was carried out on others in a Socosi Sequencer model PS-100, by the dimethylallylamine simple cleavage method. The lysine containing peptides were modified with 4-sulfophenyl isothiocyanate [10]. The phenylthiohydantoin amino acids were characterized by thin-layer chromatography, gas–liquid chromatography and with an amino acid autoanalyzer as previously described [11]. The C-terminal amino acids were determined with an autoanalyzer after digestion with carboxypeptidases A and B (0.05 M ammonium bicarbonate, 37°C, 5 or 24 h).

## 3. Results

After filtration on Sephadex G-25 of the proteolipid apoprotein P7 tryptic peptides soluble in the upper phase subsequent to the chloroform/methanol extraction, two fractions, A (long peptides) and B (shorter peptides) were characterized. After two successive chromatographies of fraction A on Sephadex G-50, peptide A2 (37 amino acids, with a C-terminal lysine residue) was purified with a yield of 13% and its terminal sequence up to the twentythird amino acid automatically established (table 1). Fraction B was chromatographed on Dowex IX2, and 11 peaks (B1–B11), were thus separated (fig.1). Their electrophoretic and chromatographic behaviours, yields, amino acid compositions and sequences are reported in table 2. Peptides B8 and B10 have the same structure but in the latter, after total hydrolysis, fatty acids were characterized by thin-layer chromatography.

## 4. Discussion

In a previous paper [6] we have demonstrated that the rat myelin proteolipids are resolved by polyacrylamide disc-gel electrophoresis in the presence of SDS in 3 bands: P7 (88%), P8M (10%) and P3 (2%).

Table 1

N-terminal sequence of peptide A2 contained in peak A after filtration on Sephadex G-25 of the tryptic digest soluble in the upper phase subsequent to the chloroform/methanol extraction of the rat proteolipid P7 apoprotein

| | 9 | 10 | | | | | | | | | | | 20 | | | | | | | | | | | 30 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Residue[a] | Cys– | Leu– | Val– | Gly– | Ala– | Pro– | Phe– | Ala– | Ser– | Leu– | Val– | Ala– | Thr– | Gly– | Leu– | *X* – | Phe– | Phe– | Gly– | Val– | Ala– | Leu– | Phe– |
| (a) | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | + | + | + | + | + | + | + |
| (b) | | 40 | 40 | 7 | 16 | 4.5 | 13 | 12 | 13 | 7 | 11 | 7 | + | 1.5 | 5 | | 4.5 | 5 | 1 | 2 | 2 | 1.5 | 1 |
| (c) | 6 | 31 | 22 | 16 | 16 | 4.5 | 10 | 9 | | 4 | 6 | 4 | | 5.0 | 2 | | 1.5 | 2 | 2 | 2 | 2 | 2.0 | 1 |

[a]The previously established N-terminal sequence of the apoprotein P7 [1] is taken into account for the numbering of the residues

Methods of identification were as follows (a) phenylthiohydantoin derivative determined by thin-layer chromatography, (b) phenylthiohydantoin derivative determined by gas–liquid chromatography, results give the percentage. (c) Amino acid determined with an Autoanalyzer after regeneration, results give the percentage. *X* = unidentified amino acid.

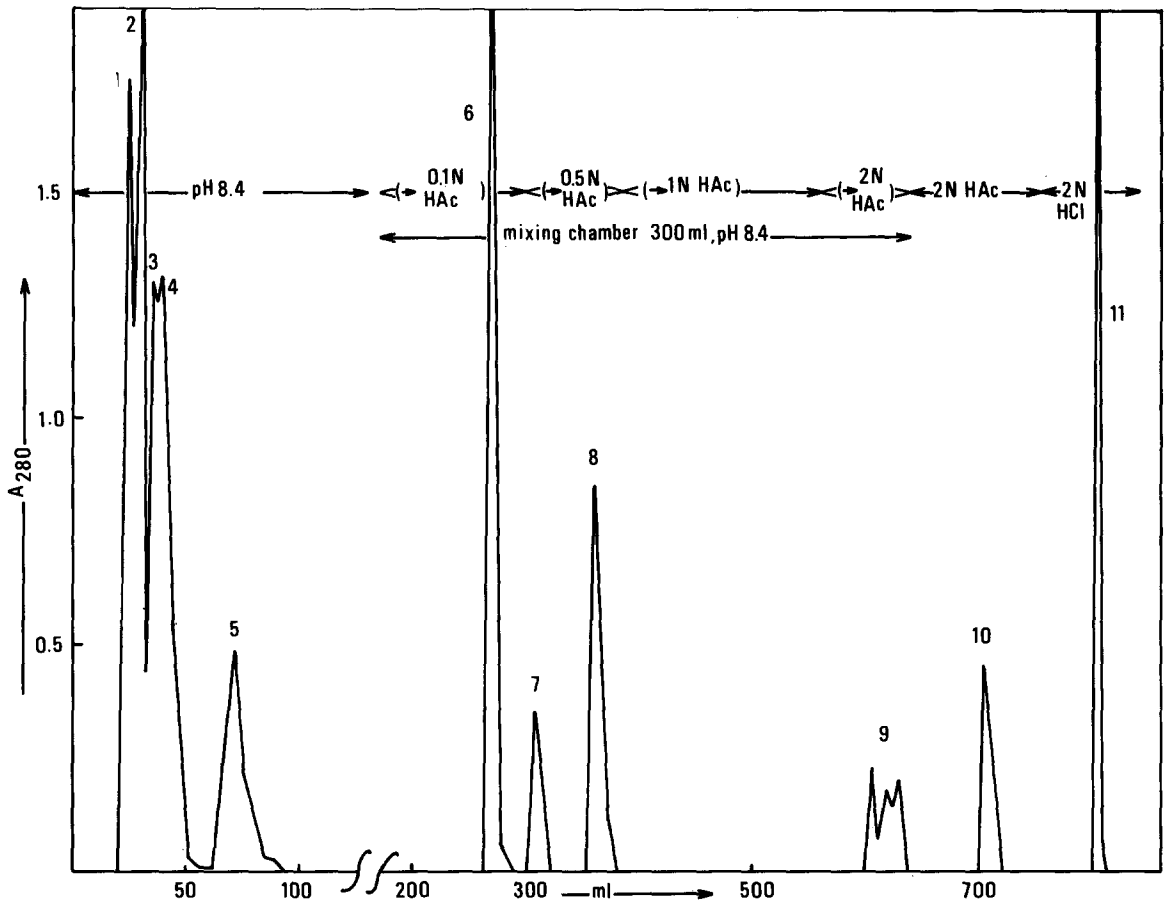


Fig. 1. Chromatography on Dowex 1 X 2 (140 X 1 cm) of the peptides contained in peak B after filtration on Sephadex G-25 of the tryptic digest soluble in the upper phase subsequent to the chloroform/methanol extraction of the rat proteolipid P7 apoprotein.

Recently, Vacher-Leprêtre et al. [3] have shown that their 20 000 component (corresponding to the P8M band) of bovine white matter proteolipids had the same N-terminal sequence as the major 25 000 one (corresponding to P7). This result was in accordance with our unpublished data showing that the sequence of the 4 first amino acids at the N-terminal side of P8M corresponded to that found for P7 apoprotein (Jollès, Jollès, Nussbaum and Mandel, unpublished data). Furthermore, it appeared that the P3 band (apparent molecular weight of 48 000) was probably due to the dimerisation of P7; this observation was supported by the fact that the level of P3 band increased by storing the myelin proteolipid apoprotein moiety after LH-20 column chromatography. Finally on the basis of a comparative study of the amino acid composition, N-terminal sequence and C-terminal amino acid of the different components of the white matter proteolipids, isolated by gel electrophoresis, Vacher-Leprêtre et al. [3] suggested that their primary sequences might be similar. They also suggested that the differences in migration rates of the different components were more likely to be related to their shapes rather than to their molecular weights.

With these conclusions, we started to investigate the tryptic peptides of the major myelin proteolipid apoprotein P7 without previous separation of the minor proteolipid apoproteins P8M and P3.

The previously proposed N-terminal sequence of apoprotein P7 was corroborated when peptides B11

Table 2

$R_F$ values (in solvents I and II), mobility $m$ (at pH 6.5), yields and structures of the peptides contained in peak B after filtration on Sephadex G-25 of the tryptic digest soluble in the upper phase subsequent to the chloroform/methanol extraction of the rat proteolipid P7 apoprotein

| Peptide | $R_F$ (I) | $R_F$ (II) | $m$[a] (pH 6.5) | Yield (%) | Structure |
|---|---|---|---|---|---|
| B 1 | 0.27 | 0.05 | + 0.30 | 37 | Gly–Leu–Ser–Ala–Thr–Val–Thr–Gly–Gly–Gln–Lys |
| B 2 | 0.20 | 0.05 | + 0.85 | 23 | Gly–Arg |
| B 3 | 0.20 | 0.05 | + 0.71 | 23 | Gly–Ser–Arg |
| B 4 | 0.40 | 0.25 | + 0.57 | 23 | Leu–Met–Gly–Arg |
| B 5 | 0.37 | 0.25 | + 0.55 | 19 | Gly–Thr–Lys–Phe |
| B 6 a | 0.20 | 0 | + 0.16 | 11 | Gly–Gln–His–Gln–Ala–His–Ser–Leu–Glu–Arg |
| B 6 b | 0.32 | 0.10 | 0 | 15 | Thr–Thr–Ile–$CySO_3H$–Gly–Lys |
| B 7 | 0.19 | 0 | | 8 | Val–$CySO_3H$–His–$CySO_3H$–Leu–Gly–Lys |
| B 8 | 0.30 | 0 | −0.20 | 14 | Thr–Ser–Ala–Ser–Ile–Gly–Ser–Leu–$CySO_3H$–Ala–Asp–Ala–Arg |
| B 9 | 0.60 | 0.50 | −0.28 | 14 | PyrGlu[c]–Asp–Phe–Ile–Gly–Tyr–Lys |
| B 10[b] | 0.43 | 0 | −0.37 | 16 | Thr–Ser–Ala–Ser–Ile–Gly–Ser–Leu–$CySO_3H$–Ala–Asp–Ala–Arg |
| B 11 | 0.36 | 0 | −0.48 | 30 | Gly–Leu–Leu–Glu–$CySO_3H$–$CySO_3H$–Ala–Arg |

[a] $m$ = 0 for Gly, $m$ = +1 for Arg, $m$ = −1 for $CySO_3H$

[b] Presence of fatty acids in the peptide

[c] Pyrrolidonecarboxylic acid

and A2 were characterized; they could be joined (B11 + A2) and the N-terminal sequence of the apoprotein was thus extended until the thirtyfirst residue. Twenty-seven out of thirthy-one amino acid residues were hydrophobic, leading to the conclusion that the N-terminal segment of the P7 apoprotein had a pronounced hydrophobic character.

Peptide B5 was a tryptic peptide devoid of a C-terminal basic amino acid; treatment of the oxidized apoprotein P7 as well as of B5 with carboxypeptidase A released only Phe. The latter seemed thus to represent the C-terminal sequence of the apoprotein P7.

Previously the arginine content of the apoprotein P7 was calculated as 6 residues/mole [1]. All the corresponding peptides were isolated. Thus the suggested molecular weight of 23 500 for the P7 apoprotein, based on amino acid analysis and sodium dodecyl sulfate polyacrylamide gel electrophoresis [1] correlated well with the number of characterized arginine peptides. Until now, 6 of the theoretical 10 lysine containing peptides [1] were recognized. On the basis of the present results, we can conclude that the previously proposed molecular weight of 12 500 [12] or the possible existence of an oligomeric series having a theoretical monomer molecular weight of 5000 [13] seem to be highly improbable.

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