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Fine mapping of the amyloid β-protein binding site on myelin basic protein

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Abstract

The assembly and deposition of amyloid β-protein (Aβ) in brain is a key pathological feature of Alzheimer's disease and related disorders. Factors have been identified that can either promote or inhibit Aβ assembly in brain. We previously reported that myelin basic protein (MBP) is a potent inhibitor of Aβ fibrillar assembly [Hoos et al. 2007 J. Biol. Chem. 282:9952–9961; Hoos et al. 2009 Biochemistry 48:4720–4727]. Moreover, the region on MBP responsible for this activity was localized to the N-terminal 64 amino acids (MBP_{1-64}) [Liao et al. 2010 J. Biol. Chem. 285:35590–35598]. In the present study we sought to further define the site on MBP_{1-64} involved in this activity. Deletion mapping studies showed that the C-terminal region (residues 54–64) is required for the ability of MBP_{1-64} to bind Aβ and inhibit fibril assembly. Alanine scanning mutagenesis revealed that amino acids K54, R55, G56 and K59 within MBP_{1-64} are important for both Aβ binding and inhibition of fibril assembly as assessed by solid phase binding, thioflavin T binding and fluorescence, and transmission electron microscopy studies. Strong spectral shifts are observed by solution NMR spectroscopy of specific N-terminal residues (E3, R5, D7, E11 and Q15) of Aβ42 upon the interaction with MBP_{1-64}. Although the C-terminal region of MBP_{1-64} is required for interactions with Aβ, a synthetic MBP_{50-64} peptide was itself devoid of activity. These studies identify key residues in MBP and Aβ involved in their interactions and provide structural insight into how MBP regulates Aβ fibrillar assembly.

Extracellular deposition of the amyloid β-protein (Aβ) in brain is a prominent pathological feature of Alzheimer's disease (AD) and a number of related disorders[1,2]. Aβ is a 39–43 amino acid peptide that exhibits a high propensity to self-assemble into β sheet-containing soluble oligomeric forms and fibrils[3,4]. Aβ peptides are proteolytically derived from a large type I integral membrane precursor protein, termed the amyloid β-protein precursor (AβPP)[5–8]. The amyloidogenic processing of AβPP initially involves a proteolytic cleavage at the amino terminus of the Aβ peptide sequence by β-secretase, an aspartyl proteinase named BACE[9–11]. Subsequent proteolytic cleavage of the remaining amyloidogenic membrane spanning AβPP carboxyl terminal fragment by γ-secretase liberates the predominant Aβ40 or Aβ42 residue peptides[12–14]. In AD, cerebral Aβ deposition occurs primarily in the form of parenchymal amyloid plaques[1,2]. The deposition of Aβ peptides

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SUPPORTING INFORMATION
^{1}H-^{15}N HSQC spectra of ^{15}N-labeled Aβ40WT with and without unlabeled MBP_{1-64} demonstrating no changes in the resonances of Aβ40WT upon addition of MBP_{1-64}. This material is available free of charge via the Internet at http://pubs.acs.org.

also occurs in cerebral blood vessels, a condition known as cerebral amyloid angiopathy (CAA)[15–17].

Aβ42 is considered to be more pathogenic due to its stronger ability to assemble into toxic species compared to Aβ40[1,2,4,18]. Further, specific mutations in Aβ, including Dutch E22Q and Iowa D23N substitutions that are associated with familial forms of CAA[19–21], exhibit greatly enhanced the fibrillogenic and pathogenic properties compared to the normal, wild-type (WT) forms of Aβ[22–26]. Monomeric Aβ peptides initially aggregate as low molecular mass oligomeric species that adopt progressive β-sheet content to assemble into higher oligomeric forms, protofibrils, and ultimately amyloid fibrils that deposit in cerebral tissues[4,27–29]. It is likely that different assemblies of Aβ can promote various pathogenic responses that collectively contribute to the syndrome of AD. For example, different soluble oligomeric species of Aβ are directly toxic to neurons, can interfere with long-term potentiation, and disrupt the integrity of cell membranes[4,29–33]. On the other hand, fibrillar assemblies of Aβ are toxic to neuronal and cerebral vascular cells, can activate complement, and can stimulate potent neuroinflammatory responses[34–39]. Understanding the assembly of Aβ is key to unraveling its pathogenesis in AD and related disorders.

A number of naturally occurring Aβ chaperone molecules have been identified in the CNS that modulate fibrillar assembly of the peptide. For example, apolipoprotein E (apoE) may either promote or inhibit Aβ fibril formation *in vitro* dependent on the isoform (E3 vs. E4) and/or the extent of lipidation[40–43]. Further *in vivo* studies in transgenic mice have demonstrated that endogenous mouse apoE facilitates Aβ fibril formation[44,45]. Similarly, apolipoprotein J, otherwise known as clusterin, is another chaperone protein that promotes Aβ fibril formation *in vitro* and *in vivo*[46,47]. Some other reported Aβ chaperones include apolipoprotein A-1[48,49] α_1-anti-chymotrypsin[50,51], transthyretin[52,53] and gangliosides[54,55]. Earlier, we identified myelin basic protein (MBP), an abundant component of the axonal myelin sheath, as a novel chaperone that can bind both WT forms of Aβ and Dutch/Iowa (D/I) CAA mutant forms of Aβ and potently inhibit their fibrillogenesis[56,57]. Detailed ultrastructural analysis showed that MBP allows the assembly of soluble oligomeric species but prevents their further maturation into larger protofibrils and amyloid fibrils[56,57]. Further analysis revealed that the N-terminal residues 1–64 of MBP contained the Aβ binding domain and inhibited Aβ fibrillogenesis in a similar manner as intact MBP[58].

Here we report the further characterization of the Aβ binding site on MBP_{1-64} involved in its fibril inhibiting activity. Deletion mapping studies showed that the C-terminal region of MBP_{1-64} (residues 54–64) is required for its ability to bind Aβ and inhibit fibril assembly. Alanine mutagenesis scanning revealed that amino acids K54, R55, G56 and K59 within MBP_{1-64} are important for both Aβ binding and inhibition of fibril assembly. We further compared the interaction of MBP_{1-64} with Aβ42WT using solution NMR spectroscopy in order to assess the mechanism of the MBP_{1-64} – Aβ interaction and to compare to small molecule inhibitors of Aβ fibrillization. The analysis reveals specific residues in Aβ42WT that shift in response to its interaction with MBP_{1-64}. Although the region MBP_{54-64} is required for interactions with Aβ a synthetic MBP_{50-64} peptide was itself devoid of activity. These studies identify precise residues in MBP that mediate its activities towards Aβ and provide structural insight into how MBP regulates Aβ fibrillogenesis.

MATERIALS AND METHODS

Reagents and Chemicals

Aβ42WT and Aβ40DI peptides were synthesized by solid-phase Fmoc (9-fluorenylmethoxycarbonyl) amino acid chemistry, purified by reverse phase HPLC, and structurally characterized as previously described[59]. Aβ peptides were initially prepared in

hexafluoroisopropanol, lyophilized, and resuspended in either dimethylsulfoxide (Me_2SO) or 100 mM NaOH as previously described[27]. MBP_{50-64} was synthesized and purified to >95% by reverse phase HPLC (China Peptides, Shanghai, China).

Recombinant MBP Peptide Expression

MBP derived peptide gene sequences (MBP_{1-64} and MBP_{1-53}) were cloned into a pTYB11 plasmid vector (New England Biolabs, Ipswich, MA) and transformed into competent *E. coli* BL21 DE3 cells by heat shock. Cells were grown at 37 °C in 1 L cultures of LB broth containing 0.1 mg/ml ampicillin until an optical density of 0.600 AU at 600 nm was reached. Expression of the fusion protein was induced with 0.3 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 26 °C for 18 h. Cells were harvested by centrifugation at 5,000 × *g* for 30 min at 4 °C and cracked in a French press in 20 mM Tris-HCl, pH 9.0/0.5 M NaCl/1 mM EDTA containing Complete Protease Inhibitor (Roche, Mannheim, Germany). Cell lysate was clarified by centrifugation and passed over Chitin Beads (New England Biolabs, Ipswich, MA) equilibrated with 20 mM Tris-HCl, pH 9.0/0.5 M NaCl/1 mM EDTA (EQ buffer). The column was washed with EQ buffer containing 0.05% Triton X-100, and the peptide cleaved and eluted from the intein fusion protein by incubation of the column in 40 mM dithiothreitol (DTT) as per manufacturers instruction. The eluate was diluted 10 fold into 50 mM glycine, pH 9.0 and passed over a CM52 column equilibrated with 50 mM glycine, pH 9.0/50 mM NaCl (CM EQ). The column was washed in CM EQ and eluted with high salt. Fractions were analyzed by SDS-PAGE, pooled, dialyzed against water, lyophilized, and stored at −70 °C.

Site-Directed Mutagenesis of Human MBP_{1-64}

The MBP_{1-64} in pTBY11 vector (plasmid DNA template) was used for alanine scanning site-directed mutagenesis, as per manufacturers instruction (Affymetrix, Santa Clara, CA), to produce MBP_{1-64} with the following mutated residues ($K^{54}A$, $R^{55}A$, $G^{56}A$, $S^{57}A$, $G^{58}A$, $D^{60}A$, $S^{61}A$, $H^{62}A$, and $H^{63}A$). The mutant MBP_{1-64} peptides were expressed and purified as described above.

Solid Phase Binding Assay

Lyophilized Aβ40D/I and Aβ42WT peptides were resuspended with Me_2SO to 2.5 mM, diluted to 12.5 μM in 50 μl μl of PBS, and then coated on flat bottom 96 well plates (Fisher Scientific, Pittsburgh, PA) by incubation at 37 °C for 18h. Each well was blocked in 100 μl of 1% BSA/PBS for 1 h at RT. Then 1.56 μM of purified recombinant MBP peptides in 50 μl PBS was added to each well and incubated at 4 °C overnight. After washing 3 × 5 min with 1% BSA/PBS/0.05% Tween20 (PBS-T), rat monoclonal antibody to MBP (1:1000; AbD Serotec, Raleigh, NC) in PBS-T was added for 1 h at RT. Wells were washed 3 × 5 min with PBS-T. Secondary horseradish peroxidase-conjugated goat anti-rat IgG was then added to each well (1:5000; GE Healthcare, Buckinghamshire, UK), which were then washed 3 × 5 min with 1% BSA/PBS-T. SureBlue TMB microwell peroxidase substrate (KPL, Gaithersburg, MD) was added, developed, and the reaction was terminated by adding 1N HCl. Absorbance of the samples was measured at a wavelength of 450 nm in a SpectraMax spectrofluorometer (Molecular Devices, Sunnyvale, CA) using SoftMax Pro control software.

Thioflavin T Fluorescence Assay

Lyophilized Aβ40D/I peptide was first resuspended with Me_2SO to 2.5 mM, diluted to 12.5 μM in PBS, and then incubated at 37 °C with shaking either alone or with 1.56 μM MBP peptides. Control samples containing 0.5% Me_2SO and 1.56 μM MBP peptides in PBS were also included. At each time point, 100 μl samples of each reaction were placed in a 96-well

microplate in triplicate and 10 μl of 100 μM thioflavin T was added. The plate was mixed for 5 sec and incubated at 25 °C in the dark for 10 min before each reading. Fluorescence was measured at 25 °C at 490 nm using an excitation wavelength of 446 nm in a SpectraMax spectrofluorometer (Molecular Devices, Sunnyvale, CA) using SoftMax Pro control software.

Transmission Electron Microscopy

Sample mixtures were deposited onto carbon-coated copper mesh grids (EM Sciences, Hatfield, PA) 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 AMT camera.

Solution NMR Spectroscopy

^{15}N-labeled Aβ42WT was dissolved in 100 mM NaOH to a concentration of 2 mM. For NMR measurements, aliquots of this stock solution were diluted to 100 μM with low salt buffer containing 10% D_2O. The pH was adjusted to 7.4 with dilute HCl, and the sample was adjusted to a total final volume of 400 μL. Lyophilized MBP_{1-64} and MBP_{50-64} were dissolved in distilled, deionized water to a concentration of 5 mM. The MBP_{1-64} and MBP_{50-64} stocks were diluted to a concentration of 100 μM in low salt buffer with 10% D_2O. The pH was adjusted to 7.4 with dilute HCl, and the sample was adjusted to a total final volume of 400 μL.

Solution NMR spectra were obtained at 4 °C on a 700 MHz Bruker Avance spectrometer. ^{1}H spectra of the 100 μM MBP_{1-64}, MBP_{50-64} and Aβ42WT samples were acquired and compared to ensure that all of the samples were at the same concentration before mixing. ^{1}H-^{15}N HSQC spectra were obtained of 50 μM ^{15}N-Aβ42WT and 50 μM MBP_{1-64} or 50 μM MBP_{50-64} after mixing of the 100 μM samples.

RESULTS

Residues 54–64 of MBP_{1-64} are required for its interactions with Aβ peptides

Previously, we reported that MBP binds Aβ peptides and inhibits their assembly into fibrils[56-58]. Moreover, we showed that the Aβ binding region on MBP resides within the N-terminal residues MBP_{1-64}[58]. To further identify the region responsible for its interactions with Aβ peptides we performed deletion analyses on MBP_{1-64}. Recombinant MBP_{1-64} and MBP_{1-53} were expressed and purified (Fig. 1A). Since our earlier studies showed that MBP interacts most strongly with highly fibrillogenic peptides such as Aβ40D/I and Aβ42WT we chose to use these in our analyses[56,57]. Solid-phase binding assays showed that compared to MBP_{1-64} the C-terminal deleted MBP_{1-53} was largely devoid of binding to immobilized Aβ40D/I and Aβ42WT peptides (Fig. 1B). Similarly, MBP_{1-53} was largely ineffective in blocking the fibrillar assembly of Aβ40D/I as assessed by thioflavin T binding and fluorescence (Fig. 1C) and TEM analysis of fibril structure (Fig. 1F). In contrast, similar studies showed that deletion of the first ten N-terminal residues of MBP_{1-64} (MBP_{11-64}) had minimal impact on its ability to inhibit Aβ fibrillar assembly (data not shown).

Residues K54, R55, G56, and K59 mediate MBP1-64 binding to Aβ and inhibition of fibril assembly

Since the region 54–64 is required for MBP_{1-64} interactions with Aβ we next performed an alanine scanning mutagenesis analysis of this region to identify specific amino acids that are important for these activities. First we conducted solid phase binding experiments to measure the interactions of MBP_{1-64} mutants with immobilized Aβ40D/I and Aβ42WT peptides as we previously described[58]. The binding data show that in MBP_{1-64} the

sequential residues K54A, R55A, and G56A as well as K59A and H63A markedly reduced binding to Aβ40D/I (Fig. 2A). On the other hand, residues S57A, G58A, D60A, S61A, and H62A had much less or no effect on MBP_{1-64} binding to Aβ40D/I. A similar pattern was observed when analyzing the binding of MBP_{1-64} mutants to Aβ42WT suggesting that the same residues in MBP are involved with binding to both of these fibrillogenic forms of Aβ.

We next investigated how each of the specific alanine mutants of MBP_{1-64} affected the inhibition of Aβ fibril assembly. In this case we focused on the Aβ40D/I peptide as it more rapidly assembles into Aβ fibrils compared to Aβ42WT. Similar to the results obtained in the binding experiments we found that in MBP_{1-64} the sequential residues K54A, R55A, and G56A as well as K59A substantially reduced its ability to inhibit Aβ fibril assembly (Fig. 2B). Although the H63A mutant showed reduced binding to immobilized Aβ peptides (Fig. 2A), it did not show a significant effect on interfering with Aβ fibril assembly. However, this may be a consequence of the different methodologies using immobilized Aβ for binding and Aβ in solution for fibril assembly.

To directly confirm the fibril inhibition results obtained in the thioflavin T binding and fluorescence experiments, we performed TEM analysis of Aβ40D/I assembly in the absence or presence of select MBP_{1-64} mutants. The K54A mutant, with markedly reduced Aβ binding and fibril assembly inhibiting activity, allowed for assembly of abundant, mature Aβ fibrils (Fig. 2D). Alternatively, the S57A mutant, with no appreciable effect on either Aβ binding or inhibition of fibril assembly, effectively blocked the assembly of mature Aβ fibrils (Fig. 2E). Similar corresponding TEM results were obtained with other MBP_{1-64} alanine mutants (data not shown). Together, these results are consistent in that they implicate MBP residues K54, R55, G56, and K59 as important for the ability of MBP to bind Aβ and inhibit fibril assembly.

Specific residues in Aβ42WT shift upon interaction with MBP_{1-64}

Solution NMR spectroscopy was undertaken to localize the positions on Aβ42WT interacting with MBP_{1-64}. Aβ42WT was chosen for this analysis as MBP_{1-64} binds to this peptide and inhibits it fibrillar assembly[57] Aβ42WT can be stabilized at low temperature (4 °C) in a largely monomeric form that associates, as the temperature is increased, to low MW oligomers in the process of forming higher MW oligomers, protofibrils and fibrils[60,61]. There is a growing body of literature describing the intermediates in the oligomerization pathway of Aβ42WT and the ability of small molecule inhibitors to interact with specific oligomers[62]. One of the striking features of the MBP_{1-64} – Aβ interaction is the ability of the MBP protein to inhibit fibril formation at sub-stoichiometric ratios of MBP to Aβ.

Fig. 3 presents the ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled Aβ42WT with (red) and without (black) unlabeled MBP_{1-64}. The largest shifts are observed in the N-terminus and central portion of Aβ42WT. In the N-terminus, shifts are observed in the resonances corresponding to negatively charged residues (E3, D7 and E11), as well as F4 and R5. In the central portion of Aβ42WT, shifts are observed in H14-L17. The shifts in Q15 and L17, as well as R5, are similar to those observed with small molecule inhibitors (curcumin and resveratrol) of Aβ assembly[63]. Resonances in the hydrophobic C-terminus of Aβ42WT are largely unaffected by binding of MBP_{1-64}. In contrast, the ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled Aβ40WT, which exhibits weak interaction with MBP[56,57], produced no appreciable shifts in residue resonances in the presence of unlabeled MBP_{1-64} (Figure S1 of the Supporting Information).

MBP_{50-64} is insufficient for Aβ binding and inhibition of fibril assembly

Since MBP residues 54–64 appear to be important for both Aβ binding and inhibition of fibril assembly as shown above we next determined if a small peptide corresponding to this

region was sufficient for these activities. A synthetic MBP_{50-64} peptide was prepared and tested for its ability to interact with Aβ and inhibit Aβ fibril assembly. MBP_{50-64} induced no appreciable changes in the ^{15}N-HSQC NMR spectrum of Aβ42WT when the two peptides were co-mixed (Fig. 4A). Similarly, MBP_{50-64} was ineffective at inhibiting Aβ fibril assembly as assessed by thioflavin T binding and fluorescence (Fig. 4B) and TEM analysis (Fig. 4D). Together, these findings indicate that although residues 54–64 are required for MBP_{1-64} to bind Aβ and inhibit fibril assembly a small peptide encompassing these residues was not sufficient to elicit these activities on its own.

DISCUSSION

The assembly and deposition of Aβ in brain is a key feature of the pathology of AD and related disorders. Thus, endogenous cerebral Aβ chaperones that influence the assembly process can have a marked impact on the pathogenesis of disease affecting both the onset and spatial location of Aβ deposition. For example, the chaperone ApoE4 can decrease the age of onset, increase the severity, and promote cerebral vascular deposition of fibrillar amyloid[64]. Therefore, elucidating the composition of Aβ chaperones in the brain and their respective mechanisms of action will provide a more complete understanding of how Aβ pathology develops and also offer opportunities for intervention.

Previously, we demonstrated that MBP is a novel brain Aβ chaperone that strongly binds Aβ peptides and potently inhibits their assembly into mature amyloid fibrils[56,57]. Subsequently, it was shown that its activity as an inhibitor was localized to the N-terminal residues 1–64 of MBP, was independent of MBP post-translational modifications, and protected cultured primary neurons from the toxic effects of Aβ[58]. Furthermore, both in human brain and in human AβPP transgenic mouse brain regions of white matter, which are rich in MBP, are largely devoid of fibrillar Aβ deposits[56,65,66,]. Thus, identifying the precise region on MBP responsible for its interactions with Aβ provides insight into its mechanism of action and establishes a framework for comparison to other modulators of Aβ assembly.

In the present study we used deletion mapping to show that the C-terminal region of MBP_{1-64} (residues 54–64) is required for its ability to bind Aβ and inhibit fibril assembly. Deletion of this region disrupted the interaction with both Aβ42WT and the familial CAA mutant Aβ40D/I, two highly fibrillogenic forms of Aβ (Fig. 1). Subsequent site-directed mutagenesis studies in this region identified residues K54, R55, G56 and K59 as important for the ability of MBP_{1-64} to bind Aβ and inhibit fibrillar assembly (Fig. 2). On the other hand, solution NMR studies identified several residues, including E3, R5, D7, E11 and Q15, on Aβ42WT that exhibit large spectral shifts upon interacting with MBP_{1-64} (Fig. 3). Together, these findings reveal specific sites on MBP and Aβ that appear key to their interactions with each other.

As its name implies, MBP is a strongly cationic protein with an isoelectric point of >11.0. Accordingly, MBP_{1-64} is also highly cationic with an isoelectric point of ≈11.5. This observation suggests that the interaction of MBP and its derived fragments with Aβ peptides may be purely ionic in nature. However, this assumption does not agree with several key findings. First, MBP and MBP_{1-64} interact most strongly with Dutch (E22Q)/Iowa (D23N) CAA mutant Aβ where there is a loss of two negatively charged amino acids increasing the isoelectric point from about 5.3 to 6.0[56]. Second, the MBP_{50-64} peptide, which has highly basic net charge (isoelectric point > 11.0) and is required for MBP_{1-64} to interact with Aβ, is itself incapable of influencing fibril assembly (Fig. 4). Finally, eosinophilic cationic protein (ECP), an unrelated protein with a size and isoelectric point very similar to MBP[67], did not inhibit Aβ assembly into fibrils (data not shown).

Nevertheless, at a certain level ionic interactions between MBP and Aβ do appear to be involved since mutation of the positively charged residues K54, R55 and K59 in MBP_{1-64} markedly impair both binding to Aβ and inhibition of fibril assembly (Fig. 2). Also, the NMR results show interaction of MBP_{1-64} with the negatively charged N-terminus of the Aβ42WT monomer stabilized at 4 °C (Fig. 3). Our earlier work showed that MBP appears to inhibit Aβ fibrillar assembly at the level of an oligomer[56–58]. Using atomic force microscopy it was demonstrated that both MBP and MBP_{1-64} allow the initial assembly of shorter low MW oligomers/protofibrils that are further stunted and capped at a height of ≈2 nm[56–58]. Importantly, the mechanism in which MBP interacts with the monomer, yet allows formation of low MW oligomers is consistent with the reported substoichiometric inhibition of Aβ assembly by MBP and its active fragments[56,57]. Although MBP and MBP_{1-64} are largely unstructured in solution, the present data suggest a model where the positively charged region MBP_{54-64} interacts with the negatively charged N-terminus of Aβ and wraps around the oligomer allowing another upstream region of MBP to cap further assembly of the oligomer into larger protofibril/fibril structures.

This type of “capping” phenomenon is also observed with designed peptides and small molecule inhibitors of Aβ fibrillar assembly[68]. Although the C-terminal region (residues 54–64) of MBP_{1-64} is required for Aβ binding and inhibition of fibril assembly, a small peptide corresponding to this region was essentially inactive (Fig. 4). This observation supports the likely need for upstream elements of MBP for these activities and suggests that the future design of peptides or other molecules with various linkers may be key to development of effective inhibitors of pathogenic Aβ assembly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Aβ	amyloid β-protein
AD	Alzheimer’s disease
MBP	myelin basic protein
AβPP	amyloid β-protein precursor
CAA	cerebral amyloid angiopathy
Aβ42WT	wild-type Aβ42 peptide
Aβ40D/I	Dutch/Iowa CAA double mutant Aβ40 peptide
PBS	phosphate-buffered saline
BSA	bovine serum albumin
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TEM	transmission electron microscopy, NMR, nuclear magnetic resonance

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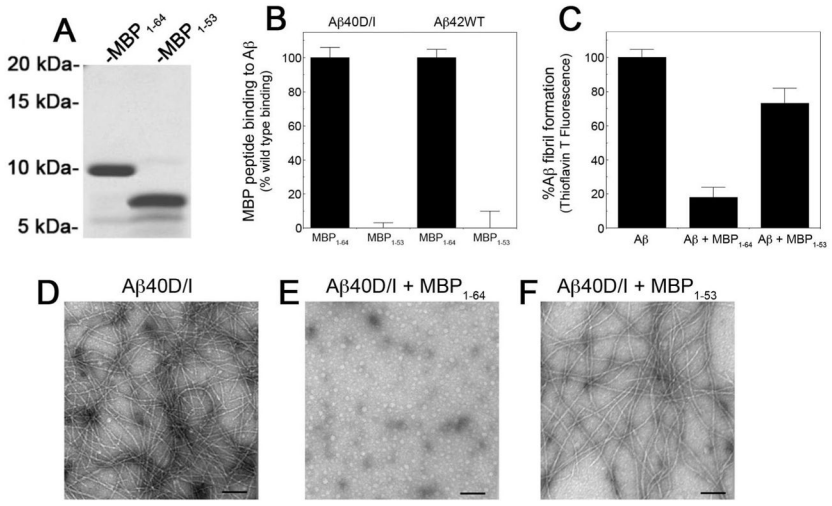

FIGURE 1.

Residues 54–64 of MBP_{1-64} are required for its interactions with Aβ peptides. **A**. MBP_{1-64} (first lane) and MBP_{1-53} (second lane) were recombinantly expressed, purified, and analyzed by SDS-PAGE. **B.** The interaction of purified MBP_{1-64} and MBP_{1-53} with Aβ40DI or Aβ42WT was analyzed by solid phase binding assay. **C.** The inhibition of Aβ40DI (12.5 μM) fibrillogenesis by purified MBP_{1-64} (1.56 μM) and MBP_{1-53} (1.56 μM) was assessed by thioflavin T binding and fluorescence. **D–F.** TEM analysis of Aβ40DI in the absence (D) or presence of purified MBP_{1-64} (**E**) or purified MBP_{1-53} (**F**). Scale bars = 100 nm. MBP_{1-64}, but not MBP_{1-53}, bound to Aβ and inhibited its fibrillar assembly.

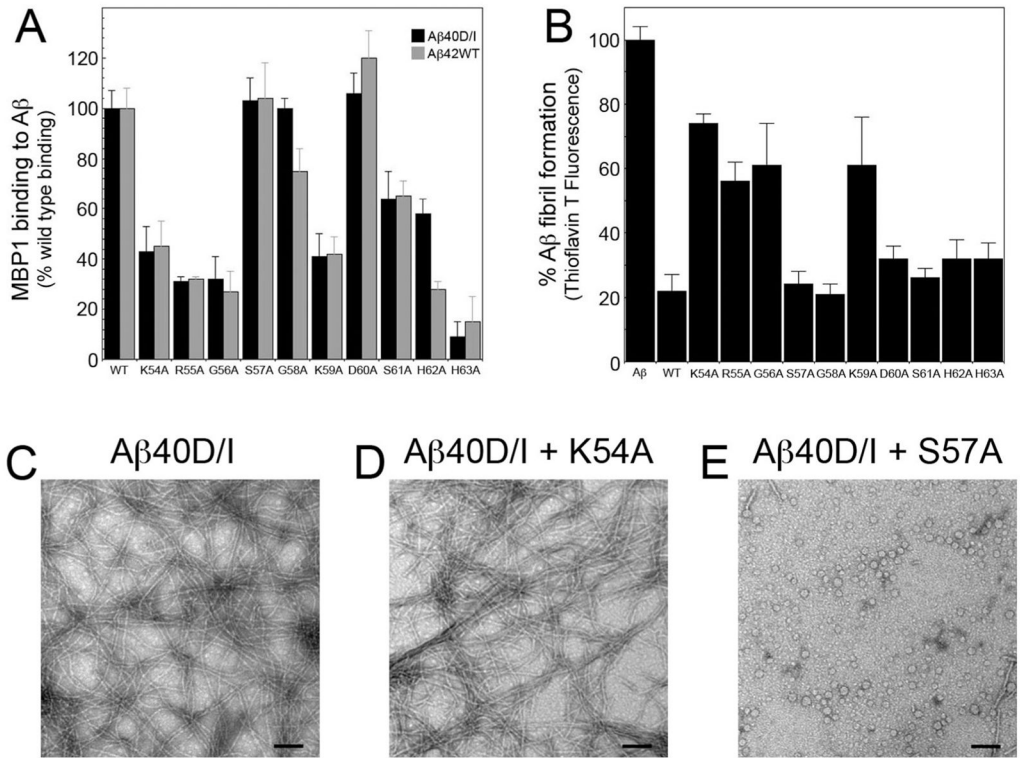

FIGURE 2.

Alanine scanning mutagenesis analysis of residues 54–64 of MBP_{1-64}. **A**. The interaction of recombinantly expressed and purified MBP_{1-64} alanine mutants with Aβ40D/I and Aβ42WT was analyzed by solid phase binding assay. **B**. The inhibition of Aβ40D/I (12.5 μM) fibrillogenesis by purified MBP_{1-64} alanine mutants (1.56 μM) was assessed by thioflavin T binding and fluorescence. **D–F**. TEM analysis of Aβ40D/I in the absence (D) or presence of purified K54A mutant MBP_{1-64} (**E**) or purified S57A mutant MBP_{1-64} (**F**). Scale bars = 100 nm. Mutation of residues K54, R55, G56 and K59 markedly impaired the ability of MBP_{1-64} to bind Aβ and inhibit its fibrillar assembly.

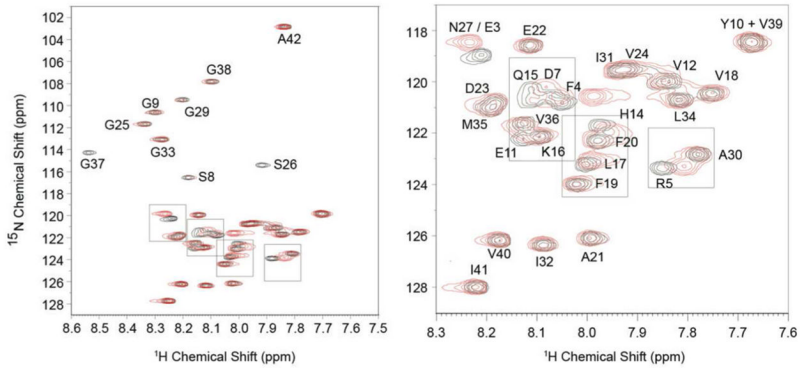

FIGURE 3.

Specific Aβ42WT residue interactions with MBP_{1-64}. ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled Aβ42WT with (red) and without (black) unlabeled MBP_{1-64}. The largest shifts are observed in three regions of Aβ42WT: the N-terminus (E3-D7 and E11) and in two regions within the central portion of the peptide (H14-L17 and S26-G28). In addition, intensity is lost in the resonance to Gly37 in the hydrophobic C-terminus of Aβ42WT.

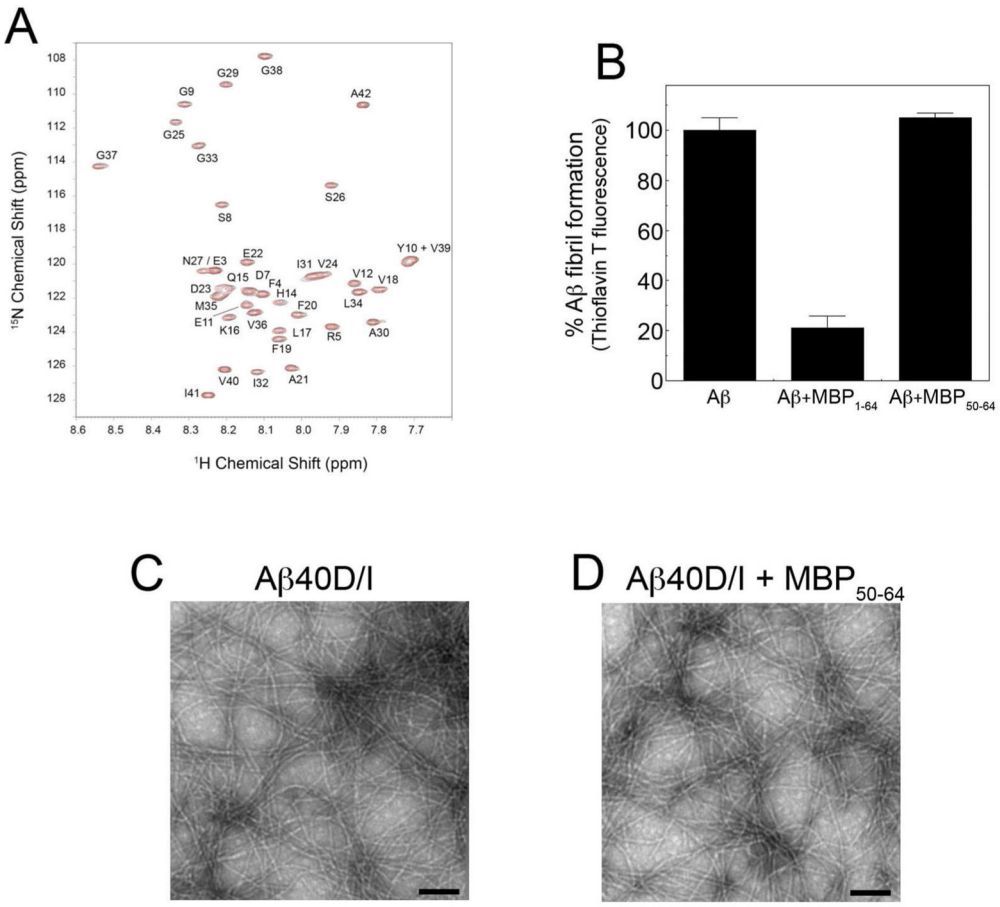

FIGURE 4.

MBP_{50-64} is insufficient for Aβ binding and inhibition of fibril assembly. **A**. ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled Aβ42WT with (red) and without (black) unlabeled MBP_{50-64}. No significant spectral shifts are observed. **B**. The inhibition of Aβ40D/I (12.5 μM) fibrillogenesis by purified MBP_{1-64} (1.56 μM) or MBP_{50-64} (1.56 μM) was assessed by thioflavin T binding and fluorescence. **C,D**. TEM analysis of Aβ40D/I in the absence (**C**) or presence of purified MBP_{50-64} (**D**). Scale bars = 100 nm. MBP_{50-64} did not bind Aβ or inhibit its fibrillar assembly.