Localization of a Fibrillar Amyloid β-Protein Binding Domain on Its Precursor*

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Deposition of fibrillar amyloid-β protein (Aβ) in senile plaques and in the walls of cerebral blood vessels is a key pathological feature of Alzheimer’s disease and certain related disorders. Fibrillar Aβ deposition is intimately associated with neuronal and cerebrovascular cell death both in vivo and in vitro. Similarly, accumulation of the Aβ protein precursor (AβPP) is also observed at sites of fibrillar Aβ deposition. Recently, we reported that fibrillar Aβ, but not unassembled Aβ, promotes the specific binding of AβPP through its cysteine-rich, amino-terminal region (Melchor, J. P., and Van Nostrand, W. E. (2000) J. Biol. Chem. 275, 9782–9791). In the present study we sought to determine the precise site on AβPP that facilitates its binding to fibrillar Aβ. A series of synthesized overlapping peptides spanning the cysteine-rich, amino-terminal region of AβPP were used as competitors for AβPP binding to fibrillar Aβ. A peptide spanning residues 105–119 of AβPP competitively inhibited AβPP binding to fibrillar Aβ in a solid-phase binding assay and on the surface of cultured human cerebrovascular smooth muscle cells. Alanine-scanning mutagenesis of residues 105–117 within glutathione S-transferase (GST)-AβPP-(18–119) revealed that His110, Val112, and Ile113 are key residues that facilitate AβPP binding to fibrillar Aβ. These specific residues belong to a common β-strand within this region of AβPP. Wild-type GST-AβPP-(18–119) protected cultured human cerebrovascular smooth muscle cells from Aβ-induced toxicity whereas H110A mutant GST-AβPP-(18–119) did not. Wild-type GST-AβPP-(18–119) bound to different isoforms of fibrillar Aβ and fibrillar amylin peptides whereas H110A mutant and I113A mutant GST-AβPP-(18–119) were substantially less efficient binding to each fibrillar peptide. We conclude that His110, Val112, and Ile113, residing in a common β-strand region within AβPP-(18–119), comprise a domain that mediates the binding of AβPP to fibrillar peptides.

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1 The abbreviations used are: Aβ, amyloid β-protein; AβPP, amyloid β-protein precursor; sAβPP, secreted amyloid β-protein precursor; HCSM cells, human cerebrovascular smooth muscle cells; KPI, Kunitz proteinase inhibitor; mAb, monoclonal antibody; GST, glutathione S-transferase; BSA, bovine serum albumin; PBS, phosphate-buffered saline.
elaborate network of fibrils on the surfaces of HCSM cells. Furthermore, fibril assembly of pathogenic Aβ on the cell surface is required for inducing downstream pathologic responses in HCSM cells, including cell-surface accumulation of sAβPPα, degradation of vascular smooth muscle cell α-actin, and ultimately an apoptotic cell death (24–27).

We have shown that the accumulation of sAβPPα is mediated by its high-affinity binding to the Aβ fibrils that assemble on the HCSM cell surface (28). This event coincides with the induction of smooth muscle cell α-actin degradation and cell death. It is noteworthy that an interaction between fibrillar Aβ and AβPP also has been implicated in neuronal cell death in vitro (29). Finally, Aβ fibril binding to Kunitz protease-inhibitory (KPI) domain containing forms of AβPP can enhance its protease-inhibitory property (30). Together, these findings indicate that interactions between fibrillar Aβ binding and AβPP may have significant physiological and pathological consequences.

In the present study, we identify the precise site in the cysteine-rich, amino-terminal region of AβPP that facilitates its binding to fibrillar forms of Aβ. Our investigations show that residues His110, Val112, and Ile113, all on a common β-strand region within AβPP, comprise a domain on AβPP that is involved with its binding to fibrillar Aβ peptides. This domain also mediates the binding of AβPP to other fibrillar peptides, suggesting that this region may participate in other biologically important interactions.

**EXPERIMENTAL PROCEDURES**

**Materials—** Aβ peptides were synthesized by solid-phase Fmoc ([N-(9-fluorenyl)methoxycarbonyl]) amino acid chemistry, purified by reverse-phase HPLC, and structurally characterized as previously described (9-fluorenyl)methoxycarbonyl) amino acid chemistry, purified by reverse-phase-HPLC, and structurally characterized as previously described (24). Amylin peptide was obtained from Bachem (San Carlos, CA). The anti-AβPP mouse monoclonal antibody (mAb) PP 119 was prepared as previously described (28). Individual oligonucleotides used in the site-directed mutagenesis were 5' phosphorylated, ~40 bases in length with 20 matched bases on either side of the point mutation, possessing a Tm of 75 °C and containing at least 40% GC content. The 50-μl PCR reaction sample included 40 ng of the pGEX-KG-human AβPP exon 2–3 fusion construct, encoding AβPP residues 18–119, as prepared previously (28). Individual amino acids from Cys105 through Cys117 within exon 3 were mutated to Ala employing single nucleotide change approach using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Briefly, complementary oligonucleotides used in the site-directed mutagenesis were 5' phosphorylated, ~40 bases in length with 20 matched bases on either side of the point mutation, possessing a Tm of 75 °C and containing at least 40% GC content. The 50-μl PCR reaction sample included 40 ng of the pGEX-KG-human AβPP exon 2–3 fusion construct and 125 ng of the sense and antisense oligonucleotides. The thermocycling profile was as follows: 20 cycles of 95 °C for 30 s, 55 °C for 1 min, 68 °C for 20 min, and 1 cycle of 72 °C for 10 min. The PCR product was then digested with DpnI for 1.5 h to remove methylated parental DNA as per the manufacturer's protocol. The digested sample was transformed into E. coli XL-1 Blue supercompetent cells and grown on LB agar plates containing 100 μg/ml ampicillin. Colonies were chosen and plasmid DNA was isolated and sequenced to confirm the presence of each mutation. In some cases, a second mutation was needed to change the codon to alanine. In these cases, sequential mutagenesis was done using two mutagenesis oligonucleotide sets, the second set with both mutations. The plasmids were sequenced to confirm the presence of the second mutation.

**Escherichia coli BL21 cells** were transformed with wild-type and mutant pGEX-KG-human AβPP exon 2–3 fusion constructs, plated on LB agar plates containing 100 μg/ml ampicillin and incubated overnight at 37 °C, and colonies were picked and used to inoculate 100 ml LB media. Protein expression was induced by the addition of isopropyl β-D-thiogalactoside to a final concentration of 1 mM for 4 h. Wild-type and mutant GST-AβPP-(18–119) fusion proteins were affinity-purified from the harvested cells using glutathione-Sepharose beads as previously described (28). The concentration of the eluted GST-AβPP-(18–119) fusion proteins was measured using the extinction coefficient of GST. The precise concentrations of wild-type and each mutant GST-AβPP-(18–119) protein were confirmed by titration using mAb 22C11 and compared with standard curves of known concentrations of purified sAβPPα. The mAb 22C11 was used for the precise titrations since its epitope on AβPP and GST-AβPP-(18–119) resides upstream of residues 105–119 and contains only two of the 11 alanine substitutions inserted in the mutant fusion proteins.

**HCSM Cell Culture—** Primary cultures of HCSM cells were established and characterized as previously described (34). Two lines of HCSM cells were used in these studies. One was derived from a 70-year-old male AD patient and the other was derived from a 37-year-old female control. All HCSM cells were used between passages 4–7 and maintained in 24-well tissue culture dishes with Dulbecco's minimum essential medium containing 10% fetal bovine serum (Gibco BRL, Grand Island, NY) and 1% non-essential amino acids, and antibiotics (Invitrogen). For experiments, near-confluent cultures of HCSM cells were placed in serum-free medium containing 0.1% BSA, non-essential amino acids, and antibiotics overnight prior to treatment. Freshly solubilized Dutch-type Aβ-(1–40) at a final concentration of 25 μM was added to the cultures in serum-free medium and incubated at 37 °C for 6 days. Cells were routinely viewed and photographed using an Olympus IX70 phase-contrast microscope. Cell viability was quantified using a fluorescent live/dead cell assay following the manufacturer's protocol (molecular Probes, Eugene, OR). The number of live and dead cells were counted from several fields (n = 4) from at least three separate wells for each experiment.

**RESULTS**

**Peptide Mapping of the Fibrillar Aβ Binding Domain in AβPP-(18–119)—** We showed that the amino-terminal region AβPP-(18–119), encoded by exons 2 and 3 of the AβPP gene, was
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mediates the binding of AβPP to fibrillar forms of Aβ (28). Here we sought to identify the precise site within this region of AβPP that is responsible for this interaction. To accomplish this, we synthesized a series of overlapping peptides of 15 amino acids starting at residue 18 of AβPP with each subsequent peptide shifting 3 amino acids toward the carboxyl-terminal end. This approach covered the entire region of AβPP-(18–119) (Fig. 1).

Each of these peptides was tested for its ability to compete against biotinylated sAβPPα for binding to fibrillar Aβ in a solid-phase binding assay. At a relatively high concentration of 1 mM, the peptide AβPP-(105–119) competed for ~80% of the biotinylated sAβPPα binding to fibrillar Aβ (Fig. 2A, lane 3). In contrast, a 1000-fold lower concentration of GST-AβPP-(18–119) completely blocked biotinylated sAβPPα binding to immobilized fibrillar Aβ (Fig. 2A, lane 2). We also found that overlapping peptide AβPP-(102–116) possessed some competing activity in the assay, although it was approximately half that of AβPP-(105–119) (data not shown). However, none of the other 15-mer peptides showed either appreciable or consistent competing activity.

We previously showed that fibrillar Aβ assembled on the surface of HCSM cells mediates the binding of endogenously produced sAβPPα on the cell surface (24, 28). Consistent with the results obtained from the solid-phase binding assay, GST-AβPP-(18–119) completely inhibited endogenous sAβPPα binding to HCSM cell surface fibrillar Aβ, whereas higher concentrations of AβPP-(105–119) could partially diminish this AβPP binding (Fig. 2, B and C). The findings that AβPP-(105–119) peptide was less effective than GST-AβPP-(18–119) and that higher concentrations of AβPP-(105–119) peptide were needed in blocking sAβPPα binding to fibrillar Aβ likely results from this region in AβPP being highly structured containing three intrachain disulfide bonds (Fig. 1).

Alanine-scanning Mutagenesis of the Putative Fibrillar Aβ Binding Domain in AβPP-(18–119)—The peptide-mapping experiments described above indicated that the sequence AβPP-(105–119) contains the fibrillar Aβ binding domain. However, this small peptide was not nearly effective as the larger, recombantly expressed AβPP-(18–119) in binding to fibrillar Aβ. Therefore, we performed alanine-scanning mutagenesis studies of the AβPP-(105–119) sequence within AβPP-(18–119) to further identify key epitopes important in facilitating its binding to fibrillar Aβ. Alanine residues were introduced from Cys105 through Cys117 of AβPP-(18–119) by site-directed mutagenesis of the AβPP-(18–119) cDNA. Each mutant AβPP-(18–119) cDNA was expressed as a GST fusion protein and purified, and the concentrations were carefully determined by quantitative immunoblotting as described in "Experimental Procedures". Each purified mutant GST-AβPP-(19–119) was tested for its ability to bind immobilized fibrillar Aβ in a solid-phase binding assay and compared with the level of binding observed with wild-type GST-AβPP-(18–119). As shown in Fig. 3 most of the alanine substitutions had modest inhibitory or enhancing effects on GST-AβPP-(18–119) binding to fibrillar Aβ. However, alanine substitutions at residues His110, Val112, or Ile113 showed highly diminished GST-AβPP-(18–119) binding to fibrillar Aβ. In particular, H110A mutant GST-AβPP-(18–119) exhibited <1% of fibrillar Aβ binding compared with wild-type GST-AβPP-(18–119). The x-ray crystal structure for this amino-terminal region of AβPP was recently reported (35).
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![Image](http://www.jbc.org/)

**Fig. 2.** $\alpha$PP-(105–119) blocks $\alpha$PP binding to fibrillar $\alpha$/$\beta$. A, two $\mu$g of fibrillar Dutch-type $\alpha$/$\beta$-(1–40) in 100 $\mu$l of PBS were dried overnight in triplicate wells of a 96-well microtiter plate. After washing, the wells were incubated with 50 $n$M biotinylated $\alpha$A/H11006 alone; and lanes 6, the wells were incubated with 50 $n$M wild-type or the different scanning alanine mutant GST-$\alpha$PP-(18–119) proteins followed by the mAb 22C11 and a sheep anti-mouse IgG horseradish peroxidase conjugate. The binding of antibody was detected using the colorimetric substrate o-phenylenediamine dihydrochloride and measured at a wavelength of 490 nm using a Molecular Devices V-max microplate reader. The GST-$\alpha$PP-(18–119) proteins tested were as follows: lane 1, wild-type; lane 2, C105A; lane 3, K106A; lane 4, T107A; lane 5, H108A; lane 6, P109A; lane 7, H110A; lane 8, F111A; lane 9, V112A; lane 10, I113A; lane 11, F114A; lane 12, Y115A; lane 13, R116A; and lane 14, C117A. Data represent the mean ± S.D. from triplicate samples from 8 to 12 independent experiments.

B and C, HCSM cells were incubated in the absence or presence of 25 $\mu$M wild-type $\alpha$A/H11001 PP-(105–119) Dutch-type. Near-confluent cultures of HCSM cells were incubated in the absence or presence of 25 $\mu$M wild-type $\alpha$A/H11001 PP-(18–119) (Fig. 4). Also observed in these studies was that the C117A mutant GST-$\alpha$PP-(18–119) showed markedly diminished binding to fibrillar $\alpha$A compared with wild-type GST-$\alpha$PP-(18–119). Residue Cys117 participates in a disulfide bond with Cys117 (Figs. 1 and 4), suggesting that this linkage is important in presenting a properly folded fibrillar $\alpha$A binding domain in $\alpha$PP-(18–119) likely involving a $\beta$-strand within this region that includes specific residues His110, Val112, and Ile113.

**H110A Mutant GST-$\alpha$PP-(18–119) Is Deficient in Blocking Cell Death in Pathogenic $\alpha$/$\beta$-treated Cultured HCSM Cells—** Incubation of HCSM cells with pathogenic Dutch-type $\alpha$A-(1–40) leads to cell death (23–26). Previous results from our lab revealed that wild-type GST-$\alpha$PP-(18–119) inhibits HCSM cell death induced by treatment with Dutch-type $\alpha$A-(1–40) (26). Therefore, we tested whether H110A mutant GST-$\alpha$PP-(18–119), which is deficient in binding to fibrillar $\alpha$A, was capable of blocking HCSM cell death induced by treatment with $\alpha$A-(1–40) Dutch-type. Near-confluent cultures of HCSM cells were incubated in the absence or presence of 25 $\mu$M Dutch-type $\alpha$A-(1–40) and in the absence or presence of 1 $\mu$M wild-type or H110A mutant GST-$\alpha$PP-(18–119) for 6 days at 37°C. After this time, HCSM cell viability was quantified as described in “Experimental Methods.” As shown in Fig. 5, wild-type GST-$\alpha$PP-(18–119) lowered $\alpha$A-induced HCSM cell death to <10% (lane 4) whereas H110A mutant GST-$\alpha$PP-(18–119) was incapable of preventing the cytotoxic effects of this peptide (lane 6). These studies demonstrate that H110A mutant GST-$\alpha$PP-(18–119) is ineffective in blocking cell death evoked by pathogenic $\alpha$A in HCSM cells.

**Deficient Binding of Mutant GST-$\alpha$PP-(18–119) Proteins to Fibrillar Amyloid Peptides—** The solid-phase binding experiments with wild-type and mutant GST-$\alpha$PP-(18–119)
A site on A showed considerably reduced binding to fibrillar wild-type A/H9262 absence of presence of 1–(18 B in Fig. 6/H9252, H110A and I113A mutant GST-A Fig. 6 A and used in solid-phase binding experiments. As shown proteins presented in Fig. 3 were performed using fibrillar Dutch-type Aβ. We determined whether key mutant GST-AβPP-(18–119) proteins similarly exhibited deficient binding to fibrillar wild-type Aβ-(1–40) and fibrillar amylin peptide. For these studies we used purified wild-type, H110A mutant, and I113A mutant GST-AβPP-(18–119) proteins as shown in Fig. 6A and used in solid-phase binding experiments. As shown proteins displayed markedly decreased binding to fibrillar Dutch-type Aβ-(1–40) compared with wild-type GST-AβPP-(18–119). Similarly, both mutant GST-AβPP-(18–119) proteins showed considerably reduced binding to fibrillar wild-type Aβ-(1–42) and fibrillar amylin peptide compared with wild-type GST-AβPP-(18–119). As a control, none of the GST-AβPP-(18–119) proteins exhibited any binding to immobilized ovalbumin. These findings suggest that His110 and Ile113, which reside on the same β-strand within this region of AβPP, are key residues for also facilitating AβPP-(18–119) binding to fibrillar wild-type Aβ and fibrillar amylin peptides. The results with fibrillar amylin further suggest that the binding of AβPP-(18–119) through this domain is not sequence-specific but appears to involve recognition of the fibrillar structure of the peptide.

DISCUSSION

Deposition of fibrillar Aβ in senile plaques and in the walls of cerebral blood vessels is a key pathological feature of AD, Down’s syndrome, and several related cerebral amyloid angiopathy disorders (1, 2). These fibrillar Aβ deposits that occur within the brain are intimately associated with neuronal and cerebrovascular cell degeneration at their respective sites (16–19). Similarly, fibrillar Aβ deposition is involved in neuronal cell and HCSM cell toxicity in vitro (20–26). Fibrillar Aβ de-

Fig. 5. H110A mutant AβPP-(18–119) does not block HCSM cell death induced by Dutch-type Aβ-(1–40). HCSM cells were incubated in the absence or presence of 25 μM Dutch-type Aβ-(1–40) in the absence of presence of 1 μM wild-type or H110A mutant GST-AβPP-(18–119) for 6 days. HCSM cell viability was measured as described under “Experimental Procedures.” Lane 1, control; lane 2, Dutch-type Aβ-(1–40); lane 3, wild-type GST-AβPP-(18–119) alone; lane 4, wild-type GST-AβPP-(18–119) + Dutch-type Aβ-(1–40); lane 5, H110A mutant GST-AβPP-(18–119) alone; and lane 6, H110A mutant GST-AβPP-(18–119) + Dutch-type Aβ-(1–40). In each case the data represent the mean ± S.D. for triplicate samples from three separate experiments.

Fig. 6. Wild-type and mutant AβPP-(18–119) proteins binding to different fibrillar peptides. A, wild-type, H110A mutant and I113A mutant GST-AβPP-(18–119) proteins were expressed, purified, and subjected to immunoblotting as described in “Experimental Procedures.” Lane 1, wild-type GST-AβPP-(18–119); lane 2, H110A mutant GST-AβPP-(18–119); and lane 3, I113A mutant GST-AβPP-(18–119). B, two μg of the different fibrillar peptides or ovalbumin in 100 μl of PBS were dried overnight in triplicate wells of a 96-well microtiter plate. After washing, the wells were incubated with 100 nM wild-type or mutant GST-AβPP-(18–119) followed by mAb 29C11 and sheep anti-mouse IgG horseradish peroxidase conjugate. The binding of antibody was detected using the colorimetric substrate o-phenylenediamine dihydrochloride and measured at a wavelength of 490 nm using a Molecular Devices V max, microplate reader. Lanes 1, 4, 7, and 10, wild-type GST-AβPP-(18–119); lanes 2, 5, 8, and 11, H110A mutant GST-AβPP-(18–119); and lanes 3, 6, 9, and 12, I113A mutant GST-AβPP-(18–119). Data represent the means ± S.D. for triplicate samples from three separate experiments.

Fig. 4. Molecular model of the putative fibrillar Aβ binding site on AβPP-(18–119). A, using the x-ray crystal structure coordinates reported by Rossjohn et al. (35), we utilized the Cn3D 3.0 program on the NCBI Website to generate a predicted structural fold of the AβPP-(18–119) region. In this model, the single α-helical region is shown in green and the β-strands are shown in red. The specific residues His110, Val112, and Ile113 are highlighted in yellow. B, sequence of AβPP-(18–119) structure shown in A identifying the single α-helix, the seven β-strand regions, and the locations of the three intrachain disulfide bonds. Note that residues His110, Val112, and Ile113 reside on a common β-strand.
Posits that occur both in vivo and in vitro cell culture models lead to accumulation of its precursor protein AβPP (20–24, 29, 40, 41). We recently reported that fibrillar Aβ mediates the pathological accumulation of AβPP on the HCSM cell surface through interaction with a domain in the cysteine-rich, amino-terminal region of AβPP (28). In this study the majority of the HCSM cell-accumulated AβPP was shown to be sAβPPα. Although sAβPPα has been postulated to be a protective molecule, the interaction between fibrillar Aβ and its precursor may have significant implications in cytotoxic mechanisms in AD and related disorders. For example, this interaction, which results in the accumulation of AβPP on the HCSM cell surface may contribute to the onset of cell death (28). Likewise, the recent study of Lorenzo et al. (29) has implicated an interaction between fibrillar Aβ and AβPP in neuronal toxicity in vitro. In light of the potential importance of these findings, we sought to determine the precise site on AβPP that facilitates its binding to fibrillar Aβ.

We previously identified the amino-terminal domain of residues 18–119 as the region on AβPP responsible for mediating its binding to fibrillar Aβ (28). Therefore, we synthesized a set of overlapping 15-amino-acid peptides that spanned this region of AβPP to further localize this site. In competition experiments, the peptide AβPP-(105–119), located at the extreme carboxyl-terminal end of this amino-terminal region of AβPP (Fig. 1), was found to compete against AβPP for binding to both immobilized fibrillar Aβ (Fig. 2A) and fibrillar Aβ assembled on the surface of HCSM cells (Fig. 2, B and C). However, AβPP-(105–119) was found to be much less effective than GST-AβPP-(18–119) in its ability to compete for AβPP binding. This disparity is likely caused by the highly structured nature of AβPP-(18–119), a region that contains three intrachain disulfide bonds (Figs. 1 and 4). Although the integrity of this structure is preserved in the recombinantly expressed GST-AβPP-(18–119), it is unlikely to be properly folded in the small synthetic AβPP-(105–119) peptide. Nevertheless, these findings suggest the involvement of this focused region on AβPP-(18–119) in binding fibrillar Aβ.

Because the peptide-competition experiments implicated the region AβPP-(105–119) as the likely site of a fibrillar Aβ binding domain, we conducted alanine-scanning mutagenesis studies in this region to determine the key residues involved. We decided to perform this analysis in recombinantly expressed GST-AβPP-(18–119) fusion proteins since the wild-type GST-AβPP-(18–119) protein faithfully recapitulates the binding characteristics of native sAβPP to fibrillar Aβ (28). Alanine substitutions were made for each amino acid from Cys105 through Cys117 of GST-AβPP-(18–119). These studies clearly identified His110, Val112, and Ile113 as key residues that facilitate GST-AβPP-(18–119) binding to fibrillar Aβ. It is noteworthy that these three particular residues reside on a predicted common β-strand within this region of AβPP (Fig. 4). It has been suggested that these particular residues, along with Phe37, Pro109, Phe111, and Tyr115 form a hydrophobic surface patch on AβPP (35). Our alanine scanning mutagenesis results indicate that P109A, F111A, and Y115A had little or no effect on GST-AβPP-(18–119) binding to fibrillar Aβ (Fig. 3). Similarly, little effect on binding was observed with a F37A mutant GST-AβPP-(18–119) (data not shown). This suggests that this putative hydrophobic surface patch is not wholly involved with mediating the binding of AβPP to fibrillar Aβ. The finding that the C117A substitution substantially affects GST-AβPP-(18–119) binding to fibrillar Aβ further supports the notion that the disulfide bond formed between Cys23 and Cys117 is important for properly presenting the β-strand of this region containing His110, Val112, and Ile113 as a functional fibrillar Aβ binding domain.

Treatment of cultured HCSM cells with pathogenic forms of Aβ results in a protracted period of cellular degeneration leading to apoptotic cell death (20, 23–27). We previously showed that GST-AβPP-(18–119), which contains a functionally active fibrillar Aβ binding domain, blocks cell death in HCSM cells treated with pathogenic Dutch-type Aβ-(1–40) (26). GST-AβPP-(18–119) may act as a dominant-negative factor containing the site for fibrillar Aβ binding but lacks other downstream regions of AβPP that mediate a cell-death response. In the present study, we show that in contrast to wild-type GST-AβPP-(18–119), the H110A mutant GST-AβPP-(18–119), which is deficient in fibrillar Aβ binding, is incapable of protecting HCSM cells from the cytotoxic effects of Dutch-type Aβ-(1–40) (Fig. 5). This finding further supports the notion that an interaction between AβPP and fibrillar Aβ contributes to the cell death response in HCSM cells.

Our earlier studies showed that biotinylated sAβPPα and GST-AβPP-(18–119) bound to fibrils formed with either Dutch-type Aβ-(1–40) or wild-type Aβ-(1–42), but not unassembled proteins, indicating that this interaction depends on fibrillar structures of the peptide. Similarly, in the present study we show that H110A mutant and I113A mutant GST-AβPP-(18–119) proteins are deficient in binding fibrils formed with either Dutch-type Aβ-(1–40) or wild-type Aβ-(1–42) (Fig. 6). It is noteworthy that the same pattern of binding was observed when fibrillar amylin peptide was used in the solid-phase binding assay. This finding suggests that the β-strand containing residues His110, Val112, and Ile113 folds into a binding site that is not specific for the Aβ amino acid sequence but rather possesses recognition for fibrillar structures. Therefore, it is possible that this domain may mediate the binding of AβPP to other fibrillar structures as well.

The binding of fibrillar Aβ, and possibly other fibrillar proteins, to AβPP through the domain identified here may have several potential consequences. For example, this interaction may help to explain the high levels of AβPP that accumulate around fibrillar Aβ present in cerebrovascular and, possibly plaque, amyloid deposits (17, 40–43). In addition, we recently showed that the binding of fibrillar Aβ to KPI-containing forms of AβPP enhances its coagulation proteinase inhibitory properties (30). The KPI domain resides downstream from AβPP-(105–119) starting at AβPP residue 289. This finding suggests that fibrillar Aβ deposits may bind, localize, and stimulate the proteinase inhibitory functions of AβPP. This activity may have implications regarding hemorrhagic stroke seen in patients with severe cerebral amyloid angiopathy. AβPP that is produced locally in the cerebral vessel wall or released by circulating activated platelets may accumulate at sites of cerebrovascular Aβ deposition. This would result in a microenvironment high in anticoagulant activity and conducive to hemorrhaging.

More germane to the present work, several studies have reported that treatment of cultured neuronal cells with the mAb 22C11 or a polyclonal antibody (both of which recognize epitopes in the amino-terminal region of AβPP not far from the identified fibrillar Aβ binding site) can stimulate G-protein activity and/or initiate cell death pathways in vitro (44–46). It is thought that these responses proceed through the dimerization of AβPP by divalent antibody binding. Also of note is the recent study of Scheuermann (47) implicating AβPP dimerization in increased Aβ production. Although antibodies to AβPP were used in these in vitro studies, clearly other more pathologically relevant agonists must exist in vivo to elicit these potential responses in situations such as AD. In this case the


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Aβ fibril, and perhaps the protofibril, may be pathological agonists that facilitate AβPP dimerization on the cell surface stimulating cell death pathways and Aβ production. In regard to the present study, GST-AβPP (18–119) may inhibit pathogenic Aβ-induced HCSM cell death by interfering with AβPP dimerization. This thought is also consistent with our finding that GST-Aβ mediated through this site may contribute to the pathologic accumulation of AβPP observed at sites of fibrillar Aβ deposition that occur in vitro or in vivo in the cultured HCSM cell surface and in vivo in the cerebral vessel walls of patients with severe cerebral amyloid angiopathy. This pathologic fibrillar Aβ-AβPP interaction may provide further insight into the mechanisms that lead to cerebrovascular and, possibly neuronal, cellular degeneration observed in AD and related disorders.

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