A Reinvestigation of the Amino Acid Sequences of Bovine, Rabbit, Monkey, and Human Myelin Basic Proteins*

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(Received for publication, August 3, 1984)

In order to determine whether bovine, rabbit, and monkey myelin basic proteins (BPs) have the sequence Gly-His or His-Gly at positions corresponding to bovine sequence 76–77, we isolated the tryptic peptides encompassing the sequence in question in these proteins and cleaved them into dipeptides with dipeptidyl aminopeptidase I (EC 3.4.14.1). Analysis by gas chromatography/mass spectrometry of the dipeptides released showed that in no case did His follow Gly or Gly precede His. The identification of peptides Ala-Gln and His-Gly (bovine BP) and Ser-His and Gly-Arg (rabbit and monkey BPs) established the His-Gly sequence. A similar sequence analysis of tryptic peptide (80–91) of human BP confirmed the sequence Thr-Gln-Asp-Glu-Asn-Pro (80–85).

Knowledge of the amino acid sequences of central nervous system BPs from a variety of animal species is obviously essential for understanding the protein’s structure, function, and immunological activity (Martenson, 1984). Complete or partial sequences of BPs from a number of species have been determined. Although several sequencing errors have been noted (Law et al., 1974; Martenson et al., 1981; Gibson et al., 1984; Kira et al., 1984) subsequent to the original publications, there has remained an apparent discrepancy at two positions. In bovine BP, residues 76–77 have been reported as Gly-His (Shapira et al., 1971; Eylar et al., 1971), and the same sequence has been assigned to the corresponding positions of rabbit, monkey, and human BPs (Shapira et al., 1971). However, since studies by other investigators on the sequences of human (Carnegie, 1971; Gibson et al., 1984), mouse (Burgess et al., 1978; Zeller et al., 1984), and pig (Kira et al., 1984) BPs have shown a His-Gly sequence at these positions, we have reinvestigated the sequence encompassing the residues in question in bovine, rabbit, and monkey BPs. We have found that in all three proteins the sequence is His-Gly. We have also corroborated the recent finding (Gibson et al., 1984) that in human BP, the sequence of residues 83–84 is Glu-Asn (Shapira et al., 1971), rather than Gin-Asp (Carnegie, 1971).

MATERIALS AND METHODS

Bovine peptide (43–88), rabbit peptide (45–87), and the homologous Rhesus monkey peptide were prepared by peptic cleavage of the parent BPs as previously described (Martenson et al., 1975, 1981). Each peptide (1.0 mg) was dissolved in 250 μl of 0.1 M NH₄HCO₃ (pH 8.3) and incubated for 22 h at 25 °C with L-1-tosylamido-2-phenyl-ethyl chloromethyl ketone-treated trypsin (Worthington) at an enzyme:peptide ratio of 1:50 by weight. After lyophilization, the digest was dissolved in 50 μl of 0.1% trifluoroacetic acid, and 10 μl (200 μg of digest) was injected onto an Ultrasphere octadecylsilane column (250 × 4.6 mm; 5-μm particle size) with a preceding guard column (20 × 4.5 mm) of the same packing. Separation of the tryptic peptides was achieved with a Varian 5000 high-performance liquid chromatography system, and the eluent was monitored at 210 nm. The following gradients of CH₃CN in 0.1% trifluoroacetic acid at 35 °C (flow rate of 1.0 ml/min) were used: 0–5 min, 0% CH₃CN; 5–75 min, 0–25% CH₃CN; 75–106 min, 25–60% CH₃CN; 106–110 min, 60% CH₃CN; 110–115 min, 60–0% CH₃CN. Bovine peptide (74–88) was eluted at 84.4 min (35.3% CH₃CN) and rabbit peptide (74–87) at 83.8 min (35.3% CH₃CN). In human and monkey BPs, Arg 78-Pro 79 (bovine numbers) is replaced by Arg 79-Thr 80 (human numbers), resulting in tryptic peptide (76–79). Monkey peptide (“76–79”) was eluted at 16.0 min (5.7% CH₃CN). The chromatogram of the tryptic digest of bovine peptide (43–88) is shown in Fig. 1.

Peptide (80–91) of human BP was purified from a similarly prepared tryptic digest of thrombic peptide (1–97) (Law et al., 1984). This tryptic peptide, Thr-Gln-Asp-Gly-Pro-Val-Val-His-Phe-Lys, is the most hydrophobic of the tryptic peptides of peptide (1–97) and is the last one eluted by the gradient described above. To reduce the peptide’s elution time in the present study, the column was equilibrated with 25% CH₃CN in 0.1% trifluoroacetic acid, and a linear gradient to 60% CH₃CN in 30 min was used. All of the other parameters were the same. Peptide (80–91) was eluted as a single peak at 16.0 min (43.6% CH₃CN). None of the other tryptic peptides were completely separated. Bovine peptide (74–88), rabbit peptide (74–87), monkey peptide (“76–79”), and human peptide (80–91) were collected, dried in a Savant Speed Vac concentrator, and subjected to sequence analysis (Krutzsch, 1983). Briefly, 5 nmol of the peptide were cleaved into dipeptides by a 4-h digestion (37 °C) with dipeptidyl aminopeptidase I (EC 3.4.14.1) (E-Y Labs, San Mateo, CA). The dipeptides were trimethylsilylated, separated by gas chromatography, and identified by mass spectrometry.

RESULTS AND DISCUSSION

Treatment of the four tryptic peptides with dipeptidyl aminopeptidase I, followed by gas chromatography of the silylated dipeptides and mass spectrometry, yielded the results shown in Table I. In all cases the dipeptides observed were

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

2 Throughout this paper, where possible, the sequence numbering refers to the actual BP in question, not to an alignment based upon sequence homologies.

those expected from the most recently published sequence data, except that in no case did His follow Gly or Gly precede His. It is clear from these results that residues 76–77 in bovine BP, and the corresponding sequences in rabbit and monkey BPs are all His-Gly. The release of dipeptides Thr-Gln, Asp-Glu, and Asn-Pro from the human tryptic peptide agrees with the sequence Thr-Gln-Asp-Glu-Asn-Pro originally reported (Shapira et al., 1971) and recently verified (Gibson et al., 1984).

Table II shows the alignment of homologous sequences (corrected where necessary) of the region in question of a number of BPs. Chimpanzee BP has been reported to be identical with human BP in this region (Westall et al., 1975) on the basis of amino acid composition data for tryptic peptides (76–79) and (80–91). All of the mammalian BPs so far examined have the invariant sequence Gln-Asp-Glu-Asn. In all of the BPs the remainder of the region is characterized either by amino acid substitutions requiring a single base change in the codon or by deletions involving Gln, His, Gly, or Gly. Since the region has now been shown to contain no Gly-His sequence in any of the BPs, there is no longer any need to suppose that two Gly-\textasciitilde His interchanges occurred or that an additional residue between the Gly and Arg residues exists in some BPs (extant or ancestral) to form a His-Gly-His sequence (Martenson, 1984).

The problems created by the use in immunological studies of synthetic BP peptides that had the wrong sequence have been pointed out (Gibson et al., 1984) in relation to an earlier study (Whitaker, 1982). Similar problems may have also occurred in studies (Day et al. 1981a, 1981b, 1981c) in which antibodies directed against synthetic peptide (64–83) of bovine BP that contained a Gly-His (rather than a His-Gly) sequence failed to cross-react with native BP.

In view of the finding that residue 83 in human BP is Glu, the residue whose \(\gamma\)-CH\(_2\) protons were found to display a chemical shift of 2.235 ppm in NMR spectra of human BP (but not of bovine, porcine, rabbit, or chicken BPs) cannot be Gln 83 as assumed (Mendez et al., 1982) on the basis of the earlier sequence data (Carnegie, 1971). A similarly “unique” resonance with chemical shift of 2.770 ppm was found in proton NMR spectra of porcine BP and thought to have arisen from a Gln residue at the corresponding position (Mendez et al., 1982). Recent studies (Kira et al., 1984), however, have established that in porcine BP this position is occupied by Glu and that an additional Gln residue not present in the other proteins examined by NMR is located in the protein’s carboxyl-terminal region. While it seems reasonable to ascribe tentatively the porcine 2.770 ppm resonance to the latter, there is no such simple explanation for the 2.235 ppm resonance observed in human BP. One possibility is that it arises from Gln 81 (in sequence Thr-Gln), whose chemical environment is likely to be somewhat different from that of the corresponding Gln in the sequence Pro-Gln of bovine, porcine, and rabbit BPs (see Table II).

**TABLE I**

<table>
<thead>
<tr>
<th>Tryptic peptide (74–88)</th>
<th>Dipeptides released by dipeptidyl aminopeptidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine (74–88)</td>
<td>Ala-Gln, His-Gly, Arg-Pro, Gly-Asp, Glu-Asn, Pro-Val, Val-His</td>
</tr>
<tr>
<td>Rabbit (74–87)</td>
<td>Ser-His, Gly-Arg, Pro-Gln</td>
</tr>
<tr>
<td>Monkey (‘76–79’)</td>
<td>Ser-His, Gly-Arg</td>
</tr>
<tr>
<td>Human (80–91)</td>
<td>Thr-Gln, Asp-Glu, Asn-Pro, Val-Val, His-Phe, Phe-Lys</td>
</tr>
</tbody>
</table>

* Strong signal.
* Weak signal.
* Very weak signal.

**TABLE II**

**Alignment of homologous sequences**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine, porcine</td>
<td>Ala-Gln-His-Gly-Arg-Pro</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Ser-( )-His-Gly-Arg-Pro</td>
</tr>
<tr>
<td>Human, monkey, chimpanzee</td>
<td>Ser-Gln-His-Gly-Arg-Thr-Gln-Asp-Glu-Asn</td>
</tr>
<tr>
<td>Mouse</td>
<td>Ser-Gln-( )-Arg-Thr</td>
</tr>
<tr>
<td>Rat</td>
<td>Ser-Gln-( )-Arg-Glu</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Ser-Gln-( )-Arg-Pro-(Asn,Asp,Gly,Amn)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Ser-Gln-His-( )-Arg-Pro</td>
</tr>
</tbody>
</table>

References: bovine, Shapira et al. (1971) and Eylar et al. (1971), corrected; porcine, Kira et al. (1984); rabbit, Shapira et al. (1971), corrected; human, Gibson et al. (1984); monkey, Shapira et al. (1971), corrected; chimpanzee, Westall et al. (1975), corrected; mouse, Burgess et al. (1978) and Zeller et al. (1984); rat, Dunkley and Carnegie (1974) and Roche et al. (1983); guinea pig, Shapira et al. (1971); chicken, Mendez et al. (1982). Parentheses denote residues deleted.
REFERENCES