Degradation of Amyloid β Protein by Purified Myelin Basic Protein*

Received for publication, July 31, 2009 Published, JBC Papers in Press, August 19, 2009, DOI 10.1074/jbc.M109.050856

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The progressive accumulation of β-amyloid (Aβ) in senile plaques and in the cerebral vasculature is the hallmark of Alzheimer disease and related disorders. Impaired clearance of Aβ from the brain likely contributes to the prevalent sporadic form of Alzheimer disease. Several major pathways for Aβ clearance include receptor-mediated cellular uptake, blood-brain barrier transport, and direct proteolytic degradation. Myelin basic protein (MBP) is the major structural protein component of myelin and plays a functional role in the formation and maintenance of the myelin sheath. MBP possesses endogenous serine protease activity and can undergo autocatalytic cleavage liberating distinct fragments. Recently, we showed that MBP binds Aβ and inhibits Aβ fibril formation (Hoos, M. D., Ahmed, M., Smith, S. O., and Van Nostrand, W. E. (2007) J. Biol. Chem. 282, 9952–9961; Hoos, M. D., Ahmed, M., Smith, S. O., and Van Nostrand, W. E. (2009) Biochemistry 48, 4720–4727). Here we show that Aβ40 and Aβ42 peptides are degraded by purified human brain MBP and recombinant human MBP, but not an MBP fragment that lacks autolytic activity. MBP-mediated Aβ degradation is inhibited by serine protease inhibitors. Similarly, Cos-1 cells expressing MBP degrade exogenous Aβ40 and Aβ42. In addition, we demonstrate that purified MBP also degrades assembled fibrillar Aβ in vitro. Mass spectrometry analysis identified distinct degradation products generated from Aβ digestion by MBP. Lastly, we demonstrate in situ that purified MBP can degrade parenchymal amyloid plaques as well as cerebral vascular amyloid that form in brain tissue of Aβ precursor protein transgenic mice. Together, these findings indicate that purified MBP possesses Aβ degrading activity in vitro.

The progressive accumulation of β-amyloid (Aβ) in senile neuritic plaques and the cerebral vasculature is the hallmark of Alzheimer disease (AD) and is widely used in the pathological diagnosis of the disease. Aβ is generated by proteolytic cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase (1, 2). The main species of Aβ are 40 and 42 amino acids in length. Aβ42 is much more amyloidogenic than Aβ40 because of its two additional hydrophobic amino acids at the carboxyl-terminal end of the peptide (3). The Aβ42 peptide is the predominant form in senile plaques, forming a β-sheet structure, which is insoluble and resistant to proteolysis.

Although increased production of Aβ has been implicated in the onset of familial forms of AD, it has been hypothesized that the more common sporadic forms of AD may be caused by the impaired clearance of Aβ peptides from the CNS. Several major pathways for Aβ clearance have been proposed including receptor-mediated cellular uptake, blood-brain barrier transport into the circulation, and direct proteolytic degradation (4–6). In the latter case, several proteinases or peptidases have been identified that are capable of degrading Aβ, including neprilysin (7, 8), insulin-degrading enzyme (9), the urokinase/tissue plasminogen activator-plasmin system (10), endothelin-converting enzyme (11), angiotensin-converting enzyme (12), gelatinase A (matrix metalloproteinase-2) (13, 14), gelatinase B (matrix metalloproteinase-9) (15), and a-lypeptide hydrolase (16). Each of these enzymes has been shown to cleave Aβ peptides at multiple sites (5). However, only neprilysin, insulin-degrading enzyme, endothelin-converting enzyme, and matrix metalloproteinase-9 have been shown to have a significant role in regulating Aβ levels in the brains of experimental animal models (8, 17, 18).

The “classic” myelin basic proteins (MBP) are major structural components of myelin sheaths accounting for 30% of total myelin protein. There are four different major isoforms generated from alternative splicing with molecular masses of 17.3, 18.5, 20.2, and 21.5 kDa. The 18.5-kDa variant, composed of 180 amino acids including 19 Arg and 12 Lys basic residues, is most abundant in mature myelin (19). One of the major functions of MBP is to hold together the cytoplasmic leaflets of myelin membranes to maintain proper compaction of the myelin sheath through the electrostatic interaction between the positive Arg and Lys residues of MBP and the negatively charged phosphate groups of the membrane lipid (20). MBP plays an important role in the pathology of multiple sclerosis, which is an autoimmune disease characterized by demyelination within white matter (21). Recently, it was reported that purified MBP exhibits autoleavage activity, generating distinct peptide fragments (22). In this study, serine 151 was
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reported as the active site serine residue involved in autocatalysis.

In the early stages of AD, appreciable and diffuse myelin breakdown in the white matter is observed (23). Also, in white matter regions there are much fewer fibrillar amyloid deposits than are commonly found in gray matter regions. Recently, our laboratory has shown that MBP strongly interacts with Aβ peptides and prevents their assembly into mature amyloid fibrils (24, 25). Through the course of these studies we observed that upon longer incubations the levels of Aβ peptides were reduced upon treatment with MBP. In light of this observation, coupled with the report that MBP possesses proteolytic activity, we hypothesized that MBP may degrade Aβ peptides. In the present study, we show that purified human brain MBP and recombinantly expressed human MBP can degrade soluble Aβ40 and Aβ42 peptides in vitro. Purified MBP also degraded fibrillar Aβ in vitro. Mass spectrometry analysis identified distinct degradation products generated from soluble and fibrillar Aβ digestion by MBP. Furthermore, purified MBP degraded parenchymal and vascular fibrillar amyloid deposits in situ in the brain tissue of APP transgenic mice. Together, these findings indicate that purified MBP possesses Aβ degrading activity in vitro.

MATERIALS AND METHODS

Reagents and Chemicals—Synthetic naïve or amino-terminal biotinylated Aβ40 and Aβ42 peptides were synthesized by solid phase Fmoc (N-(9-fluorenylethoxycarbonyl) amino acid chemistry, purified by reverse phase high performance liquid chromatography, and structurally characterized as previously described (26). Phenylmethanesulfonyl fluoride (PMSF), thioflavin-T (ThT), and thioflavin-S (ThS) were purchased from Fluka. Mass spectrometry analysis identified distinct degradation products generated from soluble and fibrillar Aβ digestion by MBP. Furthermore, purified MBP degraded parenchymal and vascular fibrillar amyloid deposits in situ in the brain tissue of APP transgenic mice. Together, these findings indicate that purified MBP possesses Aβ degrading activity in vitro.

Isolation and Purification of MBP from Normal Human Brain White Matter—MBP was purified from normal human white matter tissue as described previously (27). The predominant 18.5-kDa MBP exists as a family of charge isomers that differ in net charge and result from various post-translational modifications. To isolate the individual charge isomers of MBP, the brain homogenates were loaded onto a CM52 cation exchange column, and the components were eluted with a 0–0.2M NaCl gradient. Component 8 was found in the void volume, whereas the more cationic components (C5, C4, C3, C2, and C1) eluted with an increasing salt gradient. The components were dialyzed against water, lyophilized, and stored at −80 °C. The most abundant MBP C1 component was used in all subsequent studies.

Recombinant Human MBP Expression and Purification—The cDNA encoding the 18.5-kDa isoform of human MBP was amplified by PCR using forward (5’-GGAATTTCCATGGCGCT-CACAGAAGAGACC-3’) and reverse (5’-GACTAGTCT-CAGCGTCTAGCCATGGG-3’) oligonucleotide primers. The 516-bp fragment generated was subcloned into the vector pPROEXHT (Invitrogen). This clone allowed production of recombinant human MBP with an amino-terminal His tag (6×-hisMBP). To produce a MBP amino-terminal fragment (MBP1) containing amino acid methionine 1 to phenylalanine 64), the 192-bp fragment was generated by PCR and subcloned into the vector pPROEXHT. The integrity of the MBP1 fragment was confirmed by DNA sequencing. Following transformation into Escherichia coli BL21 (DE3) pLysS (Novagen), 5-ml cultures were grown in LB broth containing 100 μg/ml ampicillin at 37 °C for overnight and then used to inoculate 1-liter cultures for larger scale production. Expression was induced at A600 nm = 0.5 by the addition of isopropyl β-d-thiogalactopyranoside to 0.5 mM, and then growth was allowed to proceed for an additional 3 h. For recombinant MBP protein purification, the bacterial lysate was redissolved in 20 ml of a buffer containing 6 mM urea, 5 mM imidazol, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9, by stirring for 16 h at 4 °C. This material was centrifuged at 6,000 × g for 30 min and then applied to a column containing 1 ml of HisBind resin (Invitrogen). The proteins that were nonspecifically bound were removed by washing with 10 ml of lysate buffer and lysate buffer containing 20 mM imidazole sequentially. Bound recombinant hisMBP proteins were eluted with 1 M imidazole in lysate buffer. The 6×-hisMBP-containing fractions were then dialyzed against with buffer of 6 M urea and 80 mM glycine, pH 10.5 or 9, for full-length MBP or MBP1, respectively, loaded onto a Whatman CM52 column, and eluted with 80 mM glycine buffer, pH 10.5, containing 200 mM NaCl. Protein refolding was achieved by removal of denaturant and salt by slow dialysis into distilled water.

Quantitative Immunoblotting—Samples containing MBP or Aβ were added directly into SDS-PAGE sample buffer and stored at −70 °C. The aliquots were loaded onto 12% or 10–20% polyacrylamide gels, electrophoresed, and transferred onto Hybond-ECL nitrocellulose membranes (Amersham Biosciences) at 100 V for 1.5 h at room temperature. The membranes were blocked in 5% milk, PBS, 0.05% Tween 20 (PBS-T) for 1 h at room temperature. Primary antibodies were added (mAb22 for MBP; Serotec, Raleigh, NC; mAb20.1 for Aβ40 or Aβ42) for 1 h at room temperature and washed three times for 5 min with PBS-T. Secondary horseradish peroxidase-conjugated sheep anti-mouse IgG antibodies were then added to the membranes (1:5000; Amersham Biosciences), which were then washed three times for 5 min with PBS-T. The bands were visualized using the ECL detection method (Amersham Biosciences). Quantitation of MBP or Aβ bands was performed using a VersaDoc Imaging System (Bio-Rad) and the manufacturer’s Quantity OneTon software.

In Vitro Aβ Degradation—Aβ peptides were initially prepared in hexafluoroisopropanol, dried, and resuspended in MeSO to a concentration of 1 mg/ml. 1 μM Aβ40 or Aβ42 was incubated in the absence of presence of 250 mM of purified MBP, hisMBP, or hisMBP1 in PBS buffer with 1 mg/ml of bovine serum albumin at 37 °C for 24 h. The Aβ samples were then analyzed by quantitative immunoblotting as described above.
were collected, and the cell lysates were prepared. The level in biotinylated Aβ peptides in the cell culture media or cell lysate samples was quantitatively analyzed by immunoblotting using streptavidin-horseradish peroxidase. The cell culture lysates were analyzed for MBP expression by immunoblotting as described above.

**In Vitro Fibril Aβ Degradation**—To prepare amyloid fibrils, 5 mM Aβ42 in Me2SO was diluted in PBS to 100 μM, vortexed for 30 s, and incubated at 37 °C for 5 days (28). Triplicate samples of 10 μM of aged fibrillar Aβ were incubated with purified MBP in PBS containing 1 mg/ml bovine serum albumin at 37 °C for 48 h. After digestion, the remaining fibrillar Aβ was quantitated using a ThT fluorescence assay. Briefly, 5 μl of 100 μM ThT was added to 100 μl of sample, mixed, and incubated at room temperature in the dark for 10 min. ThT fluorescence was measured using a ThT fluorescence assay.

**Electron Microscopy**—Sample mixtures were deposited onto carbon-coated copper mesh grids and negatively stained with 2% (w/v) uranyl acetate. The samples were viewed with a FEI Tecnai 12 BioTwin transmission electron microscope, and digital images were taken with an Advanced Microscopy Techniques camera.

**Mass Spectrometry**—Purified soluble MBP or aged fibrillar Aβ42 (10 μM) were incubated in the absence or presence of purified MBP in PBS at 37 °C for 5 days. After incubation, the samples were centrifuged at 14,000 × g for 30 min, and the supernatants were removed, and the pellets were dissolved in 100 μl of PBS. Fluorescence in the pellet, corresponding to remaining fibrillar Aβ, was measured at λex of 480 nm and λem of 515 nm.

Medium supplemented with 10% fetal bovine serum (Gemini Bio-products, Woodland, CA) in 24-well tissue culture plates. Triplicate nearly confluent cultures were infected with Ad-MBP (multiplicity of infection, 250), followed by the addition of 2 μg/ml of amino-terminal biotinylated Aβ40 or Aβ42 in serum-free medium for 48 h. The culture media samples
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**FIGURE 3.** Autolytic activity of recombinant hisMBP and hisMBP1 and in vitro Aβ degradation. A, purified recombinant hisMBP and hisMBP1 were analyzed by SDS-PAGE and Coomassie Blue staining. B, purified recombinant hisMBP and hisMBP1 were incubated at 37 °C for 72 h. The aliquots were analyzed by immunoblotting using the anti-MBP antibody mAb22.

**TOF** mass spectrometer system operated in the reflector mode unless otherwise indicated. The mass scale (m/z 500 – 5000) was calibrated with a mixture of peptides or internal calibration saline and snap-frozen on dry ice. Five-month-old Tg2576 mice after perfusion with cold saline and stained with thioflavin-S, and then imaged with fluorescence microscopy. The parenchymal plaque amyloid or vascular amyloid area of ThS fluorescence was determined using image analysis software (Image J). The fractional area was compared between paired sections.

**Statistical Analysis**—The data were analyzed by Student’s *t* test at the 0.05 significance level.

**RESULTS**

**MBP Autolysis Involves Serine Proteinase Activity**—Recently, purified MBP was reported to possess serine proteinase autolytic activity (22). To confirm this finding, MBP was purified from normal human white matter as described under “Materials and Methods.” For the most abundant MBP C1 component, a high degree of purity was obtained yielding a single protein species of ~18.5 kDa as detected by SDS-PAGE and silver staining (Fig. 1A) and by mass spectrometry (Fig. 1B). To confirm the previously reported serine proteinase autolytic activity, purified human MBP was incubated at 37 °C in the absence or presence of the serine proteinase inhibitors PMSF for 72 h, and aliquots were removed and analyzed by immunoblotting using the anti-MBP antibody mAB22.

As shown in Fig. 1C, MBP exhibited autolysis, which was completely inhibited by treatment with PMSF. These experiments confirmed that purified human brain MBP exhibits serine proteinase autolytic activity. Other purified MBP isoforms, C2 and C3, also exhibited autolytic activity as shown for C1 (data not shown). The MBP C1 isoform was used in all of the subsequent experiments.

**In Vitro Aβ40 and Aβ42 Degradation by Purified MBP**—We have recently shown that MBP strongly binds Aβ peptides and inhibits Aβ fibril formation (24). In light of the endogenous proteinase activity of MBP, we sought to determine whether it could proteolytically degrade Aβ peptides in vitro. Quantitative immunoblotting analysis presented in Fig. 2 shows that incubation with purified MBP resulted in degradation of soluble synthetic Aβ40 and Aβ42 peptides. One nm MBP degraded ~2 nm Aβ40 and ~3 nm Aβ42 per hour. Although the rate of Aβ

**Paired adjacent sections (one incubated with PBS containing 1 mg/ml bovine serum albumin, and the other incubated with 5 μM purified MBP in absence or presence of 1 mM PMSF) in triplicate were incubated at 37 °C for 2 days, stained with thioflavin-S, and then imaged with fluorescence microscopy.**
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hydrolysis was relatively slow, Aβ42 was degraded faster than Aβ40 in the same time period.

Proteolytic Activity and Aβ Degradation Activity of Recombinant 6×-hisMBP—The serine 151 in MBP was reported to be the active site serine involved in its autolysis (22). To test whether serine 151 is the active site involved in autolysis as well as in the Aβ degradation, we produced recombinant wild type and S151A mutant human 6×-hisMBP. The purified recombinant 6×-hisMBP wild type (hisMBP) exhibited similar autolysis and Aβ degrading activity as purified human brain MBP (Fig. 3). However, recombinant 6×-hisMBP-S151A showed similar autolysis and Aβ degrading activity (data not shown), suggesting that this is not the proteolytic active site. To further define the proteolytic activity of MBP, we prepared and purified a recombinant amino-terminal fragment of MBP containing residues 1–64 (hisMBP1). Recent work in our laboratory demonstrated that this region harbors the Aβ binding domain on MBP.4 However, this fragment possesses neither endogenous proteinase activity nor Aβ degrading activity (Fig. 3).

Exogenous Aβ40 and Aβ42 Degradation in Cos-1 Cells Expressing Human MBP—We next determined whether MBP expressed in a cell-based system could degrade soluble Aβ peptides. To do this, we used an adenoviral vector (Ad-MBP) to express MBP in Cos-1 cells, a cell type that does not normally express MBP protein. Immunoblotting confirmed that Cos-1 cells infected with Ad-MBP express MBP, whereas uninfected and adenovirus with green fluorescent protein-infected Cos-1 cells showed no MBP expression (Fig. 4A, top panel). To analyze exogenous Aβ degradation in this system, we used amino-terminally biotinylated Aβ peptides to increase the detection sensitivity. Post infection, 2 μg/ml of soluble biotinylated Aβ40 or Aβ42 was added into the culture media and then incubated for 48 h. Detection of Aβ peptides in the culture media samples using streptavidin-horseradish peroxidase showed that MBP expression in Cos-1 cells resulted in the loss of both Aβ40 and Aβ42 (Fig. 4A, middle and bottom panels, respectively). Quantitation of biotinylated Aβ40 or Aβ42 levels in the culture media of Ad-MBP infected cells showed that they were significantly decreased compared with uninfected or adenovirus with green fluorescent protein infected Cos-1 cells (Fig. 4, B and C). Essentially the same results were obtained using unlabeled Aβ40 and Aβ42 peptides that were detected by quantitative immunoblotting or enzyme-linked immunosorbent assay (data not shown). Finally, quantitation of cell-associated biotinylated Aβ showed no difference in the levels of biotinylated Aβ40 or Aβ42 with the Cos-1 cells under any condition (Fig. 4, D–F). Together, these results suggest that MBP expressed in Cos-1 cells can promote Aβ degradation.

In Vitro Aβ Fibril Degradation—We next determined whether MBP could degrade fibrillar Aβ. As a first approach, 10

4 M. D. Hoos and W. E. Van Nostrand, unpublished data.
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**FIGURE 5. In vitro fibrillar Aβ degradation by purified MBP.** A, fibrillar Aβ42 was incubated in the absence or presence of purified MBP at 37 °C for 48 h. The remaining fibrillar in the samples was quantitated using a ThT (Th-T) fluorescence assay. The data shown are the means ± S.D. of three separate determinations (**, p < 0.01). B, the fluorescence in the pellet of FITC-labeled fibrillar Aβ42 was prepared and incubated in the absence or presence of purified MBP. The remaining fluorescent fibrillar Aβ was pelleted by centrifugation and measured in a fluorimeter. The data shown are the means ± S.D. of three separate determinations, *, p < 0.05. C and D, fibrillar Aβ42 was incubated in the absence (C) or presence (D) of purified MBP at 37 °C for 48 h. The samples were then visualized by transmission electron microscopy. Scale bars, 100 nm.

μM of aged fibrillar Aβ42 was incubated with or without 1 μM purified MBP at 37 °C for 48 h, and the remaining fibrillar Aβ was measured using the ThT fluorescence binding assay. A shown in Fig. 5A, the remaining ThT fluorescence signal was decreased by nearly 50% in the presence of purified MBP. As an alternative approach, fibrillar Aβ was prepared by aging FITC-labeled Aβ42 with unlabeled Aβ42 (at a 1:9 molar ratio) and then incubated with or without 1 μM purified MBP at 37 °C for 48 h. The remaining amyloid fibrils were collected by centrifugation and measured by fluorescence spectroscopy. Similar to the ThT fluorescence binding assay, when incubated with purified MBP fibrillar Aβ decreased by nearly 50% (Fig. 5B). Finally, fibrillar Aβ was incubated with or without purified MBP for 48 h, and then transmission electron microscopy was performed to visualize the remaining fibrillar Aβ at the ultrastructural level. Incubation with purified MBP markedly reduced the extent of fibrillar Aβ (Fig. 5C). Together, these data strongly suggest that MBP can degrade fibrillar Aβ.

**MBP-generated Cleavage Products of Aβ**—Fibrillar Aβ42 was digested with purified MBP to determine specific cleavage products. Aβ fragments were isolated and analyzed by MALDI-TOF mass spectrometry. Nine specific Aβ degradation fragments (Fig. 6C) were observed that were not present in the supernatant of the postincubation fibrillar Aβ preparation alone (Fig. 6A). Similarly, these nine specific Aβ fragments were not present in the postincubation of purified MBP alone, although MBP-specific fragments were observed resulting from MBP autolysis (Fig. 6B). Fig. 6D shows the multiple cleavage sites on fibrillar Aβ for digestion with MBP. We also determined soluble Aβ42 degradation products by MBP and analyzed by MALDI-TOF. For soluble Aβ42, four of the same specific fibrillar Aβ42 degradation peaks were observed; however, the signals obtained were much lower (data not shown).

**MBP Degrades Parenchymal and Vascular Amyloid Deposits in Situ**—The above data showed that purified MBP was capable of degrading soluble and fibrillar synthetic Aβ peptides in vitro. We next determined whether purified MBP could degrade actual amyloid deposits that form in the brains of APP transgenic mice. To do this, adjacent brain slices of aged Tg2576 mice were incubated at 37 °C for 48 h with buffer alone or purified MBP treated with PMSF or left untreated. After incubation the sections were stained with ThS, and the area of fluorescence between matching plaques or vascular amyloid deposits from adjacent sections was measured. The area of ThS fluorescence of adjacent brain sections did not show a difference when incubated with buffer alone, whereas the area of parenchymal and cerebral vascular amyloid deposits was significantly decreased in the brain sections incubated with purified MBP (Figs. 7 and 8, respectively). However, the fibrillar amyloid degradation by purified human MBP was completely inhibited by pretreatment with PMSF. These results demonstrate that purified MBP is capable of degrading fibrillar amyloid deposits in brain and that serine proteinase activity of MBP was involved.

**DISCUSSION**

In the present study, we have identified a novel function of purified MBP as an Aβ peptide degrading enzyme in vitro. First, we confirmed that highly purified MBP from normal human white matter or purified recombinant 6×-hisMBP exhibit serine proteinase autolytic activity. Subsequent experiments revealed that both soluble Aβ40 and Aβ42 peptides were degraded by purified MBP in vitro and in Cos-1 cells expressing MBP. Further, we demonstrated by several means that purified MBP could degrade fibrillar Aβ as well. Mass spectrometry analysis identified multiple MBP cleav-
We showed that purified human MBP could degrade parenchymal and fibrillar amyloid deposits in brain tissue sections of APP transgenic mice. Several lines of evidence argue against the possibility of a contaminating proteinase activity in our purified MBP preparations. First, we purified MBP from normal human white matter with a very high degree of purity as assessed by mass spectrometry and by the observation of a single protein band by high pressure liquid chromatography analysis using SDS-PAGE and silver staining (Fig. 1, A and B). Second, MBP purified from a variety of highly diverse sources including human and mouse brain tissues, Ad-MBP-infected Cos-1 cells, and recombinant expression in E. coli all exhibited similar autolytic and Aβ degrading activity. Lastly, the purified recombinant 6×-hisMBP amino-terminal fragment exhibited a loss in autolysis and Aβ degrading activity. Taken together, these data strongly suggest that contamination of purified MBP with another Aβ-degrading enzyme is highly unlikely.

Serine residue 151 of MBP was previously reported as the active site serine for its proteinase activity (22). However, a purified 6×-hisMBP-S151A mutant still exhibited autolysis and Aβ degrading activity (data not shown). This indicates that Ser151 is likely not the proteolytic active site. Further work is needed to determine the precise proteolytic active site of MBP autolytic and Aβ degrading activity.

Compared with other known Aβ-degrading enzymes such as insulin-degrading enzyme, neprilysin, endothelin-converting enzyme, and plasmin, the Aβ degrading activity of MBP in vitro appears to be quite modest. For example, it was reported that 25 μM Aβ40 was degraded by 50 nM plasmin within 4 h (30). In comparison, we observed that 250 nM MBP could degrade 1 μM Aβ40 or Aβ42 in 24 h (Fig. 2). Perhaps the conditions used in our in vitro Aβ degradation assays do not reflect the optimum environment for MBP to exert its proteolytic activity. MBP is one of the major components of CNS myelin produced by oligodendrocytes in brain (31). Although the total amount of MBP in brain is

**FIGURE 6.** MALDI-TOF mass spectrometry analysis of Aβ fragments released from fibrillar Aβ digestion by purified MBP. A–C, fibrillar Aβ42 alone (A), purified MBP alone (B), or fibrillar Aβ42 and MBP together (C) were incubated 37 °C for 5 days. After incubation the supernatants from the samples were analyzed by MALDI-TOF mass spectrometry. Comparing with fibrillar Aβ42 alone or MBP alone, nine specific peaks were identified as Aβ fragments (reflector mode). D, summary of the MBP cleavage sites on Aβ42. ▲, cleavage sites identified on fibrillar Aβ42; △, cleavage sites identified on soluble Aβ42.
orders of magnitude higher than other Aβ-degrading enzymes, whether MBP exhibits Aβ degrading activity as a component of myelin is presently unknown. However, when demyelination occurs, as in AD and other neurodegenerative conditions, this opportunity may release soluble MBP that is capable of degrading Aβ.

Fibrillar Aβ in plaques and vascular deposits is highly resistant to proteolysis because of its inherent structural nature and modifications that occur such as oxidation and cross-linking (32). Many Aβ-degrading enzymes such as endothelin-converting enzyme, insulin-degrading enzyme, and neprilysin exhibit...
 little or no degradative activity toward fibrillar Aβ. In contrast, plasmin and matrix metalloproteinase-9 have been shown to degrade Aβ fibrils in vitro and amyloid plaques in situ (28). Likewise, we demonstrate that purified MBP also exhibits fibrillar Aβ degradative activity and in situ plaque degradation. Moreover, MALDI-TOF mass spectrometry analysis identified numerous fibrillar Aβ degradation fragments generated by purified MBP consistent with other known Aβ-degrading enzymes that exhibit multiple cleavage sites on Aβ.

MBP is the main structural protein of the myelin sheath and is associated with myelin membrane through interaction between the positive Arg and Lys residues and the negatively charged phosphate groups of the membrane phospholipids (33). It was reported that MBP still undergoes autolysis in the presence of lipid (22). Here we show that MBP expressed in Cos-1 cells could still degrade Aβ40 and Aβ42. Together, these findings suggest that MBP still retains its proteolytic activity in association with lipids or cell membranes, although the detailed enzymatic activity of MBP in vivo needs to be further investigated.

AD is largely considered as a disease of the brain gray matter (34). However, widespread and diffuse myelin breakdown has been reported in AD patients, and white matter deficits are observed in the early stage of the disease (23). It has been reported that MBP levels were significantly decreased in the white matter of AD patients (36, 37). It is noteworthy that the reported that MBP levels were significantly decreased in the white matter of AD patients, and white matter deficits are exhibited in AD patients (35). In any case, potential multiple functions of MBP in inhibition of Aβ fibril assembly, Aβ degradation, and modulation of Aβ mediated cytotoxicity that may play a role in the pathogenesis of amyloid deposition.

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