Amino Acid Sequence of Human Myelin Basic Protein Peptide 45–89
As Determined by Mass Spectrometry*

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In order to resolve the uncertainties about the primary structure of human myelin basic protein at residues 45–89, the sequence of this peptide and its tryptic fragments were reinvestigated by fast atom bombardment mass spectrometry. The sequence at positions 77–78 was found to be His-Gly and the sequence at positions 83–84 was shown to be Glu-Asn. The Ser at position 56 was not phosphorylated, whereas the residue at position 46 or 47 showed a heterogeneity of Gly and Ser in this peptide fragment in one of two protein preparations from different patients. These results demonstrate the usefulness of fast atom bombardment mass spectrometry for primary structure information. The corrected sequence of human basic protein peptide 45–89 will permit a more detailed immunochemical analysis of this peptide and its in vivo degradation products.

Myelin basic protein is a cationic protein of 169 amino acids with an approximate molecular weight of 18,500; BP1 constitutes 30% of central nervous system myelin proteins (Carnegie and Moore, 1980; Norton, 1981). BP has been extensively studied because it induces experimental allergic encephalomyelitis (Hashim, 1978). The encephalitogenic determinants of BP for inducing experimental allergic encephalomyelitis differ among different laboratory animals. For example, the peptide encompassing residues 45 through 89 of human BP contains major encephalitogenic sites for the Lewis rat (Kibler et al., 1972), rabbit (Shapira et al., 1971a), and monkey (Kibler et al., 1972). BP is very susceptible to degradation, and fragments of BP, notably material cross-reactive with peptide 45–89, enter the cerebrospinal fluid of humans with acute central nervous system myelin injury as it occurs in exacerbation of multiple sclerosis (Whitaker, 1977; 1980). Due to their potential for inducing an autoimmune response, the subsequent clearance and catabolism of released BP peptides may have disease implications for recurrent neurological disorders such as multiple sclerosis. In attempting to develop immunoassays for the detection of small fragments of human BP peptide 45–89, synthetic small peptides, the structures of which were based on the reported sequence of this peptide (Carnegie, 1971; Shapira et al., 1971b; Carnegie and Moore, 1980), were prepared (Whitaker, 1982). The near “peptide-specificity” of these antibodies (Whitaker, 1982) prompted a re-examination of the primary structure of human BP peptide 45–89.

The complete amino acid sequence for human (Carnegie, 1971) and bovine (Eylar et al., 1971; Brostoff et al., 1974) BP and the partial sequence of BP from other species (Shapira et al., 1971b) have been reported. In human BP peptide 45–89, four areas in the sequence require clarification. The residues at positions 77–78 have been identified as His-Gly (Carnegie, 1971) or Gly-His (Shapira et al., 1971b), and the residues at 83–84 have been reported to be Glu-Asn (Shapira et al., 1971b) or Gln-Asp (Carnegie, 1971). Questions also remain as to the frequency and site of a Gly and Ser heterogeneity at residues 46 or 47 (Chou et al., 1978) and whether the Ser at position 56 is phosphorylated (Carnegie et al., 1974). The present study was undertaken to resolve these uncertainties in the amino acid sequence in human BP peptide 45–89.

MATERIALS AND METHODS AND EXPERIMENTS AND RESULTS

DISCUSSION

Knowledge of the structure of BP has made it possible to examine a number of biological and immunological features of this molecule. Differences in the primary structure of BP may have a marked effect on these biological and immunological properties. For example, certain changes within the encephalitogenic determinant of bovine BP peptide 113–121 recognized by the guinea pig diminish or abolish the encephalitogenic activity whereas modifications elsewhere in the molecule do not (Eylar, 1971). A single amino acid substitution in guinea pig BP of Thr for Ser at residue 79, which is

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** The abbreviation used is: BP, myelin basic protein.

1 The numbering system used is: BP, myelin basic protein.

2 Portions of this paper (including "MATERIALS AND METHODS," "EXPERIMENTS AND RESULTS," Figs. 1–5, and Table 1) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the JBC. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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within the encephalitogenic determinant for the Lewis rat, is associated with a marked decrease in encephalitogenic activity of this peptide region (Kibler et al., 1977). These examples illustrate the importance of defining the primary structure of BP and the present investigation has added information relevant to four areas within human BP peptide 45-89. The variations or heterogeneity in the sequence previously noted (Chou et al., 1978) in peptide 44-49 of a Ser and Gly exchange has been identified to occur at residues 46 or 47 in one of two patients. Thus, some 50% of the peptide isolated from one of two human brains had a Ser at positions 46 or 47, whereas the remaining had the usual Gly. No evidence for phosphorylation of the Ser at position 56 could be found. This residue was reported being phosphorylated by in vivo protein kinases (Carnegie et al., 1974), but studies on the three major microheterogeneous components of bovine BP indicated that phosphorylation occurred in vivo only at Thr 97 and Ser 165 (Chou et al., 1976). BP has a number of sites which can be phosphorylated, and it is a substrate for cyclic AMP-dependent and independent as well as calcium-stimulated phosphorylation (Agrawal et al., 1981; Carnegie et al., 1974; Daile and Carnegie, 1974; Petrali et al., 1980; Turner et al., 1982). Although an endogenously phosphorylated residue was not identified, it is conceivable that BP peptides in vivo may still be phosphorylated. In a previous study unusual features of the elution of tyrosine in the amino acid analysis were recognized (Gilliom et al., 1983), but the mass spectrometric data revealed no evidence for a tyrosine modification. The possibility of a phosphorylated tyrosine was examined, but convincing evidence for such a modification was not obtained.

The most important finding of this study on the primary structure of BP peptide 45-89 are the correct sequences of residues 77-78 and 83-84 of His-Gly and Glu-Asn, respectively. This makes it possible to more carefully examine the immunochemical reactivities of peptides from the large fragments so that immunoassays may be developed for elucidating the metabolism of BP peptides in the body fluids. The corrected sequences at residues 77-78 and 83-84 have a marked influence on the immunochemical reactivities of human BP peptides 69-81 and 80-89 with certain antiserum. These relatively minor corrections of the detailed sequence studies carried out over 10 years ago (Shapiro et al., 1971a, 1971b; Carnegie, 1971) point out that reliance on the composition of tryptic peptides may not permit one to detect a rearrangement of amino acids within peptides and that amide/acid assignments may escape detection. Thus, the information obtained by fast atom bombardment mass spectrometry helps to resolve the primary structure of human BP peptide 45-89 by using techniques heretofore not available (Barber et al., 1981; Gibson and Biemann, 1982; Biemann, 1982). The BP synthesized by two cDNA clones from rat brain contains the glutamic acid-asparagine at residues 83-84 (Roach et al., 1983). The histidine-glycine residues at positions 77-78 are deleted in rat BP (Roach et al., 1983).

These studies of sequences may also have implications regarding further considerations of the conformation and in vivo modification of BP peptides or BP itself. It is apparent that different regions of the BP molecule may serve different roles. For example, in the myelin sheath it has been predicted that the carboxyl half of BP will interact with lipids better than the amino half (Jones and Rumby, 1977). In addition, although the BP molecule itself may have little stable molecular organization (Martenson, 1978), there are regions that seem to have a more developed conformation (Mendz et al., 1986). One of these regions of bovine BP is in the area of residues 79-88, a region whose amino acid sequence had been particularly uncertain. This portion contains areas which are shown by NMR to have greater conformational development than the remainder of the molecule and also to have an antigenic determinant that is either inaccessible or not present in the intact molecule (Chapman and Moore, 1976; Whitaker et al., 1977). Since BP peptides appear to have biological effects such as the stimulation of fibroblasts that are not detected with the BP peptides isolated by the usual acid extraction procedures because of the acid lability of the BP-related growth factors (Gospodarowicz et al., 1982), it is possible that short-lived or labile derivatives of BP and BP peptides exist. With the proper extraction and purification procedures, fast atom bombardment mass spectrometry may afford a means for recognizing these alterations of BP and provide a clearer picture of the accurate in vivo structure of this important myelin protein.

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REFERENCES


* J. N. Whitaker and O. F. Smith, unpublished results.
**SUPPLEMENTARY MATERIAL**

**Amino Acid Sequence of Human Myelin Basic Protein Peptide 45-89**

Determined by Mass Spectrometry

by

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**MATERIALS AND METHODS**

**Sequences**

All chemical reagents were obtained commercial and were spectrally analyzed grade. Trifluoroacetic acid and glycerol were distilled prior to use.

**Preparation of Human BP Peptide 45-89**

Enzymes.

Homogenization of post-mortem tissue was accomplished by frozen and powdered post-mortem tissue from two adult males both of whom had died from non-neurological causes. Myelin was isolated by salt precipitation and extraction at pH 7 followed by ion exchange chromatography on carboxymethyl cellulose at pH 10.6 (Whitaker et al., 1974). A mixture of microheterogeneous components one-three (Chou et al., 1974; Whitaker, 1977) was digested with bovine brain contaminating 2 (Whitaker and Seyer, 1973). BP peptide 45-89 was isolated from the digestion mixture by ion exchange chromatography on carboxymethyl cellulose at pH 3.6, gel filtration on Sephadex G-50 superfine and reversed phase-HPLC (Waters Asaoe.) HPLC column. A linear binary gradient elution program was started with 0.08% trifluoroacetic acid in H2O and increased to 30% CH3CN over 40 min at 1.3 ml/min. The progress of the digestion was monitored by monitoring the absorbance of the effluent at 215 nm, 0.1 absorbance units full scale. At completion the trypsin hydrolysate was precipitated and then dissolved in 10 ml of 50% acetic acid and eluted from a Sephadex G-50 superfine column. The eluate was lyophilized and then dissolved in 10 ml of 50% acetic acid and then in 4N HCl. The digestion mixture was hydrolyzed with trypsin and digested with TFA to isolate the amine active region of 1-2000 at 8°C. The resulting sample was then diluted with 300 ml of 0.08% trifluoroacetic acid and then aspirated into a quartz cuvette and then injected into the HPLC. Three separate fractions were collected and dried in a vacuum centrifuge. The peptide residue was transferred to 3 ml vials and dissolved in 500 cHCl acid and glycerol. PABMS spectra were scanned over a mass range of 1-3000 at 8°C.

**Preparation of 100 µg of human BP peptide 45-89 from the second patient**

was hydrolyzed with trypsin as described above. In this case the subsequent separation by HPLC was optimized using 0.088 trifluoroacetic acid in H2O and eluting the peptides with a linear gradient of 0-30% CH3CN over 40 min at 1.6 ml/min. Sufficient resolution was obtained under these conditions to isolate individual peptides at single peaks to be analyzed by PABMS.

**Molecular Weights of Tryptic Peptides of Human BP Peptide 45-89 as Determined by PABMS**

<table>
<thead>
<tr>
<th>MW</th>
<th>Np</th>
<th>Peptide</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>551</td>
<td>550</td>
<td>Tyr-Leu-Arg</td>
<td>1</td>
</tr>
<tr>
<td>618</td>
<td>600</td>
<td>Unidentified</td>
<td>1</td>
</tr>
<tr>
<td>904</td>
<td>903</td>
<td>Tyr-Arg-Leu</td>
<td>2</td>
</tr>
<tr>
<td>916</td>
<td>910</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>2</td>
</tr>
<tr>
<td>1105</td>
<td>1100</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>2</td>
</tr>
<tr>
<td>1185</td>
<td>1180</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>3</td>
</tr>
<tr>
<td>1200</td>
<td>1200</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>3</td>
</tr>
<tr>
<td>1360</td>
<td>1360</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>1</td>
</tr>
<tr>
<td>1358</td>
<td>1350</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>2</td>
</tr>
<tr>
<td>1400</td>
<td>1400</td>
<td>Tyr-Arg-Leu-Arg</td>
<td>3</td>
</tr>
</tbody>
</table>

PABMS number refers to the peptide designated in Figure 2.

**Figure 2.** The published sequences of human BP peptide 45-89 indicating the sequence of disparity at positions 77-78 and 83-84. The tryptic peptides (Table I; Fraction 1: 1, Fraction 2: 2, Fraction 3: 3) identified from their molecular weight by PABMS are indicated.

The presence of MW=130, Arg(80)-Arg(64), and the absence of an MW=130 ion (Table I; spectrum not shown) which would be expected if Ser(56) were phosphorylated, clearly shows that the Ser(56) is not modified to any appreciable extent. It should be noted that we were unable to isolate a phosphorylated peptide by PABMS that was purified by HPLC using the same conditions as used for isolating fragments from BP peptide 45-89. In that case the

**EXPERIMENTS AND RESULTS**

The PABMS spectra of the total digest from the first patient, separated into three components and detailed by HPLC, showed a number of abundant phosphorylated molecular ions (MW) which were easily correlated with the predicted tryptic peptides (Table I, Fig. 1). These tryptic peptides covered the entire sequence as previously determined (Shapira et al., 1978; Carnegie, 1979) and confirmed the presence of Tyr (69) in its normal (unmodified) form. However, the PABMS spectra provided some additional data which permitted us to resolve the ambiguities in the sequence (Shapira et al., 1978; Carnegie, 1979) and the question of a postulated phosphoserine residue at position 56 (Carnegie et al., 1977).
The amino acid sequence of human myelin basic protein peptide 45-89 is shown on the right. The sequence is presented in a tabular format, indicating the presence of each amino acid residue in the sequence.

The presence of residues 77 and 78 was confirmed by the overlap of the sequence with the peptide sequence of the intact protein. This discrepancy was resolved by re-analysis of the sequence using a different method, which resulted in the correct sequence being determined.

The molecular weight of the peptide was found to be 1184 and 1621, with the dominant fragment ion being labeled as Figure 5. This was further confirmed by the overlap with the sequence of the intact protein, indicating the correct sequence being identified.

In conclusion, the correct sequence of the human myelin basic protein peptide 45-89 has been determined, resolving the discrepancy in the previously published sequence.

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5 B. W. Gibson and K. Biemann, unpublished results.
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