Biochem. J. (1990) 268, 105–110 (Printed in Great Britain)

Myelin basic protein is affected by reduced synthesis of myelin proteolipid protein in the *jimpy* mouse

Allison M. FANNON* and Mario A. MOSCARELLO

Research Institute, The Hospital for Sick Children, and Department of Clinical Biochemistry, University of Toronto, Toronto, Ont. Canada M5G 1X8

Myelin basic proteins (MBPs) from 6-day-old, 10-day-old, 20-day-old and adult normal mouse brain were compared with those from 20-day-old *jimpy* (dysmyelinating mutant) mouse brain to determine the effect of reduced levels of proteolipid protein (PLP) on MBPs. Alkaline-urea-gel electrophoresis showed that 6-day-old and 10-day-old normal and *jimpy* MBPs lacked charge microheterogeneity, since C8 (the least cationic of the components; not be confused with complement component C8) was the only charge isomer present. In contrast, MBPs from 20-day-old and adult normal mouse brain displayed extensive charge microheterogeneity, having at least eight components. A 32 kDa MBP was the major isoform observed on immunoblots of acid-soluble protein from 6-day-old and 10-day-old normal and 20-day-old *jimpy* mouse brain. There were eight bands present in 20-day-old and adult normal mouse brain. Purified human MBP charge heteromers C1, C2, C3 and C4 reacted strongly with rat 14 kDa MBP antiserum, whereas the reaction with human C8 was weak. This suggested that MBPs from early-myelinating and *jimpy* mice did not react to MBP antisera because C8 was the major charge isomer in these animals. Purification of MBPs from normal and *jimpy* brain by alkaline-gel electrophoresis showed that both normal and *jimpy* MBPs have size heterogeneity when subjected to SDS/PAGE. However, the size isoforms in normal mouse brain (32, 21, 18.5, 17 and 14 kDa) differed from those in *jimpy* brain (32, 21, 20, 17, 15 and 14 kDa) in both size and relative amounts. Amino acid analyses of MBPs from *jimpy* brain showed an increase in glutamic acid, alanine and ornithine, and a decrease in histidine, arginine and proline. The changes in glutamic acid, ornithine and arginine are characteristic of the differences observed in human C8 when compared with C1.

INTRODUCTION

The murine dysmyelinating mutants, such as *jimpy*, provide an excellent tool for the study of developmental aspects of myelin formation. *jimpy* Mice produce only a few whorls of uncompacted myelin (Hogan & Greenfield, 1984) as a result of the premature oligodendrocyte death (Knapp *et al.*, 1986). A single base change (A to G) in the myelin proteolipid protein (PLP) gene, which causes a reduction in the synthesis of PLP (Roussel *et al.*, 1987; Ghandour & Skoff, 1988), has been identified as the primary defect in *jimpy* mice (Macklin *et al.*, 1987; Nave *et al.*, 1987). Via an unidentified mechanism, the defective PLP gene pleiotropically affects both myelin lipid metabolism and protein synthesis, in the latter case reflected by decreased levels of myelin-associated glycoprotein (MAG), 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) and myelin basic protein (MBP) (Campagnoni & Macklin, 1988). The levels of polysomal and nuclear *jimpy* MBP mRNA are reduced to 20–25% of normal (Sorg *et al.*, 1987), correlating with a decreased synthesis *in vitro* of MBP of 26% of normal. Yet immunoblots of *jimpy* brain homogenates and myelin detected 0 and 8% MBP respectively of wild-type levels (Sorg *et al.*, 1986), suggesting rapid turnover of MBP before incorporation into myelin in *jimpy* brain. In addition to specific effects on protein and lipid synthesis, the *jimpy* mutation has generalized effects resulting in an abnormal oligodendrocyte-cell cycle (Knapp & Skoff, 1987) and astrocytic gliosis.

The MBPs account for 30% of the total protein in normal central-nervous-system myelin [reviewed by Campagnoni (1988) and Campagnoni & Macklin (1988)]. They are a group of protein isoforms having both size and charge heterogeneity. A single gene for the MBPs has been localized to chromosome 18 and consists of a total of seven exons spread out over 30–35 kb of DNA. Seven size isoforms of MBP having molecular masses of 32, 23, 21.5, 18.5, 17.25, 17.22 and 14 kDa have been reported in rodent brain. Five of these (21.5–14 kDa) have been shown to arise from the alternative splicing of one or two exons from the MBP gene (Newman *et al.*, 1987). The 21.5, 18.5, 17.22 and 14 kDa MBPs are the major isoforms in rodents, whereas the 23 (Agrawal *et al.*, 1986) and 32 kDa (Chanderkar *et al.*, 1986) variants have been reported in young rodent brain before the active stage of myelination which, in rodents, is 10–20 days. Studies on the developmental expression of MBPs have shown that each size isoform appears to have its own developmental pattern (Barbarese *et al.*, 1978). The significance of developmental regulation of MBP isoforms in myelinogenesis is yet to be understood.

In addition to the size isoforms, MBPs also display charge microheterogeneity which can be observed by an alkaline-urea/gel electrophoresis (Martenson & Gaitonde, 1969). The most cationic charge microheteromer (i.e. has the fastest mobility in this gel system) has been termed component 1 or C1, whereas the least cationic component (i.e. the slowest mobility) has been named C8 (these should not be confused with complement component C8 etc.). Those with intermediate mobilities have been termed C2, C3, C4 etc. C1 is considered to be the least modified component, with the other charge heteromers arising from post-translational modifications, including deamidation, phosphorylation, loss of *C*-terminal arginine, deimination of arginine and oxidation of methionine to methionine sulphoxide (Chou *et al.*, 1976, 1977; Cheifetz *et al.*, 1984; Wood & Moscarello, 1989). Despite extensive characterization of MBP

Abbreviations used: C8, component 8, the least cationic MBP charge isomer (not to be confused with complement component C8); CNPase, 2′3′-cyclic nucleotide 3′-phosphodiesterase; MBP, myelin basic protein; MAG, myelin-associated glycoprotein; PLP, myelin proteolipid protein; TEMED, *NNN′N′*-tetramethylethylenediamine.

* To whom correspondence and reprint requests should be addressed.

charge isomers, their significance in myelinogenesis is only beginning to be examined.

Previous reports characterizing MBPs in *jimpy* mice have focused on MBP synthesis and gene expression. Here we show that the *jimpy* mutation arrests myelination of 20-day-old *jimpy* mice at an age equivalent to 6–10 days in the normal animal. The charge microheterogeneity, size isoforms and the immunoreactivity of MBPs in young (6- and 10-day old) and *jimpy* mice differ from those in mature animals. Whereas 20-day-old and normal adult mouse MBPs display extensive charge microheterogeneity similar to that observed in human MBP, MBPs from early-myelinating mice lack charge microheterogeneity, since C8 was the only charge isomer detected by our methods. ('Early-myelinating' is used to describe those animals which have not reached the age of rapid myelin accumulation and compaction, which in rodents is 10 to 20 days of age. It is not meant to imply that these animals have not yet begun the processes involved in myelination.) Purification of *jimpy* C8 from alkaline gels shows that C8 in *jimpy* contains at least six size isoforms. In addition, amino acid analyses and immunoblots indicate that C8 in early-myelinating mice is similar to human C8. Although MBPs play a major role in myelin stabilization through interactions with myelin lipids, PLP and perhaps among themselves, the function of the various MBP isoforms and charge isomers is not understood.

MATERIALS AND METHODS

Isolation of acid-soluble brain protein

Whole mouse brain was homogenized in 19 vol. of chloroform/methanol (2:1, v/v). After being stirred at 4 °C for 30 min, the suspension was filtered through Whatman no. 1 paper under vacuum. The residue was washed with acetone, then homogenized in 1–2 ml of 0.05 M-H_2SO_4 per brain. The homogenate was stirred overnight at 4 °C to extract completely the acid-soluble protein. After centrifuging at 6350 *g* for 15 min in a JA-20 rotor (Beckman), the supernatant was dialysed against 6 litres of distilled water. The contents of the dialysis bag were filtered through a 0.45 μm-pore-size Pre-Disc Sample Filter (Bio-Rad) attached to a syringe to remove insoluble substances and freeze-dried.

Purification of MBP from acid extract

Alkaline-urea/gel electrophoresis (Deibler *et al.*, 1972) was used to purify MBPs from 6-day- and 10-day-old normal mouse brain and 20-day-old *jimpy* mouse brain. Owing to the pH of the gel systems (10.6), the only acid-soluble proteins which migrated into the gel were the MBPs. The portion of the gel containing the MBPs was homogenized in 1 ml of water; the homogenized gel suspension was removed by centrifuging, whereas the urea and glycine were removed by extensive dialysis against water. Finally the MBPs were concentrated by freeze-drying.

In order to purify MBP from adult mouse brain, a modified alkaline-gel-electrophoresis system was devised. This was necessary because MBPs from adult mouse brain migrate farther into the gel than those from early-myelinating brains. The preparative tube gels consisted of 10% acrylamide, 0.3% bisacrylamide, 10 mM-sodium glycinate, pH 10.6, 0.01% ammonium persulphate and 0.3% *NNN'N'*-tetramethylethylenediamine (TEMED). A 1 mg portion of acid extract, dissolved in 200 μl of 4 M-urea, was applied to each gel and electrophoresed. After electrophoresis for 30 min at 3.75 mA/tube, the top 1.5 cm of the gel, which contained most of the MBPs, was homogenized in 2 ml of distilled water/gel. The gel suspension was clarified by centrifuging, the supernatant was dialysed against 4 l of distilled water and the retentate was freeze-dried.

Other methods

SDS/PAGE was performed by the method of Laemmli (1970) with a Hoefer Mighty Small II gel apparatus. For immunoblotting, protein was transferred to nitrocellulose in a TE Series Transphor Electrophoresis Unit (Hoefer) at 300 mA for 60 min. The protocol outlined in the Bio-Rad Immuno-Blot assay kit was followed. Non-fat milk powder (5%) in 20 mM-Tris/HCl-buffered saline, pH 7.0, was used for blocking. The blot was overlayed for 1 h with a polyclonal antibody IgG fraction (30 mg/ml) against rat 14 kDa MBP (a gift from Dr. J. M. Boggs of the Research Institute, The Hospital for Sick Children). Alkaline phosphatase-conjugated secondary antibody (1:5000 dilution), 5-bromo-4-chloro-3-indolyl phosphate toluidine salt and *p*-nitroblue tetrazolium chloride were used for detection. The BCA protein assay (Pierce) was used for protein determination. For amino acid analyses, samples were hydrolysed in 5.7 M-HCl at 110 °C for 24 h and analysed on the Beckman 7300 physiological system or the Waters PicoTag system.

RESULTS

C8 is the only charge isomer in *jimpy* and early-myelinating mouse brain MBPs

Acid-soluble proteins, extracted from 6-day-old, 10-day-old, 20-day-old and adult normal mouse brain, as well as 20-day-old *jimpy* mouse brain, were subjected to alkaline-urea/gel electrophoresis. Owing to the pH of this system, the MBPs are the only proteins sufficiently cationic to migrate into the gel. Therefore it was not necessary to purify the MBPs from the other acid-soluble proteins before using these gels. The alkaline-urea gels showed that 20-day-old and adult normal mouse MBPs have extensive charge microheterogeneity (Fig. 1, gels 4 and 5), similar to that observed in human MBPs.

In young mice (days 6 and 10 postnatally) that have not yet reached the age of rapid myelin synthesis, only the C8 charge isomer was synthesized (Fig. 1, gels 1 and 2). However, by 10 days of age, trace amounts of MBP charge components begin to appear (Fig. 1, gel 2). Whereas two darkly staining bands were observed in the upper part of the gels at day 6 postnatally, the slower-migrating one was greatly reduced by 10 days. MBPs isolated from 20 day *jimpy* mouse brain also lack charge

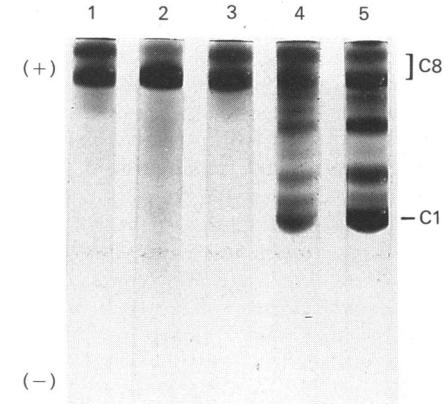

Fig. 1. Charge microheterogeneity of normal and *jimpy* mouse MBPs

Acid-soluble proteins (200 μg) from 6-day-old (gel 1), 10-day-old (gel 2), 20-day-old (gel 4) and adult (gel 5) normal mouse brain and 18–20-day-old *jimpy* mouse brain (gel 3) were separated on alkaline-urea gels. Of the acid-soluble proteins, only MBPs migrate into these gels.

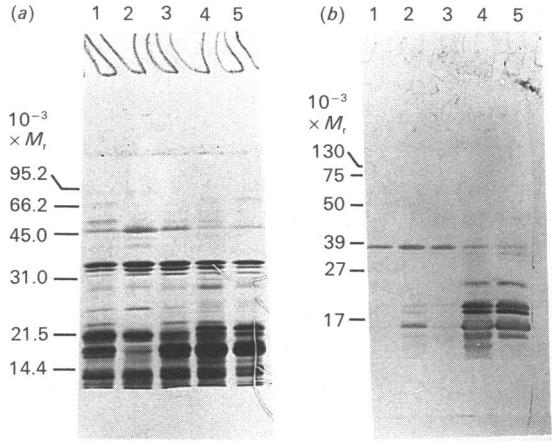

Fig. 2. Immunoreactivity of *jimpy* MBP to rat 14 kDa MBP antibodies

(*a*) A portion (10 μg) of acid-soluble proteins from 6-day-old (lane 1), 10-day-old (lane 2), 20-day-old (lane 4) and adult (lane 5) normal mouse brain and 18–20-day-old *jimpy* mouse brain (lane 3) was separated on a 15% Laemmli gel. (*b*) Immunoblot using an antiserum to rat 14 kDa MBP.

microheterogeneity (Fig. 1, gel 3). In fact MBPs from *jimpy* mouse brain give the same pattern on alkaline-urea gels as those from 6-day-old normal mouse brain (Fig. 1, gel 1).

Immunoreactivity to MBP antisera is reduced in early-myelinating and *jimpy* acid-soluble proteins

Acid-soluble proteins from 6-day-old, 10-day-old, 20-day-old and adult normal mouse brain and 20-day-old *jimpy* mouse brain were separated in a 15%-(w/v)-acrylamide Laemmli (1970) gel (Fig. 2*a*), transferred to nitrocellulose and overlaid with rat 14 kDa MBP antibodies (Fig. 2*b*). In Fig. 2(*a*) the unfractionated acid-soluble proteins from mouse brain showed a number of bands in addition to several proteins in the 14–32 kDa range. Fig. 2(*b*) shows that predominantly one band of about 32 kDa reacts with antibodies to rat 14 kDa MBP in 6-day-old normal (lane 1) and 20-day-old *jimpy* MBPs (lane 3), whereas approximately eight bands are apparent in 20-day-old and adult normal mouse MBPs (lanes 4 and 5). At 10 days of age (lane 2), and to some extent in 20-day-old *jimpy* mice (lane 3), the 18, 17 and 14 kDa MPBs are visible. In addition, the immunoblot shows that the immunoreactivity of the 32 kDa isoform appears to decrease in 20-day-old and adult normal mice, although the amounts appear to be relatively constant when compared after Coomassie Blue staining (Fig. 2*a*). This experiment, using acid-soluble protein from 18–20-day-old *jimpy* and normal adult mouse brain, was repeated with antibodies prepared against bovine, chicken and monkey MBPs with identical results. To test whether the lack of immunoreactivity of *jimpy* MBPs to MBP antisera could be related to the lack of charge microheterogeneity in *jimpy* MBPs, human MBPs, fractionated into charge isomers by CM-52 cation-exchange chromatography (Wood & Moscarello, 1989), were immunoblotted. Fig. 3(*a*) shows a 15% Laemmli gel of purified human MBP charge isomers, C1, C2, C3, C4, C8 and unfractionated MBPs respectively; Fig. 3(*b*) is the corresponding immunoblot. C8 (Figs. 3*a* and 3*b*, lane 5) was applied in greater quantities (5 μg) on the gel than the other components (lanes 1–4, $\approx$ 1 μg each), because C8 does not stain as intensely with Coomassie Blue as do the other components. The immunoblot (Fig. 3*b*) showed that the immunoreactivity of C8 was significantly reduced compared with other MBP components (C1, C2, C3 and C4). The 17 kDa MBP, which is present in C3 and C4, also reacts with the antiserum (Figs. 3*a* and 3*b*, lanes 3 and 4). Thus the presence of only C8 in *jimpy* MBPs may account for the decrease in immunoreactivity of MBPs from *jimpy* and early-myelinating mice to MBP antisera (Fig. 2*b*, lane 2), although further studies are necessary to substantiate this observation.

Purification of MBPs from acid-soluble brain protein

Normal (6- and 10-day-old) and *jimpy* (20-day-old) MBPs were purified from other acid-soluble brain proteins by using alkaline-urea/gel electrophoresis as described in the Materials and methods section. SDS/PAGE of purified MBPs from normal mouse brain showed that only MBPs (Fig. 4*a*, lane 2) were purified from the acid-soluble protein fraction (Fig. 4*a*, lane 1). Comparison of an immunoblot showing the relative amounts of MBPs in the acid extract (Fig. 2*b*, lane 1) and SDS/PAGE of the purified MBPs (Fig. 4*a*, lane 2), indicated that the preparative alkaline gels purified the 21, 18.5, 17 and 14 kDa MBPs in approximately the same relative amounts as found originally in the acid-soluble protein fraction. In addition to the four major

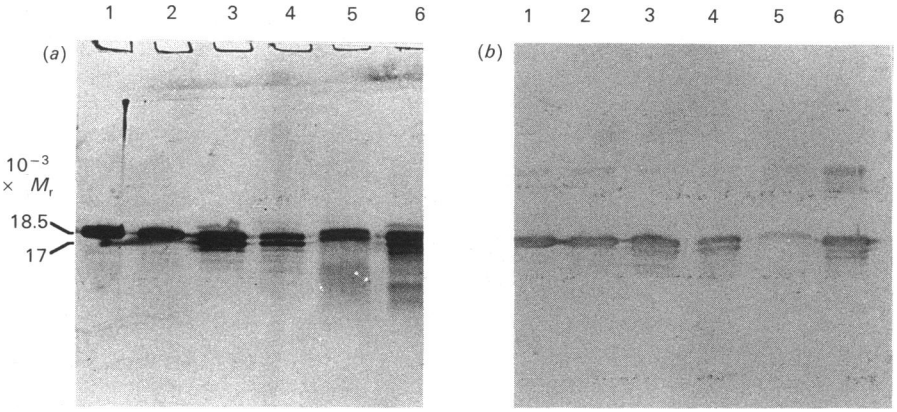

Fig. 3. Immunoreactivity of human MBP charge isomers to MBP antisera

The charge isomers of MBP, purified by CM-52 ion-exchange chromatography, were subjected to electrophoresis on a 15% Laemmli gel (*a*), transferred to nitrocellulose and overlaid with rat 14 kDa MBP antibodies (*b*). Lane 1, C1, 1 μg; lane 2, C2, 1 μg; lane 3, C3, 1.25 μg; lane 4, C4, 1.25 μg; lane 5, C8, 5 μg; lane 6, unfractionated MBP, 3 μg.

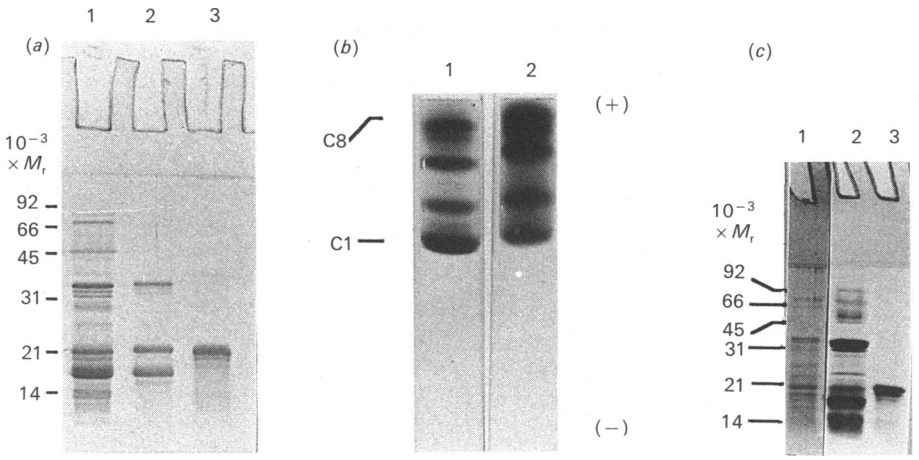

Fig. 4. Purification of *jimpy* mouse MBPs by alkaline-gel electrophoresis

Acid-soluble proteins from *jimpy* mouse brain were purified by alkaline-urea/gel electrophoresis. The upper portion of the gel was homogenized in water, dialysed against water to remove glycine and urea and freeze-dried. (*a*) SDS/15%-PAGE of normal adult mouse MBPs (lane 2, 4 μg) after purification from acid-soluble protein fraction (lane 1, 10 μg) by a modified method of alkaline-gel electrophoresis (see the Materials and methods section). Purified human MBPs (3 μg) are shown in lane 3, showing the dominant 18.5 kDa isoform. (*b*) Analytical alkaline-urea gels of acid-soluble protein (gel 1, 150 μg) and purified MBPs (gel 2, 50 μg) from normal mouse brain showing that the charge microheterogeneity of MBPs is retained after purification. (*c*) SDS/15%-PAGE of 18–20-day-old *jimpy* MBPs (lane 1, 1.5 μg) purified by alkaline-urea/gel electrophoresis; lane 2, *jimpy* acid-soluble protein fraction (10 μg); lane 3, purified human MBPs (3 μg).

isoforms of MBP, the 32 kDa isoform described by Chanderkar *et al.* (1986) was also purified by this procedure.

The charge microheterogeneity was retained when MBPs from normal mouse brain were purified by this method, as shown in the analytical alkaline-urea/gel system of Deibler *et al.* (1972) (Fig. 4*b*). The components of purified normal mouse MBP (Fig. 4*b*, gel 2) were nearly identical with those present in the acid-soluble extract (Fig. 4*b*, gel 1), with the exception that some C8 may have been lost owing to the short electrophoresis time used for the preparative gels. Thus the modified alkaline-gel-electrophoresis system purified normal MBPs from the acid-soluble brain protein fraction without affecting the relative amounts of MBP size isoforms or charge isomers.

Purification of *jimpy* MBPs from acid-soluble brain protein fraction by alkaline-urea/gel electrophoresis resulted in purified C8, since *jimpy* MBPs consist of this charge isomer only. However, SDS/PAGE of the purified *jimpy* C8 (Fig. 4*c*, lane 1) shows that size heterogeneity is present. The size isomers differed from those observed in normal MBPs (Fig. 4*a*, lane 2). Protein bands corresponding to 32, 21, 20, 17, 15 and a small amount of 14 kDa isoforms (Fig. 4*c*, lane 1) are evident in *jimpy* C8, whereas the 32, 21, 18.5, 17 and 14 kDa MBPs are present in normal C8 (Fig. 2*a*, lane 2). In normal 20-day-old mouse brain the 14 kDa isoform is the major MBP size isoform. The reduction in the amount of 14 kDa MBP in *jimpy* mice correlates with the finding by Campagnoni *et al.* (1984) that the level of 14 kDa MBP mRNA was more severely diminished than that of the other MBP mRNAs. In addition, a 15 kDa MBP size variant is present in *jimpy* MBP which is not found in normal MBP. Sorg *et al.* (1986) also noted a 15 kDa MBP size isoform in *jimpy* mice using a cell-free translation system. The 21, 20 and 17 kDa MBP isoform from *jimpy* mice are not present on the immunoblot shown in Fig. 2(*b*). This is most likely due to the weak recognition of C8 by the MBP antibodies. The presence of the abnormal size isoforms in *jimpy* MBPs suggests one of two possibilities: the isoform ratios of *jimpy* MBP reflect those in normal mouse at a very early stage of myelinogenesis or the developmental regulation of MBP isoforms in *jimpy* is abnormal, the former being the most likely.

Amino acid composition of *jimpy* MBPs

Purified MBPs from 6-day-old, 10-day-old, 20-day-old and adult normal and *jimpy* mouse brain were subjected to amino acid analyses in a Beckman 7300 physiological amino acid analyser or the Waters PicoTag system. The amino acid compositions of normal adult MBPs, which represent all charge isomers, and *jimpy* MBPs, which is representative of C8, are shown in Table 1. Changes in the glutamic acid and arginine

Table 1. Amino acid composition of MBPs purified from *jimpy* and normal adult mouse brain

The analyses were performed on the Beckman 7300 physiological amino acid analyser after liquid hydrolysis in 5.7 M-HCl for 24 h at 110 °C. The values for *jimpy* C8 are means ± S.D. for three determinations. Two independent determinations (A and B) for normal MBPs are given.

Amino acid	Composition (residues/100 residues)		
		Adult normal	
	jimpy	A	B
Asp	7.2±3.6	7.0	7.2
Glu	14.6±1.5	8.1	8.3
Ser	9.6±3.8	12.1	11.7
Gly	13.0±5.3	13.8	14.3
His	1.5±0.2	4.0	3.8
Arg	3.6±0.7	9.7	9.6
Thr	5.3±0.8	5.8	6.2
Ala	11.9±2.8	8.2	8.6
Pro	4.0±1.8	9.5	7.6
Tyr	1.9±0.8	1.8	1.8
Val	4.9±0.8	1.8	2.0
Met	1.0±0.4	1.2	1.1
Cys	0.6±0.4	0.0	0.0
Ile	2.5±0.8	1.9	1.9
Leu	6.6±2.6	5.2	5.0
Phe	2.3±1.3	3.9	4.0
Lys	8.5±1.3	5.2	5.9
Orn	1.3±1.5	0.9	0.8

contents are the most significant findings which correlate with differences found between human C1 and C8 (Wood & Moscarello, 1989). The molar percentage of glutamic acid in *jimpy* is increased by approx. 6%, whereas the arginine is reduced by approx. 6%. Ornithine is present in both normal and *jimpy* MBPs, but is somewhat higher in *jimpy* MBPs. Other less pronounced differences between normal and *jimpy* MBPs include an increase in alanine, valine and lysine, and a decrease in proline and histidine. Amino acid analyses of gel-purified MBPs from 6-day-old and 10-day-old normal mice were similar to those for the *jimpy* mouse.

DISCUSSION

Recently the MBP charge isomer, C8, from human myelin was characterized in our laboratory (Wood & Moscarello, 1989). C8 is the least cationic MBP charge isomer, as shown by alkaline-urea/gel electrophoresis and CM-52 cation-exchange chromatography. Specifically, sequence data showed that six arginine residues were deiminated, generating citrulline. In addition to the decreased arginine, an unexplained increase in glutamic acid was observed. These changes decrease the overall positive charge on C8, which was shown to have marked effects on the ability of this MBP to aggregate lipid vesicles (Wood & Moscarello, 1989).

In *jimpy* mice, the PLP gene has a point mutation resulting in the synthesis of greatly reduced amounts of PLP and an arrest of myelination. Here we show that the *jimpy* mutation arrests myelination at an age equivalent to 6 and 10 days of age in the normal animal, affecting MBP synthesis such that only one MBP charge isomer, C8, is formed. Evidence for these observations was provided by amino acid analyses, alkaline-urea/gel electrophoresis and immunoblots.

The amino acid composition of purified *jimpy* C8 has similar changes in arginine and glutamic acid residues to those reported for human C8. Although citrulline was not observed in *jimpy* mouse C8, ornithine was found. Ornithine results from the oxidation of citrulline to ornithine during acid hydrolysis. The high level of glutamic acid found by amino acid analyses is particularly noteworthy, since the changes observed in other amino acids (arginine, proline, histidine and glutamic acid) in *jimpy* MBPs occur in metabolically related ones, that is, arginine, proline, histidine and glutamine are all part of the 'C:5' biosynthetic family, which are converted into glutamate *en route* to α-oxoglutarate. Arginine can be converted into citrulline and ornithine *en route* to glutamic semialdehyde and finally to glutamate.

Additional evidence, which is in agreement with the observation that C8 is the only charge isomer in *jimpy* MBPs, is the weak immunoreactivity of *jimpy* MBPs to MBP antisera. By using purified MBP charge isomers from human brain, we showed that C8 reacted weakly with MBP antisera, whereas C1, C2, C3 and C4 reacted strongly. This observation was supported by recent findings in our laboratory. Antisera to peptide consisting of residues 24–33 of the human MBP sequence, in which the arginine residues at positions 25 and 31 are replaced by citrulline residues, that is, the sequence found in human C8, was strongly reactive with C8, but poorly reactive with C1 (J. McLaurin, personal communication). Finally, MBP from *jimpy* mouse brain migrates to the same position as human C8 by alkaline-urea/gel electrophoresis (results not shown). The data from amino acid analysis, immunoblots and alkaline-urea gel electrophoresis provide convincing evidence that C8 is the only MBP charge isomer synthesized in *jimpy* mouse brain.

The location of the components during myelination is not known. Since 6-day-old and 10-day-old normal and *jimpy* mice have negligible amounts of compact myelin, the C8 in these animals is probably present in oligodendrocytes. However C8 is present in fully compacted myelin in the human (Wood & Moscarello, 1989). It is not understood whether the other charge isomers are derived from C8, for example, through post-translational modifications, or whether they are produced independently. However, it seems unlikely that C1 is derived from membrane-bound C8, because there is no known enzyme that can convert citrulline into arginine when it is in peptide-bonded form.

A number of other studies strongly suggest that C8 is the predominant MBP charge isomer in young animals. MBP isolated from neonatal rats consists entirely of C8 (J. Ramwani, unpublished work). Also, the isolation and characterization of less compact, more dense, myelin fractions revealed that they were enriched in C8 and deficient in the more cationic components C1, C2 and C3 (Cruz & Moscarello, 1985). These two findings suggest that C8 is the major MBP charge isomer in immature and poorly compacted myelin.

The decrease in cationic nature of C8 suggests that the electrostatic and hydrophobic interactions of C8 differ uniquely from those of the other MBP charge isomers. For example, lipid–protein-interaction studies showed that human C8 was capable of inducing aggregation of proteoliposomes containing phosphatidylcholine and lipophilin (human PLP), and that the aggregation was dependent upon the presence of lipophilin (Wood & Moscarello, 1989). Components C1, C2, and C3 failed to induce vesicle aggregation under identical conditions. The MBP–lipophilin interactions in the vesicle-aggregation studies seemed to be hydrophobic in nature. The presence of six citrulline residues in human C8 removes one positive charge at each site, resulting in long stretches of hydrophobic or apolar regions in C8 through which PLP–C8 interactions are possible. In other studies, when lipophilin was purified from white matter, some MBP co-purified with it (Wood & Moscarello, 1989). Characterization of this small amount of MBP by alkaline-urea/gel electrophoresis and column chromatography showed that it behaved like C8 in these systems. In earlier cross-linking studies with the bifunctional reagent, difluorodinitrobenzene, a dimer of the 18.5 kDa MBP and PLP was identified in cat spinal cord by immunochemical methods (Golds & Braun, 1978). Another study by Edwards *et al.* (1989) showed a specific association of proteolipid protein and MBP in ligand-blot-overlay technique. Although the charge isomer of MBP involved was not identified in these studies, it is tempting to speculate that it was C8. Taken together, the abovementioned studies demonstrate that PLP and C8 interaction is not only possible, but probable.

Therefore we suggest that, in normal myelination, the interaction of C8 and PLP could result in a cell-signalling event necessary for myelination. Bartlett *et al.* (1987) showed that *jimpy* oligodendrocytes *in vitro* will revert to a normal phenotype if supplemented with conditioned media from normal oligodendrocyte cultures. In addition, Puckett *et al.* (1987) showed that PLP is present in Schwann cell bodies but not peripheral-nervous-system myelin. The presence of 'central-nervous-system-specific' PLP in the PNS is another indicator that PLP may have an additional role in myelination. The proposed interaction between C8 and PLP, although highly speculative, provides a starting point for the consideration of the functions of the many MBP charge isomers with respect to myelin development.

This work was supported by an operating grant from the Multiple Sclerosis Society of Canada and a studentship, also from the Multiple Sclerosis Society of Canada, to A.M.F.

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Received 30 November 1989; accepted 12 December 1989