The NH₂-terminal Region of the P2 Protein From Rabbit Sciatic Nerve Myelin*

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The reaction of the P2 protein from rabbit sciatic nerve with CNBr produced four peptides; a 20-residue peptide (CN3) containing tryptophan which occupies the blocked NH₂ terminus; Peptide CN1, a large peptide comprising over 70% of the P2 molecule; Peptide CN2, a fraction containing two tightly bound peptides having 2 half-cystine residues and comprising the COOH terminus; and Peptide CN4, a nonapeptide of the CN3 region obtained by abnormal CNBr cleavages of the bonds between Trp-8 and Lys-9 and Asp-17 and Asp-18, respectively. The complete sequence of Peptide CN3 was determined by sequenator and dansyl-monitored Edman procedures; the NH₂-terminal sequence was shown to be N-Ac-Ser-Asn-Lys. Peptide CN3 is joined to Peptide CN1 by a Met-Lys linkage; an overlap peptide obtained by BNPS-skatole cleavage of the P2 protein between Trp-8 and Lys-9 proved invaluable in linking Peptides CN3 and CN1. We determined 44 and 33 residues, respectively, for Peptide CN1 and the BNPS-skatole peptide with a sequenator. These studies may bear on our understanding of allergic neuritis and Guillain-Barre syndrome since the P2 protein is an antigenic target in these diseases. Peptides CN1, CN2, and CN3 add up to 138 amino acids and account for the total P2 protein; the sequence of the first 64 residues of the P2 protein is: N-Ac-Ser-Asn-Lys-Phe-Leu-Gly-Thry- Trp-Lys-Leu-Val-Ser-Arg-Glu-Leu-Gly-Leu-Ala-Thr-Arg-Glu-Leu-Asn-Leu-Asn-Val-Ser-Val-Ile-ILE-Ser- Lys-Lys-Gly-Arg-Thr-ILE-Arg-Glu-Ser-Gly-Phe-Lys-Asn-Thr-Glu-Ile-Thr-Phe.

The P2 protein (1) has received particular attention as an example of an extrinsic membrane protein found in peripheral nerve myelin but not in central nerve myelin (2-4). It is a small basic protein of $M_r = 14,000$ that is distinguished from the myelin basic protein (present in both peripheral and central myelin) by its high degree of definable secondary structure, primarily $\beta$ structure (2, 5). While not definitely established, evidence favors the P2 protein as the causative factor in peripheral nerve myelin that is responsible for experimental allergic neuritis, an autoimmune demyelinating disease of the peripheral nervous system (1, 2, 6, 7). Peripheral nerve myelin induces a severe form of allergic neuritis in monkeys (8) remarkably similar to the Guillain-Barre syndrome in humans. In view of the recent finding that the P2 protein, when formed in a complex with phosphatidylinerine, is a potent neuritogen in rats (9), and in view of the work of Brostoff et al. (10) who obtained a peptide from the bovine P2 protein which induced allergic neuritis in rabbits, it appears that the P2 protein is indeed the antigenic focus in allergic neuritis. The peptide of Brostoff et al. (10) appears to be derived from the NH₂ terminus of the P2 protein. Thus, knowledge of the amino acid sequence of this important region may prove valuable in elucidating a major disease-inducing site.

Many questions concerning molecular parameters involved with allergic neuritis and its relation to its human counterpart, the Guillain-Barre syndrome, can be approached via knowledge of the amino acid sequence. The use of synthetic peptides has proven invaluable in elucidating the essential details of the antigenic and disease-inducing domains of the myelin basic protein (11-14). Previously, we reported (15) that the rabbit P2 protein contains 2 half-cystine and 3 methionine residues and isolated four peptide fragments following CNBr treatment. Now we can report the amino acid sequence of the NH₂-terminal region of the P2 protein (64 residues) and the position of the CNBr peptides. The NH₂-terminal sequence, which begins with N-Ac-serine, 1 appears to define uniquely this membrane protein which serves as an exclusive marker for peripheral nerve myelin as shown by recent fluorescent antibody studies. 2

METHODS

Cyanogen Bromide Peptides—Rabbit sciatic nerve P2 protein, which appeared homogeneous by polyacrylamide gel electrophoresis in dodecyl sulfate was prepared as described previously (2, 15) and was reacted with cyanogen bromide according to the procedure described earlier (15). The resulting four peptides were resolved on a Sephadex G-25 (superfine) column (114 X 45 cm), using 0.5 M acetic acid as the eluting buffer. The peptide profile was followed at 235 nm and 280 nm; four peaks were obtained. The fractions under each peak were pooled and lyophilized. When 3H-alkylated material (see below) was used, radioactivity in each tube was determined with an Intertechnique SL32 liquid scintillation counter. The peptide yields from each peak were 140 mg (Peak I); 17.3 mg (Peak II); 21.4 mg (Peak III); and 12 mg (Peak IV).

Purification of Peptides—The material from Peak I was further purified by gel filtration on a Sephadex G-75 (fine) column (65 cm X 3.5 cm), using 0.2 M acetic acid as the eluting buffer. Fractions of 5 ml were collected and the elution profile was followed at 280 nm; two peaks were clearly resolved. The first peak, at the void volume, represented 10% of the original material and appeared to be ungraded or only partially degraded P2 protein. The major portion (referred to as CN1 peptide), eluted in the second peak and was shown by disc gel electrophoresis (15) to be homogeneous. It was recovered in a 70% yield.

The material from Peaks II and III was purified on Bio-Gel P6-P10

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1 The abbreviations used are: Ac, acetyl; CM, carboxymethyl; PTH, phenylthiohydantoin; BNPS-skatole, the brominated adduct of 2-(2-nitrophenylsulfenyl)-3-methylindoline.

columns (70 x 2.5 cm and 59 x 2 cm, respectively). The peptides, eluted with 0.2 M acetic acid, were monitored at 205 nm and 280 nm. In both cases, only one major peak was eluted. Appropriate fractions were pooled and lyophilized. By high voltage electrophoresis at pH 4.7 the major peaks from these fractions, referred to as Peptide CN2 and Peptide CN3, were found to be over 95% pure. Peptides CN2 and CN3 were recovered in yields of 45% and 38% theoretical, respectively. Small peaks, barely recorded by ascending chromatography in n-butyl alcohol/acetic acid/pyridine/H₂O (12:2:3:8 v/v) using Whatman No. 3MM paper. Peptide material in n-butyl alcohol/acetic acid/pyridine/H₂O (122:38:19:151 v/v) stained in 10% formic acid, and then lyophilized. A nonapeptide referred to as Peptide CN4, Peak IV material, was recovered in 25% yield.

Purity of Peptides—Purity of peptides was checked as described previously (15) using high voltage electrophoresis, paper chromatography, amino acid analysis, and in some cases, polyacrylamide gel electrophoresis.

Localization of Half-Cystine in P2 Protein—P2 protein (20 mg) was reduced and alkylated according to the method of Hirs (16) as described previously (15), using iodol-1-¹⁴C]acetamide. The carboxymethylated protein was then treated with cyanogen bromide (15), and the resulting peptides separated on a Sephadex G-25 (superfine) column (45 x 2 cm). Fractions (2 ml) were collected; the radioactivity in 0.05-ml aliquots from each tube was measured in an Intertechnique SL2 liquid scintillation counter.

Trypsin Digestion—Peptide CN3 was digested with trypsin in enzyme:peptide molar ratio of (1:20) for 3 to 5 h at 37°C, and subjected to high voltage electrophoresis at pH 4.7. The digested material (2 to 10 mg) was dissolved in 0.2 M acetic acid (20 to 30 mg/ml) and applied to Whatman No. 3MM paper (0.5 mg/inch). Electrophoresis was carried out in pyridine/acetic acid buffer (2.5:2.5 v/v) buffer, pH 4.7, for 3 h at 1700 to 3000 V and 150 to 250 mA. After the paper was air-dried, the peptide bands were located and then eluted as described above for chromatography. Three peptides were recovered migrating at 0.04, 0.23, and 0.44 with respect to methyl green, and these are referred to as Peptides CN3a, CN3b, and CN3b, respectively.

Amino Acid Analysis—Peptides were hydrolyzed in constant boiling 6 N HCl for 24 to 48 h at 105-110°C and analyzed in a Beckman or Durrum amino acid analyzer (15).

Carboxypeptidase Treatment—Peptides (0.5 to 1.0 mg) were incubated with 20 to 30 µg of carboxypeptidase A and B (Worthington) in 0.2 M ammonium bicarbonate buffer for 0.5 to 24 h at 37°C under toluene, lyophilized, and then applied to an amino acid analyzer. In the case of Peptide CN3a, following 24 h incubation, the mixture was subjected to high voltage electrophoresis. The N-acetylserine was detected using bromocresol blue (17).

Identification of Acetylated NH₂-Terminal Residue—Mass spectrometric analysis of the NH₂-terminal Peptide CN3a was carried out according to the method of Morris et al. (23, 24); approximately 150 nmol of peptide was first permethylated (24).

RESULTS

Isolation of Peptides—The [¹⁴C]carboxymethylated rabbit P2 protein, treated with CNBr in 70% formic acid, was applied to a column of Sephadex G-25 as shown in Fig. 1. Since the P2 protein has a total of 3 methionine residues (15), four peptides are anticipated from CNBr cleavage. Previously, Sephadex G-50 had been used, but in that case, the smaller peaks did not resolve clearly (15). With Sephadex G-25, four main peaks are seen, referred to as Peaks I, II, III, and IV (Fig. 1). Nearly all of the radioactivity is associated with Peak II. We obtained an identical elution pattern (i.e. four peaks) on Sephadex G-25 when the CNBr digest of the unmodified P2 protein was examined.

We showed previously (15) that the first peak is composed primarily of a large peptide (CN1) of approximately 100 residues, which is easily purified by gel filtration (Table I). Peptide CN1 comprises over 70% of the total P2 protein chain of approximately 127 to 130 residues. When treated with carboxypeptidase A, a homoserine residue in peptide CN1 (Table I), a residue missed previously (15). Thus, peptide CN1 is not at the COOH terminus of the P2 molecule, but must occupy an intermediate position.

In order to determine whether peaks II, III, and IV comprise the expected small peptides, they were subjected to gel filtration and high voltage electrophoresis. In each case, a major peptide accounted for each peak. Peak II contained over 90% of the radioactivity and thus accounts for the 2 half-cystine

FIG. 1. The elution pattern of the CNBr digest of the rabbit P2 protein on a Sephadex G-25 column (45 x 2.5 cm) is shown in 0.5 M acetic acid. The protein was first alkylated with [¹⁴C]-iodoacetamide (15). The absorbance was determined at 235 nm (closed circles). The radioactivity (open circles) was determined in each tube with a liquid scintillation counter. The absorption profile showed four peaks (I, II, III, and IV).
The NH\textsubscript{2} Terminal Region of the P2 Protein

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Peptide\textsuperscript{a} CN1</th>
<th>Peptide\textsuperscript{a} CN2</th>
<th>Peptide\textsuperscript{a} CN3</th>
<th>Peptide\textsuperscript{a} CN4</th>
<th>Peptide\textsuperscript{a} P2 protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>12.4 (12)</td>
<td>3.6 (4)</td>
<td>1.5 (2)</td>
<td>1.0 (1)</td>
<td>16</td>
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<tr>
<td>Arginine</td>
<td>5.3 (5)</td>
<td>0.9 (1)</td>
<td>0</td>
<td>0</td>
<td>6</td>
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<tr>
<td>Aspartic acid</td>
<td>10.1 (10)</td>
<td>3.7 (4)</td>
<td>1.6 (2)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>11.5 (12)</td>
<td>0.95 (1)</td>
<td>0.9 (1)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>4.7 (5)</td>
<td>3.0 (3)</td>
<td>1.7 (2)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>12.1 (12)</td>
<td>2.4 (2)</td>
<td>0.9 (1)</td>
<td>13</td>
<td></td>
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<tr>
<td>Proline</td>
<td>2.1 (2)</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Glycine</td>
<td>9.7 (10)</td>
<td>1.1 (1)</td>
<td>1.0 (1)</td>
<td>11</td>
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<tr>
<td>Alanine</td>
<td>6.1 (6)</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
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<tr>
<td>Half-cystine</td>
<td>0</td>
<td>+ (2)</td>
<td>0</td>
<td>2</td>
<td></td>
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<tr>
<td>Valine</td>
<td>4.2 (4)</td>
<td>4.8 (5)</td>
<td>1.0 (1)</td>
<td>1.3 (1)</td>
<td>9</td>
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<tr>
<td>Isolucine</td>
<td>7.3 (7)</td>
<td>0.97 (1)</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>8.7 (9)</td>
<td>1.8 (2)</td>
<td>1.1 (1)</td>
<td>11</td>
<td></td>
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<tr>
<td>Tyrosine</td>
<td>0</td>
<td>0.5 (1)</td>
<td>0.8 (1)</td>
<td>2</td>
<td></td>
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<tr>
<td>Phenylalanine</td>
<td>3.3 (3)</td>
<td>1.7 (2)</td>
<td>1.0 (1)</td>
<td>6</td>
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<tr>
<td>Homoserine</td>
<td>0.5 (1)</td>
<td>0.73 (1)</td>
<td>0.9 (1)</td>
<td>3 (Met)</td>
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<tr>
<td>Tryptophan</td>
<td>1.0 (1)</td>
<td>+ (1)</td>
<td>0</td>
<td>2</td>
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</tr>
<tr>
<td>Carboxymethylcysteine</td>
<td>0</td>
<td>1.7 (2)</td>
<td>0</td>
<td>0</td>
<td></td>
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</tbody>
</table>

Total 99 9 20 9 127

\textsuperscript{a} The single homoserine residue in Peptide CN1 was found by carboxypeptidase treatment. The tryptic peptide CN1 is based on absorbance at 280 nm; tryptophan was detected in Peptide CN3 with the Ehrlich reagent.

\textsuperscript{b} The half-cystine content was determined as carboxymethylcysteine by amino acid analyzer following alkylation of the P2 protein.

\textsuperscript{c} Taken from Ref. 15.

residues of the P2 protein. This peptide, referred to as CN2, was further purified by gel filtration on a Bio-Gel P6-P10 column, and appeared homogeneous by high voltage electrophoresis and amino acid analysis. The composition of Peptide CN2 (Table I) showed a high valine and lysine content, the expected 2 half-cystines, 1 tyrosine, and a homoserine residue. It was found that the CN2 peptide fraction is composed of two peptides as shown by sequenator analysis reported elsewhere (25).

Peak III material was fractionated as described above for Peak III. It was slightly contaminated with Peak II material, but upon purification appeared homogeneous by high voltage electrophoresis and amino acid analysis (less than 5% contaminating residues such as arginine and alanine). Importantly, Peptide CN3 has a blocked NH\textsubscript{2}-terminal residue as shown by its unreactivity in the Edman or dansyl procedures. This result is compatible with studies on the intact P2 protein which also shows a blocked NH\textsubscript{2}-terminal residue (15). Peptide CN3 has 1 homoserine residue, and a total of 20 residues. The composition of peptide CN3 (Table I) shows more amino acids than material prepared previously by high voltage electrophoresis (15), and appears more highly purified. One residue not noted previously (15) is tryptophan, which was detected with the Ehrlich reagent on paper (15). However, the reaction gave a greenish color in contrast to the typical bluish color normally observed for tryptophan-containing peptides. The CNBr treatment may have resulted in oxidation of the tryptophan residue to an oxindole derivative.

The Peak IV material was also further purified by high voltage electrophoresis. The dominant material, referred to as Peptide CN4, appears to be a small peptide of 9 residues (Table I).

The Structure of Peptide CN3—Tryptsin treatment of Peptide CN3 gave three peptides as expected, a tripeptide (CN3a), a hexapeptide (CN3b), and a undecapeptide (CN3c) separable by either gel filtration on Sephadex G-10 or high voltage electrophoresis. The tripeptide CN3a has a blocked NH\textsubscript{2} terminus as shown by the dansyl-procedure, and COOH-terminal lysine inferred from the specificity of trypsin. When peptide CN3a was treated with carboxypeptidase A and B for 24 h, lysine and asparagine (1.0 and 0.4 mol/mol) were found by amino acid analysis (Table II). Thus, the sequence of the tripeptide is: serine-asparagine-lysine. The serine residue is blocked at the amino position. High voltage electrophoresis of the carboxypeptidase mixture also showed asparagine and lysine, and a spot co-migrating with N-acetylserine.

Analysis of Peptide CN3a was made with the mass spectograph which gave signals at m/e of 126 and 158 corresponding to the ions of N-acetyldehydroalanine and N-acetylserine, respectively. It was concluded that the amino-blocking group is an acetyl moiety and that the P2 protein begins with the tripeptide region: N-acetylserine-asparagine-lysine.

Peptide CN3b contains six amino acids including tryptophan (Table II). Following high voltage electrophoresis, the most rapidly migrating peptide (migration 0.44 with respect to the dye) was Peptide CN3b which stained green with the Ehrlich reagent whereas Peptides CN3a and CN3c were negative. It also showed a strong fluorescence on paper when examined by UV light. The tryptophan residue (for its degradation product) occupies position 5 in Peptide CN3b as shown by the dansyl-Edman procedure which gave the sequence of the COOH-terminal region as Phe-Leu-Gly-Thr-Lys.

The composition and sequence of Peptide CN3

<table>
<thead>
<tr>
<th>Derived tryptic peptide</th>
<th>Composition\textsuperscript{a}</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN3a</td>
<td>Lys 1.0 Ser 0.9 Asp 0.9</td>
<td>N-Ac-Ser\textsuperscript{a}-Asn-Lys</td>
</tr>
<tr>
<td>CN3b</td>
<td>Lys 1.0 Thr 0.9 Gly 1.0 Phe 0.9 Trp +</td>
<td>Phe-Leu-Gly-Thr-Lys\textsuperscript{a}</td>
</tr>
<tr>
<td>CN3c</td>
<td>Asp 2.6 (3) Leu 0.7 (1)</td>
<td>Leu-Val-Ser-Ser-Glx-Asx-Phe Ser 1.6 (2) Tyr 0.5 (1) Glu 1.0 Phe 1.6 Val 0.8 (1) Hse 0.7 (1)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Carboxypeptidase treatment (24 h) gave 1.0 mol of lysine and 0.4 mol of asparagine for Peptide CN3a and 1.0 mol of homoserine and 0.9 mol of tyrosine for Peptide CN3c.

\textsuperscript{b} Determined by carboxypeptidase treatment and mass spectroscopy.

\textsuperscript{c} Determined by dansyl-Edman degradation.

\textsuperscript{d} Determined by sequenator.

The amino acid sequence of the first 64 residues of the Rabbit P2 protein

![Graph](http://www.jbc.org/)

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Stable amino acids were calculated by quantitative gas-liquid chromatography. The peptides analyzed are triptic peptides isolated from the P2 protein noted previously (15) which eluted in a retarded position on gel filtration on Sephadex G-25. Peptide CN3b must occupy a position between Peptides CN3a and CN3c since Peptide CN3a has a blocked NH2 terminus and Peptide CN3c terminates in homoserine. Peptides CN3a and CN3c, respectively, were completed as shown in Table II and Fig. 2. It is of interest that even at Step 10 tyrosine was obtained on back hydrolysis in a yield of 40% that of leucine (Step 1). Thus this peptide when combined with cytochrome c is especially suited for sequenator analysis. The acidic character of the peptide (2 Asp, 1 Glu) may contribute to its insolubility during sequencing and accounts for its slow migration on electrophoresis. The COOH-terminal homoserine (methionine) was confirmed with carboxypeptidase, which showed Tyr-Hse. The sequence studies show 20 residues for peptide CN3, in agreement with the amino acid analysis. Thus the sequence of Peptide CN3, the NH2-terminal CNBr peptide, is completely defined as shown in Table III.

Sequence of Peptide CN4—Three peptides from Peak IV material were resolved on electrophoresis; all of them had essentially the same amino acid composition (Table I) and the 11 remaining residues of Peptide CN3 and 23 residues of the NH2-terminal region of the PO protein were produced since the yield of the NHe-terminal region of the PO protein begins with a prominent hydrophobic region (residue 40 to 51) containing 5 isoleucine residues. At this stage, no other feature appears immediately evident that this sequence is identical with a region of Peptide CN3, but we were mystified since it lacks homoserine. It appeared that Peptide CN4 must be derived from the P2 protein by a process other than oxidative cleavage at a methionine residue; a process apparently involving tryptophan and aspartic acid residues. It seems that the peptide bond between Trp-8 and Lys-9 is cleaved on one end, and the Asp-17 and Asp-18 bond at the other end. Since certain peptide linkages involving aspartic acid are labile, it is possible that the cleavage at Asp-17 and Asp-18 was due to the acidic conditions used, although attempts to cleave this linkage by incubating P2 protein in 70% formic acid were unsuccessful. Although Peptide CN4 does not originate from cleavage at a methionyl residue, it is produced in significant yields as judged from the recovery of Peptide CN4 in approximately 20% of the theoretical yield.

Peptide CN1—When Peptide CN1 was analyzed by sequenator, the sequence of 44 residues was determined in three separate experiments. The results are shown in Fig. 2 and Table III. Since Peptide CN1 was highly purified, only 1 amino acid residue was found at each step by the sequenator. The repetitive yield was found to be 92%. In each case, the residue determined by gas chromatography of the PTH-derivative was confirmed by back-hydrolysis in HCl. The first 44 residues of Peptide CN1 were established (Table III).

BNPS-Skatole Peptide—The finding of tryptophan in Peptide CN3 prompted us to use BNPS-skatole, a reagent which we found previously (26) very effective in cleaving the tryptophan peptide linkage in the myelin basic protein. A large peptide, obtained by gel filtration of the reaction mixture, was examined directly by sequenator and 33 residues were clearly discerned as shown in Fig. 2 and Table III. It is evident that the bond between Trp-8 and Lys-9 had reacted with BNPS-skatole since the sequence begins precisely with residue 3 (lysine) of Peptide CN3 (Table III). Significant quantities of the BNPS-skatole peptide were produced since the yield of valine (3rd residue) was 36% of the theoretical yield based on the amount of peptide analyzed (Fig. 2).

The sequenator results of the BNPS-skatole peptide cover the 11 remaining residues of Peptide CN3 and 23 residues of Peptide CN1 (Table III). Thus this BNPS-skatole peptide provides an important bridge between Peptides CN3 and CN1.

**DISCUSSION**

The results of this study establish the amino acid sequence of the NH2-terminal region of the P2 protein from rabbit sciatic nerve over 64 residues as shown in Table III. The NH2-terminal residue, blocked as noted previously (15), is N-acetylseryine. The NH2-terminal region is indeed basic, containing 10 basic residues (8 lysine, 2 arginine) and 5 acidic residues (3 aspartic, 2 glutamic acid). Thus the sequence over nearly half the P2 molecule is established. It reveals a prominent hydrophobic region (residue 40 to 51) containing 5 isoleucine and 1 valine residues. At this stage, no other feature appears obviously remarkable. Upon comparison with the myelin basic protein, also found in peripheral nerve myelin where it is referred to as P1 protein (27), no sequence homology is evident. Nor is any sequence homology evident between the P2 protein and partial sequences of the PO protein (28). These data rule out the possibility that the P2 protein could be derived from the PO protein as proposed from other studies (29). Moreover, the acetylated serine residue would render a relationship between the P2 and PO proteins highly improbable; the NH2-terminal region of the PO protein begins with isoleucine and differs markedly from the P2 protein sequence (28).
As in the case of the myelin basic protein (26), the use of BNPS-skatole was extremely helpful in cleaving a tryptophanyl linkage and providing a useful overlapping sequence. The use of BNPS-skatole in effect deblocked the NH₂-terminal region and permitted the sequenator analysis over the region bridging Peptides CN3 and CN1 (Table III). Thus, the large Peptide CN1 is situated between the NH₂-terminal CN3 peptide and the peptides of the CN2 fraction which are localized at the COOH terminus. These studies therefore provide a perspective on the P2 molecule (Table III) in which the 3 methionine residues are localized at the terminal regions; 1 methionine terminates the 20-residue CN3 peptide in the NH₂-terminal region while the other 2 methionyl residues are placed in the COOH-terminal region where the 2 half-cystines also reside. It is now evident that the P2 protein contains approximately 138 residues based on the sum of residues in Peptides CN1, CN2, and CN3 (Table I). A previous estimate (15) showing 127 to 132 residues based on amino acid analysis of the P2 protein and CNBr peptides appears to be slightly low.

Although the P2 protein does not induce allergic encephalomyelitis in guinea pigs (2), it is of interest to compare its tryptophan domain with that in the rabbit basic protein. They are:

(Basic Protein)phe-ser-trp-gly-ala-glu-gly-gln-lys
(P2 Protein)gly-trp-lys-lys-leu-val-ser-ser-glu

It is well established (11) that the 9-residue tryptophan domain of the basic protein shown above contains all of the essential residues for induction of clinical allergic encephalomyelitis in guinea pigs; particularly the tryptophan, glutamine, and lysine residues are crucial (12). It is apparent that these stringent requirements are not met by this tryptophan segment of the P2 protein, since the Gln-Lys is replaced by Ser-Glu in addition to other changes. There is, in fact, no obvious homology. Although the P2 protein contains an additional tryptophan residue, it is evident that the NH₂-terminal region is not an encephalitogenic region for the guinea pig. The P2 protein now becomes one of the few membrane proteins in which details of the amino acid sequence are known. The fairly polar character of the P2 protein, indicated by the 15 charged amino acids over the initial 64 residues, is consistent with the positioning of the P2 protein in the myelin bilayer where it behaves as an extrinsic protein, not penetrating deeply into the lipid leaflet since it can be extracted in part from intact myelin by aqueous solvent. Even the highly polar myelin basic protein requires disruption of the lipid structure by defatting before appreciable extraction is possible. The P2 protein exists in a highly compact conformation having up to 70% β structure (2) and some nonpolar regions, but with considerable polar character which likely limits penetration into the lipid bilayer.

The most important ramifications of the P2 sequence elucidation may be its relevance to allergic neuritis and the human counterpart, the Guillain-Barre syndrome. These diseases (8) show similar clinical signs (limb weakness, paralysis, etc.) and histologic signs such as mononuclear cell infiltration and demyelination found exclusively in the peripheral nervous system. Thus, allergic neuritis is considered an accurate model of the feared, sometimes fatal, human disease particularly as induced in monkeys (8) and more recently in rats (9, 30). A bridge between the two diseases was established in 1974 by Sheremata et al. (31), who demonstrated that peripheral blood lymphocytes in patients with Guillain-Barre syndrome are sensitized to P2 protein as shown by the migration inhibition test, but not to basic protein as found in multiple sclerosis. Thus the P2 protein may play a crucial autoantigenic role in the human disease as do in allergic neuritis. In the latter, we found that the highly purified P2 protein induces severe allergic neuritis in rats but only when injected as a complex with phospholipids, notably phosphatidylserine (9). This finding establishes the P2 protein as the crucial antigen in allergic neuritis, and now permits exploitation of this model to investigate immunologic and chemical parameters of the animal and human diseases.

Brostoff et al. (10) recently reported that a 21-residue CNBr fragment, originating from the NH₂-terminal region of the bovine P2 protein because of a blocked NH₂ terminus, induced both clinical and histologic signs of allergic neuritis in the peripheral nervous system of three out of three rabbits at 0.5-3 mg doses. This peptide appeared free of possible contamination from peptides derived from the myelin basic protein. This "neurotogenic" peptide might correspond to our Peptide CN3, the peptide of 20 residues occupying the NH₂ terminus of the P2 protein. While there is a slight difference in size between the Brostoff peptide and our Peptide CN3, a primary disease-inducing sequence may be localized in this region. Based on the sequence reported here, we anticipate the synthesis of peptides potentially capable of inducing allergic neuritis. Such peptides should prove useful in understanding mechanisms operating in the animal disease, and could have relevance to the investigation of treatment of the human disease.

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