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Fine Mapping of the Amyloid β-Protein Binding Site on Myelin Basic Protein

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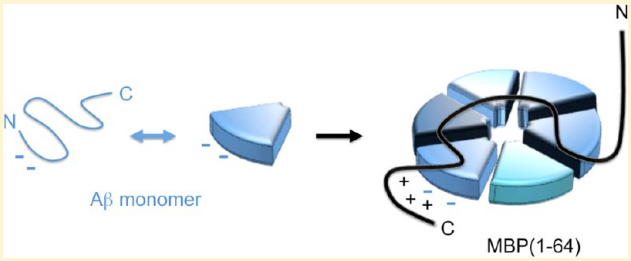
Supporting Information

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, M. D., et al. (2007) *J. Biol. Chem. 282*, 9952−9961; Hoos, M. D., et al. (2009) *Biochemistry 48*, 4720−4727]. Moreover, the region on MBP responsible for this activity was localized to the 64 N-terminal amino acids (MBP_{1-64}) [Liao, M. C., et al. (2010) *J. Biol. Chem. 285*, 35590−35598]. In the study presented here, 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 nuclear magnetic resonance 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 peptides.[12−14] In AD, cerebral Aβ deposition occurs primarily in the form of parenchymal amyloid plaques.[1,2] The deposition of Aβ peptides also occurs in cerebral blood vessels, a condition known as cerebral amyloid angiopathy (CAA).[15−17]

Aβ42 is considered to be more pathogenic because of its stronger ability to assemble into toxic species compared to that of 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] greatly enhanced the fibrillogenic and pathogenic properties compared to those of 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-order oligomeric forms, protofibrils, and ultimately amyloid fibrils that are deposited 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 central nervous system that modulate fibrillar assembly of the peptide. For example, apolipoprotein E (apoE) may either promote or inhibit Aβ fibril formation in vitro depending on the isoform (E3 vs E4)

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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 N-terminal residues 1−64 of MBP contained the Aβ binding domain and inhibited Aβ fibrillogenesis in a manner similar to that of 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 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. We further compared the interaction of MBP_{1-64} with Aβ42WT using solution NMR spectroscopy to assess the mechanism of the MBP_{1-64}−Aβ interaction and to compare the compound 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 toward 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 high-performance liquid chromatography (HPLC), and structurally characterized as previously described.[59] Aβ peptides were initially prepared in hexafluoro-2-propanol, lyophilized, and resuspended in either dimethyl sulfoxide (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 *Escherichia 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 5000*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, and 1 mM EDTA containing Complete Protease Inhibitor (Roche, Mannheim, Germany). The cell lysate was clarified by centrifugation and passed over Chitin Beads (New England Biolabs) equilibrated with 20 mM Tris-HCl (pH 9.0), 0.5 M NaCl, and 1 mM EDTA (EQ buffer). The column was washed with EQ buffer containing 0.05% Triton X-100, and the peptide was cleaved and eluted from the intein fusion protein by incubation of the column in 40 mM dithiothreitol (DTT) as per the manufacturer's instructions. 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) and 50 mM NaCl (CM EQ). The column was washed in CM EQ and eluted with a high salt concentration. Fractions were analyzed by SDS−PAGE, pooled, dialyzed against water, lyophilized, and stored at −70 °C.

Site-Directed Mutagenesis of Human MBP_{1-64}. MBP_{1-64} in the pTBY11 vector (plasmid DNA template) was used for alanine scanning site-directed mutagenesis, as per the manufacturer's instructions (Affymetrix, Santa Clara, CA), to produce MBP_{1-64} with the following mutated residues: K54A, R55A, G56A, S57A, G58A, D60A, S61A, H62A, and H63A). 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 a final concentration of 2.5 mM, diluted to 12.5 μM in 50 μL of PBS, and then coated on flat bottom 96-well plates (Fisher Scientific, Pittsburgh, PA) by incubation at 37 °C for 18 h. Each well was blocked in 100 μL of a 1% BSA/PBS mixture for 1 h at room temperature. Then purified recombinant MBP peptides (1.56 μM) in 50 μL of PBS were added to each well and incubated at 4 °C overnight. After the samples had been washed for 3 × 5 min with a 1% BSA/PBS/0.05% Tween 20 mixture (PBS-T), the rat monoclonal antibody to MBP (1:1000; AbD Serotec, Raleigh, NC) in PBS-T was added for 1 h at room temperature. Wells were washed for 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, U.K.), and the wells were then washed for 3 × 5 min with a 1% BSA/PBS-T mixture. SureBlue TMB microwell peroxidase substrate (KPL, Gaithersburg, MD) was added, the mixture developed, and the reaction terminated by adding 1 N HCl. The 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 a final concentration of 2.5 mM, diluted to 12.5 μM in PBS, and then incubated at 37 °C while being shaken 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 contents of the plate were mixed for 5 s and incubated at 25 °C in the dark for 10 min before each reading. Fluorescence was measured at 25 °C and 490 nm using an excitation wavelength of 446 nm in a SpectraMax spectrofluorometer (Molecular Devices) 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 an 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

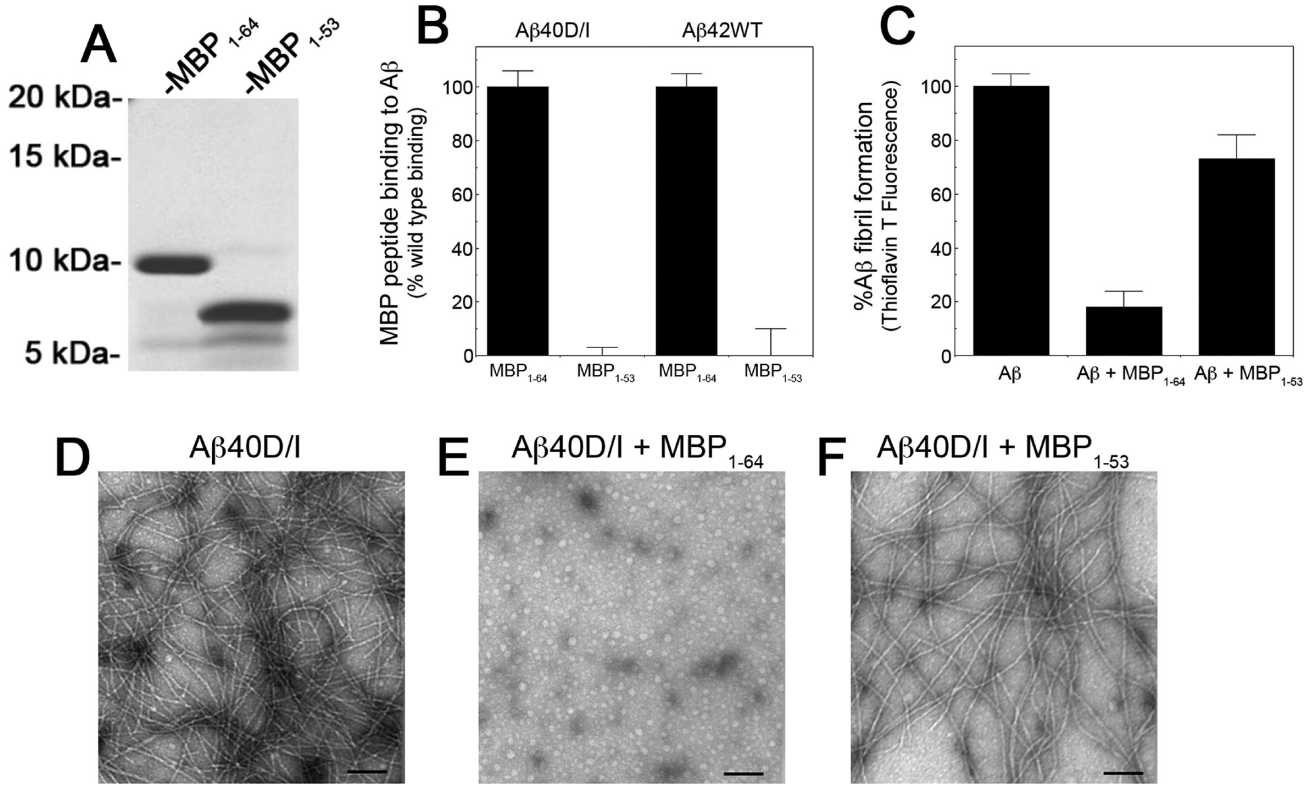

Figure 1. Residues 54−64 of MBP_{1-64} are required for its interactions with Aβ peptides. (A) MBP_{1-64} (first lane) and MBP_{1-53} (second lane) were recombinantly expressed, purified, and analyzed by SDS−PAGE. (B) The interaction of purified MBP_{1-64} and MBP_{1-53} with Aβ40DI or Aβ42WT was analyzed by a 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 are 100 nm. MBP_{1-64}, but not MBP_{1-53}, bound to Aβ and inhibited its fibrillar assembly.

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. ^1H 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 being mixed. $^1H-^{15}N$ HSQC spectra for 50 μM [^{15}N]Aβ42WT and 50 μM MBP_{1-64} or 50 μM MBP_{50-64} were recorded after the 100 μM samples had been mixed.

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-terminus of 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 (Figure 1A). Because 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}, MBP_{1-53} with a C-terminal deletion was largely devoid of binding to immobilized Aβ40D/I and Aβ42WT peptides (Figure 1B). Similarly, MBP_{1-53} was largely ineffective in blocking the fibrillar assembly of Aβ40D/I as assessed by thioflavin T binding and fluorescence (Figure 1C) and TEM analysis of fibril structure (Figure 1F). In contrast, similar studies showed that deletion of the first 10 N-terminal residues of MBP_{1-64} (MBP_{11-64}) had a minimal impact on its ability to inhibit Aβ fibrillar assembly (data not shown).

Residues K54, R55, G56, and K59 Mediate Binding of MBP_{1-64} to Aβ and Inhibition of Fibril Assembly. Because the region of residues 54−64 is required for interactions of MBP_{1-64} 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 mutation of sequential residues (K54A, R55A, and G56A) as well as K59A and H63A mutations markedly reduced the level of binding to Aβ40D/I (Figure 2A). On the other hand, mutations S57A, G58A, D60A, S61A, and H62A had a much smaller or no effect on binding of MBP_{1-64} 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 in binding to both of these fibrillogenic forms of Aβ.

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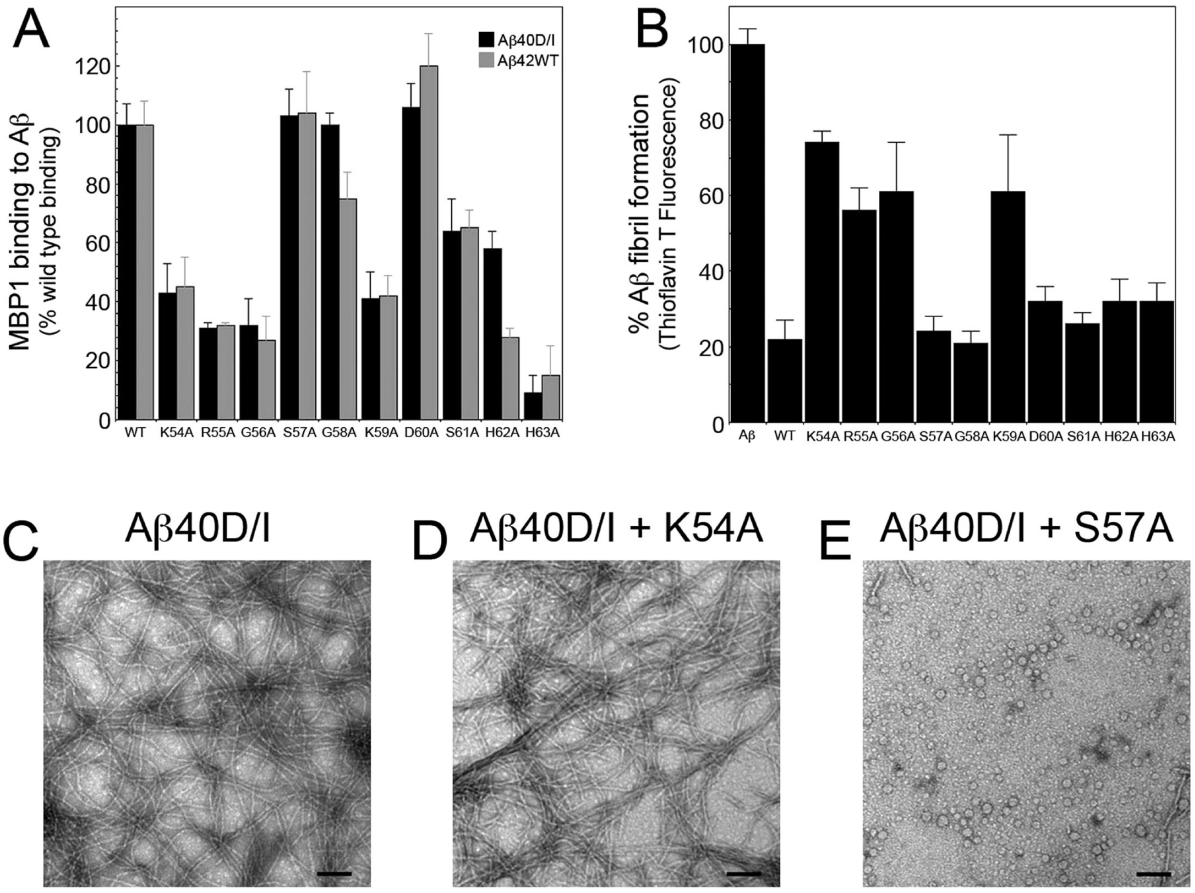

Figure 2. Alanine scanning mutagenesis analysis of residues 54−64 of MBP_{1-64}. (A) The interaction of recombinantly expressed and purified MBP_{1-64} alanine mutants with Aβ40D/I and Aβ42WT was analyzed by a solid phase binding assay. (B) The inhibition of Aβ40D/I (12.5 μM) fibrillogenesis by purified MBP_{1-64} alanine mutants (1.56 μM) was assessed by thioflavin T binding and fluorescence. (C−E) TEM analysis of Aβ40D/I in the absence (C) or presence of purified K54A mutant MBP_{1-64} (D) or purified S57A mutant MBP_{1-64} (E). Scale bars are 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.

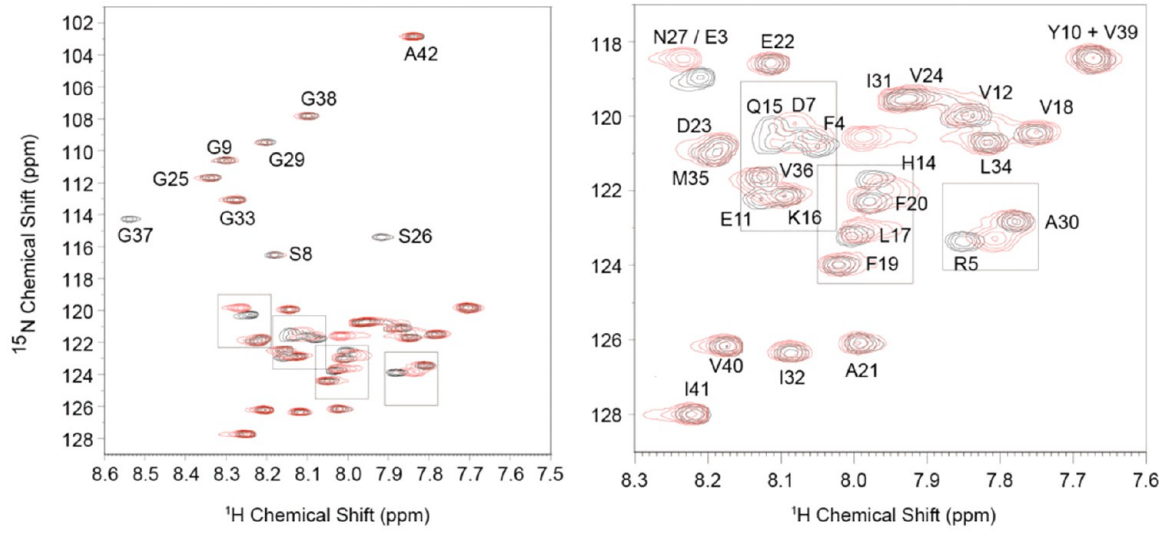

Figure 3. Interactions of specific Aβ42WT residues with MBP_{1-64}. ^{1}H−^{15}N HSQC spectra of ^{15}N-labeled Aβ42WT with (red) and without (black) unlabeled MBP_{1-64}. The largest shifts are observed in three regions of Aβ42WT: the N-terminus (E3−D7 and E11) and two regions within the central portion 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.

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. Like the results obtained in the binding experiments, we found that in MBP_{1-64} the sequential mutations K54A,

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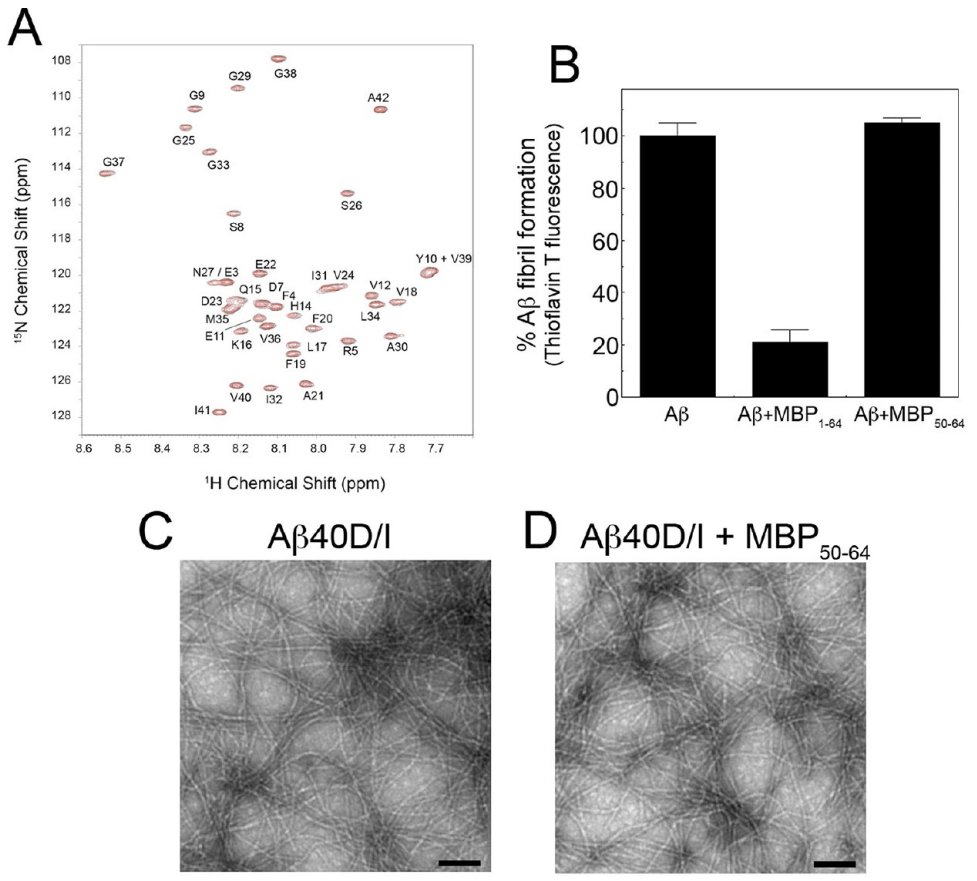

Figure 4. MBP_{50-64} is insufficient for Aβ binding and inhibition of fibril assembly. (A) $^{1}H-^{15}N$ HSQC spectra of ^{15}N-labeled Aβ42WT with (red) and without (black) unlabeled MBP_{50-64}. No significant spectral shifts are observed. (B) The inhibition of Aβ40D/I (12.5 μM) fibrillogenesis by purified MBP_{1-64} (1.56 μM) or MBP_{50-64} (1.56 μM) was assessed by thioflavin T binding and fluorescence. (C and D) TEM analysis of Aβ40D/I in the absence (C) or presence (D) of purified MBP_{50-64}. Scale bars are 100 nm. MBP_{50-64} did not bind Aβ or inhibit its fibrillar assembly.

R55A, and G56A as well as K59A substantially weakened its ability to inhibit Aβ fibril assembly (Figure 2B). Although the H63A mutant showed a reduced level of binding to immobilized Aβ peptides (Figure 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 (Figure 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 (Figure 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 indentify MBP residues K54, R55, G56, and K59 as being 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 a low temperature (4 °C) in a largely monomeric form that associates, as the temperature is increased, to low-molecular weight (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 on 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 substoichiometric MBP:Aβ ratios.

Figure 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 in H14–L17 are observed. 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. Because 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

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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 comixed (Figure 4A). Similarly, MBP_{50-64} was ineffective at inhibiting Aβ fibril assembly as assessed by thioflavin T binding and fluorescence (Figure 4B) and TEM analysis (Figure 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 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 this 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 (Figure 1). Subsequent site-directed mutagenesis studies in this region identified residues K54, R55, G56, and K59 as being important for the ability of MBP_{1-64} to bind Aβ and inhibit fibrillar assembly (Figure 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} (Figure 3). Together, these findings reveal specific sites on MBP and Aβ that appear to be 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 ∼5.3 to 6.0.[56] Second, the MBP_{50-64} peptide, which has a highly basic net charge (isoelectric point of >11.0) and is required for the interaction of MBP_{1-64} with Aβ, is itself incapable of influencing fibril assembly (Figure 4). Finally, eosinophilic cationic protein (ECP), an unrelated protein with a size and isoelectric point very similar to those of MBP,[67] did not inhibit assembly of Aβ into fibrils (data not shown).

Nevertheless, at a certain level, ionic interactions between MBP and Aβ do appear to be involved because mutation of the positively charged residues K54, R55, and K59 in MBP_{1-64} markedly impaired both binding to Aβ and inhibition of fibril assembly (Figure 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 (Figure 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 and/or 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, these data suggest a model in which the positively charged MBP_{54-64} region 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 (Figure 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 the development of effective inhibitors of pathogenic Aβ assembly.

ASSOCIATED CONTENT

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.

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Notes

The authors declare no competing financial interest.

■ 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.

■ REFERENCES

(1) Selkoe, D. J. (2001) Alzheimer's disease: Genes, proteins, and therapy. *Physiol. Rev. 81*, 741−766.

(2) Hardy, J., and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science 297*, 353−356.

(3) Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., and Beyreuther, K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A. 82*, 4245−4269.

(4) De Felice, F. G., Viera, M. N. N., Saraiva, L. M., Figueroa-Villar, J. D., Garci-Abreu, J., Liu, R., Chang, L., Klein, W. L., and Ferreira, S. T. (2004) Targeting the neurotoxic species in Alzheimer's disease: Inhibitors of Aβ oligomerization. *FASEB J. 18*, 1366−1372.

(5) Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature 325*, 733−736.

(6) Goldgaber, D., Lerman, M. I., McBride, O. W., Saffiotti, U., and Gajdusek, D. C. (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science 235*, 877−880.

(7) Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A., St George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M., and Neve, R. L. (1987) Amyloid β protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science 235*, 880−884.

(8) Robakis, N. K., Ramakrishna, N., Wolfe, G., and Wisniewski, H. M. (1987) Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. *Proc. Natl. Acad. Sci. U.S.A. 84*, 4190−4194.

(9) Vassar, R., Bennett, B. D., Babu-Khan, S., Khan, S., Mendiaz, E., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Louis, J.-C., Collins, F., Treanor, J., Rogers, G., and Citron, M. (1999) β-Secretase cleavage of the Alzheimer's precursor protein by the transmembrane aspartic protease BACE. *Science 286*, 735−741.

(10) Sinha, S., Anderson, J. P., Barbour, R., Basi, G. S., Caccavello, R., Davis, D., Doan, M., Dovey, H. F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberberg, I., Power, M., Tanh, H., Tatsuno, G., Ting, J., Schenk, D., Suomensaari, S. M., Wang, S., Walker, D., John, V., et al. (1999) Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature 402*, 537−540.

(11) Beck, J. P., Bienkowski, M. J., Sinha, S., and Heinrikson, R. L. (2003) Human β-secretase (BACE) and BACE inhibitors. *J. Med. Chem. 46*, 4625−4630.

(12) De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature 391*, 387−390.

(13) Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature 398*, 513−517.

(14) Edbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., and Haass, C. (2003) Reconstitution of γ-secretase activity. *Nat. Cell Biol. 5*, 486−488.

(15) Jellinger, K. A. (2002) Alzheimer's disease and cerebrovascular pathology: An update. *J. Neural Transm. 109*, 813−836.

(16) Rensink, A. A., de Waal, R. M., Kremer, B., and Verbeek, M. M. (2003) Pathogenesis of cerebral amyloid angiopathy. *Brain Res. Brain Res. Rev. 43*, 207−223.

(17) Attems, J., Jellinger, K., Thal, D. R., and Van Nostrand, W. (2011) Sporadic cerebral amyloid angiopathy. *Neuropathol. Appl. Neurobiol. 37*, 75−93.

(18) Findeis, M. A. (2007) The role of amyloid β peptide 42 in Alzheimer's disease. *Pharmacol. Ther. 116*, 266−286.

(19) Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., van Duinen, S. G., Bots, G. T., Luyendijk, W., and Frangione, B. (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science 248*, 1124−1126.

(20) Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J. A., Van Hul, W., Wehnert, A., Vegter-Van der Vlis, M., and Roos, R. A. (1990) Amyloid β protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). *Science 248*, 1120−1122.

(21) Grabowski, T. J., Cho, H. S., Vonsattel, J. P., Rebeck, G. W., and Greenberg, S. M. (2001) Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann. Neurol. 49*, 697−705.

(22) Davis, J., and Van Nostrand, W. E. (1996) Enhanced pathologic properties of Dutch-type mutant amyloid β-protein. *Proc. Natl. Acad. Sci. U.S.A. 93*, 2996−3000.

(23) Verbeek, M. M., de Waal, R. M., Schipper, J. J., and Van Nostrand, W. E. (1997) Rapid degeneration of cultured human brain pericytes by amyloid β protein. *J. Neurochem. 68*, 1135−1141.

(24) Miravalle, L., Tokuda, T., Chiarle, R., Giaccone, G., Bugiani, O., Tagliavini, F., Frangione, B., and Ghiso, J. (2000) Substitutions at codon 22 of Alzheimer's Aβ peptide induce diverse conformational changes and apoptotic effects in human cerebral endothelial cells. *J. Biol. Chem. 275*, 27110−27116.

(25) Melchor, J. P., McVoy, L., and Van Nostrand, W. E. (2000) Charge alterations of E22 enhance the pathogenic properties of the amyloid β-protein. *J. Neurochem. 74*, 2209−2212.

(26) Van Nostrand, W. E., Melchor, J. P., Cho, H. S., Greenberg, S. M., and Rebeck, G. W. (2001) Pathogenic effects of D23N Iowa mutant amyloid β-protein. *J. Biol. Chem. 276*, 32860−32866.

(27) Stine, W. B., Jr., Dahlgren, K. N., Krafft, G. A., and LaDu, M. J. (2003) In vitro characterization of conditions for amyloid-β peptide oligomerization and fibrillogenesis. *J. Biol. Chem. 278*, 11612−11622.

(28) Paravastua, A. K., Leapman, R. D., Yau, W. M., and Tycko, R. (2008) Molecular structural basis for polymorphism in Alzheimer's β-amyloid fibrils. *Proc. Natl. Acad. Sci. U.S.A. 105*, 18349−18354.

(29) Ahmed, M., Davis, J., Aucoin, D., Sato, T., Ahuja, S., Aimoto, S., Elliott, J. I., Van Nostrand, W. E., and Smith, S. O. (2010) Structural conversion of neurotoxic amyloid-β(1−42) oligomers to fibrils. *Nat. Struct. Mol. Biol. 17*, 561−567.

(30) Ono, K., Condron, M. M., and Teplow, D. B. (2009) Structure-neurotoxicity relationships of amyloid β-protein oligomers. *Proc. Natl. Acad. Sci. U.S.A. 106*, 14745−14750.

(31) Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., and Selkoe, D. J. (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature 416*, 535−539.

(32) Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Brett, F. M., Farrell, M. A., Rowan, M. J., Lemere, C. A., Regan, C. M., Walsh, D. M., Sabatini, B. L., and Selkoe, D. J. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med. 14*, 837−842.

(33) Glabe, C. G. (2006) Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. *Neurobiol. Aging 27*, 570−575.
(34) Urbanc, B., Cruz, J., Le, R., Sanders, J., Hsiao Ashe, K., Duff, K., Stanley, H. E., Irizarry, M. C., and Hyman, B. T. (2002) Neurotoxic effects of thioflavin S-positive amyloid deposits in transgenic mice and Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A. 99*, 13990−13995.
(35) Van Nostrand, W. E., Melchor, J., and Ruffini, L. (1998) Pathogenic cell surface amyloid β-protein fibril assembly in cultured human cerebrovascular smooth muscle cells. *J. Neurochem. 70*, 216−223.
(36) Rogers, J., Cooper, N. R., Webster, S., Schulz, J., McGeer, P. L., Styren, S. D., Civin, W. H., Brachova, L., Bradt, B., and Lieberburg, I. (1992) Complement activation by β-amyloid in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A. 89*, 10016−10020.
(37) Tacnet-Delorme, P., Chevallier, S., and Arlaud, G. J. (2001) β-Amyloid fibrils activate the C1 complex of complement under physiological conditions: Evidence for a binding site for Aβ on the C1q globular regions. *J. Immunol. 167*, 6374−6381.
(38) Rozemuller, A. J., van Gool, W. A., and Eikelenboom, P. (2005) The neuroinflammatory response in plaques and amyloid angiopathy in Alzheimer's disease: Therapeutic implications. *Curr. Drug Targets: CNS Neurol. Disord. 4*, 223−233.
(39) Reed-Geaghan, E. G., Savage, J. C., Hise, A. G., and Landreth, G. E. (2009) CD14 and toll-like receptors 2 and are required for fibrillar Aβ-stimulated microglial activation. *J. Neurosci. 29*, 11982−11992.
(40) Strittmatter, W. J., Weisgraber, K. H., Huang, D. Y., Dong, L. M., Salvesen, G. S., Pericak-Vance, M., Schemchel, D., Saunders, A. M., Goldgaber, D., and Roses, A. D. (1993) Binding of human apolipoprotein E to synthetic amyloid β peptide: Isoform-specific effects and implications for late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A. 90*, 8098−8102.
(41) LaDu, M. J., Falduto, M. T., Manelli, A. M., Reardon, C. A., Getz, G. S., and Frail, D. E. (1994) Isoform-specific binding of apolipoprotein E to β-amyloid. *J. Biol. Chem. 269*, 23403−23406.
(42) Wisniewski, T., Castano, E. M., Golabek, A., Vogel, T., and Frangione, B. (1994) Acceleration of Alzheimer's fibril formation by apolipoprotein-E in vitro. *Am. J. Pathol. 145*, 1030−1035.
(43) Manelli, A. M., Stine, W. B., van Eldik, L. J., and LaDu, M. J. (2004) ApoE and Aβ42 interactions: Effects of isoform and conformation on structure and function. *J. Mol. Neurosci. 23*, 235−246.
(44) Bales, K. R., Verina, T., Cummins, D. J., Du, Y., Dodel, R. C., Saura, J., Fishman, C. E., DeLong, C. A., Piccardo, P., Petegnief, V., Ghetti, B., and Paul, S. M. (1999) Apolipoprotein E is essential for amyloid deposition in the APPV717F transgenic mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A. 96*, 15233−15238.
(45) Costa, D. A., Nilsson, L. N., Bales, K. R., Paul, S. M., and Potter, H. (2004) Apolipoprotein E is required for the formation of filamentous amyloid, but not for amorphous Aβ deposition, in an AβPP/PS double transgenic mouse model of Alzheimer's disease. *J. Alzheimer's Dis. 6*, 509−514.
(46) Matsubara, E., Fragnione, B., and Ghiso, J. (1995) Characterization of Apolipoprotein J-Alzheimers Aβ interaction. *J. Biol. Chem. 270*, 7563−7567.
(47) DeMattos, R. B., O'dell, M. A., Parsadanian, M., Taylor, J. W., Harmony, J. A., Bales, K. R., Paul, S. M., Aronow, B. J., and Holtzman, D. M. (2002) Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A. 99*, 10843−10848.
(48) Lefterov, I., Fitz, N. F., Cronican, A. A., Fogg, A., Lefterov, P., Kodali, R., Wetzel, R., and Koldamova, R. (2010) Apolipoprotein A-1 deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1dE9 mice. *J. Biol. Chem. 285*, 36945−36957.
(49) Lewis, T. L., Cao, D., Lu, H., Mans, R. A., Su, Y. R., Jungbauer, L., Linton, M. F., Fazio, S., LaDu, M. J., and Li, L. (2010) Overexpression of human apolipoprotein A-I preserves cognition and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer's disease. *J. Biol. Chem. 285*, 36958−36968.
(50) Aksenova, M. V., Aksenova, M. Y., Butterfield, D. A., and Carney, J. M. (1996) α-1-Antichymotrypsin interaction with Aβ(1−40) inhibits fibril formation but does not affect the peptide toxicity. *Neurosci. Lett. 211*, 45−48.
(51) Potter, H., Wefes, I. M., and Nilsson, L. N. (2001) The inflammation-induced pathological chaperones ACT and apoE are necessary catalysts of Alzheimer amyloid formation. *Neurobiol. Aging 22*, 923−930.
(52) Schwarzman, A. L., Tsiper, M., Wente, H., Wang, A., Vitek, M. P., Vasiliev, V., and Goldgaber, D. (2004) Amyloidogenic and anti-amyloidogenic properties of recombinant transthyretin variants. *Amyloid 11*, 1−9.
(53) Buxbaum, J. N., Ye, Z., Reixach, N., Friske, L., Levy, C., Das, P., Golde, T., Masliah, E., Roberts, A. R., and Bartfai, T. (2008) Transthyretin protects Alzheimer's mice from the behavioral and biochemical effects of Aβ toxicity. *Proc. Natl. Acad. Sci. U.S.A. 105*, 2681−2686.
(54) Kakio, A., Nishimoto, S. I., Yanagisawa, K., Kozutsumi, Y., and Matsuzaki, K. (2001) Cholesterol-dependent formation of GM1 ganglioside-bound amyloid β-protein, an endogenous seed for Alzheimer amyloid. *J. Biol. Chem. 276*, 24985−24990.
(55) Yamamoto, N., Hirabayashi, Y., Amari, M., Yamaguchi, H., Romanov, G., Van Nostrand, W. E., and Yanagisawa, K. (2005) Assembly of hereditary amyloid β-protein variants in the presence of favorable gangliosides. *FEBS Lett. 579*, 2185−2190.
(56) Hoos, M. D., Ahmed, M., Smith, S. O., and Van Nostrand, W. E. (2007) Inhibition of familial cerebral amyloid angiopathy mutant amyloid β-protein fibril assembly by myelin basic protein. *J. Biol. Chem. 282*, 9952−9961.
(57) Hoos, M. D., Ahmed, M., Smith, S. O., and Van Nostrand, W. E. (2009) Myelin basic protein binds to and inhibits the fibrillar assembly of Aβ42 in vitro. *Biochemistry 48*, 4720−4727.
(58) Liao, M. C., Hoos, M. D., Aucoin, D., Ahmed, M., Davis, J., Smith, S. O., and Van Nostrand, W. E. (2010) Amino terminal domain of myelin basic protein inhibits amyloid β-protein fibril assembly. *J. Biol. Chem. 285*, 35590−35598.
(59) Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C., and Glabe, C. (1992) Assembly and aggregation properties of synthetic Alzheimer's A4/β amyloid peptide analogs. *J. Biol. Chem. 267*, 546−554.
(60) Hou, L. M., Shao, H., Zhang, Y., Li, H., Menon, N. K., Neuhaus, E. B., Brewer, J. M., Byeon, I. J. L., Ray, D. G., Vitek, M. P., Iwashita, T., Makula, R. A., Przybyla, A. B., and Zagorski, M. G. (2004) Solution NMR studies of the Aβ(1−40) and Aβ(1−42) peptides establish that the met35 oxidation state affects the mechanism of amyloid formation. *J. Am. Chem. Soc. 126*, 1992−2005.
(61) Fawzi, N. L., Ying, J., Ghirlando, R., Torchia, D. A., and Clore, G. M. (2011) Atomic-resolution dynamics on the surface of amyloid-β protofibrils probed by solution NMR. *Nature 480*, 268−272.
(62) Ladiwala, A. R. A., Lin, J. C., Bale, S. S., Marcelino-Cruz, A. M., Bhattacharya, M., Dordick, J. S., and Tessier, P. M. (2010) Resveratrol selectively remodels soluble oligomers and fibrils of amyloid Aβ into off-pathway conformers. *J. Biol. Chem. 285*, 24228−24237.
(63) Ono, K., Li, L., Takamura, Y., Yoshiike, Y., Zhu, L., Han, F., Mao, X., Ikeda, T., Takasaki, J., Nishijo, H., Takashima, A., Teplow, D., Zagorski, M., and Yamada, M. (2012) Phenolic compounds prevent amyloid β-protein oligomerization and synaptic dysfunction by site specific binding. *J. Biol. Chem. 287*, 14631−14643.
(64) Liu, C. C., Kanekiyo, T., Xu, H., and Bu, G. (2013) Apolipoprotein E and Alzheimer's disease: Risk, mechanisms and therapy. *Nat. Rev. Neurol. 9*, 106−118.
(65) Behrouz, N., Defossez, A., Delacourte, A., and Mazzuca, M. (1991) The immunohistochemical evidence of amyloid diffuse deposits as a pathological hallmark in Alzheimer's disease. *J. Gerontol. 46*, B209−B212.
(66) Wisniewski, H. M., Bancher, C., Barcikowska, M., Wen, G. Y., and Currie, J. (1989) Spectrum of morphological appearance of amyloid deposits in Alzheimer's disease. *Acta Neuropathol. 78*, 337−347.

(67) Barker, R. L., Loegering, D. A., Ten, R. M., Hamann, K. J., Pease, L. R., and Gleich, G. J. (1989) Eosinophil cationic protein cDNA. Comparison with other toxic cationic proteins and ribonucleases. *J. Immunol. 143*, 952−959.
(68) Mastrangelo, I. A., Ahmed, M., Sato, T., Liu, W., Wang, C., Hough, P., and Smith, S. O. (2006) High-resolution atomic force microscopy of soluble Aβ42 oligomers. *J. Mol. Biol. 358*, 106−119.