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Peptide and Protein Mimetics Inhibiting Amyloid β-Peptide Aggregation

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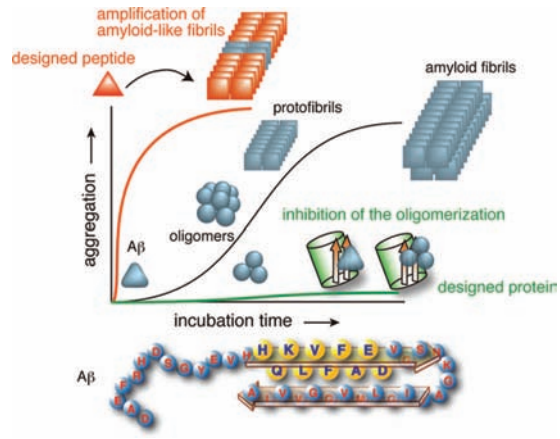
CONSPECTUS

Protein misfolding is related to some fatal diseases including Alzheimer's disease (AD). Amyloid β-peptide (Aβ) generated from amyloid precursor protein can aggregate into amyloid fibrils, which are known to be a major component of Aβ deposits (senile plaques). The fibril formation of Aβ is typical of a nucleation-dependent process through self-recognition. Moreover, during fibrillization, several metastable intermediates such as soluble oligomers, including Aβ-derived diffusible ligands (ADDLs) and Aβ*56, are produced, which are thought to be the most toxic species to neuronal cells. Therefore, construction of molecules that decrease the Aβ aggregates, including soluble oligomers, protofibrils, and amyloid fibrils, might further our understanding of the mechanism(s) behind fibril formation and enable targeted drug discovery against AD. To this aim, various peptides and peptide derivatives have been constructed using the "Aβ binding element" based on the structural models of Aβ amyloid fibrils and the mechanisms of self-assembly. The central hydrophobic amino acid sequence, LVFF, of Aβ is a key sequence to self-assemble into amyloid fibrils. By combination of this core sequence with a hydrophobic or hydrophilic moiety, such as cholic acid or aminoethoxy ethoxy acetic acid units, respectively, good inhibitors of Aβ aggregation can be designed and synthesized.



A peptide, LF, consisting of the sequence Ac-KQKLLLFLEE-NH_2, was designed based on the core sequence of Aβ but with a simplified amino acid sequence. The LF peptide can form amyloid-like fibrils that efficiently coassemble with mature Aβ1−42 fibrils. The LF peptide was also observed to immediately transform the soluble oligomers of Aβ1−42, which are thought to pose toxicity in AD, into amyloid-like fibrils. On the other hand, two Aβ-like β-strands with a parallel orientation were embedded in green fluorescent protein (GFP), comprised of a β-barrel structure, to make pseudo-Aβ β-sheets on its surface. The GFP variant P13H binds to Aβ1−42 and inhibits Aβ1−42 oligomerization effectively in a substoichiometric condition. Thus, molecules capable of binding to Aβ can be designed based on structural similarities with the Aβ molecule. The peptide and protein mimetics based on the structural features of Aβ might lead to the development of drug candidates against AD.

1. Introduction

Protein misfolding has been implicated in a number of fatal diseases, including Alzheimer's disease (AD), Parkinson's disease, and Huntington's disease.[1−4] AD is the most common form of dementia and is characterized by the deposition of the amyloid β-peptide (Aβ) in senile plaques and intracellular neurofibrillary tangles consisting of abnormally hyperphosphorylated tau protein.[1] Although the molecular mechanisms of AD pathogenesis have not been clearly understood due to its complexity, recent advances have demonstrated that pathological assembly of Aβ is a causal factor in AD.[5] Aβ is generated from amy-

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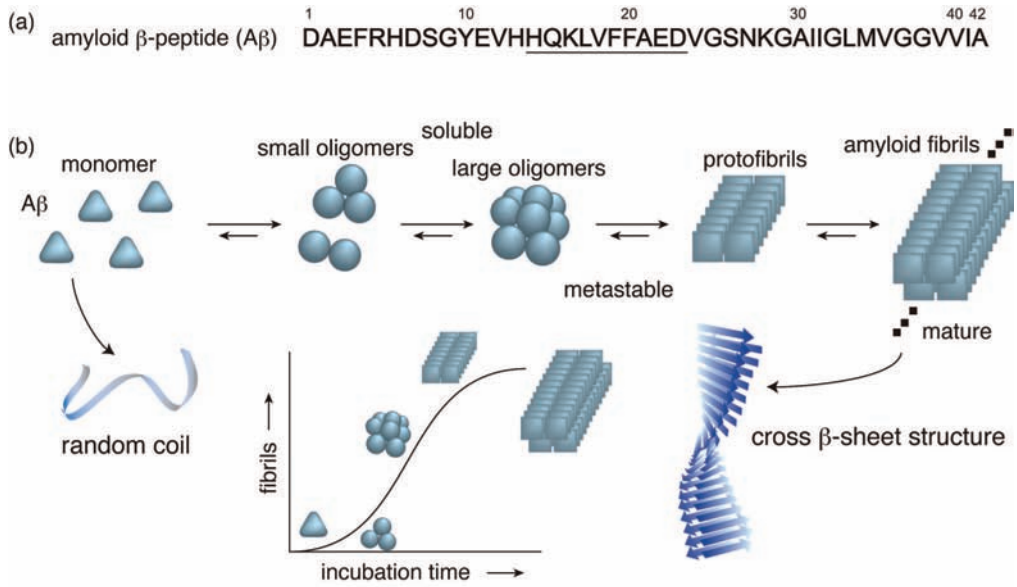

FIGURE 1. (a) The amino acid sequence of Aβ1−42 peptide and (b) schematic representation of the aggregation of Aβ monomer forming a random coil structure into amyloid fibrils composed of cross β-sheet structures via several metastable oligomers (small and large oligomers and protofibrils).

loid precursor protein (APP) by β- and γ-secretase enzymes[6] and usually contains either 40 or 42 amino acids (Aβ1−40 and Aβ1−42, respectively).[7] Aβ1−40 is the major product of APP processing, while Aβ1−42 is the predominant component of senile plaques.[8,9] Mutations causing AD are seen in genes encoding the APP and secretase enzymes.[10,11] Some mutations in genes encoding presenilins (PS1 and PS2) participating in the catalytic complex of γ-secretase are functionally associated with an increase in the production of Aβ1−42.[11]

Since the aggregation of Aβ correlates with the toxicity to the neuronal cells, self-assembly of Aβ peptides *in vitro* has been evaluated. *In vitro* studies revealed that the monomeric Aβ can self-assemble into amyloid fibrils with facile conversion in physiological conditions. Amyloid fibril formation is a nucleation-dependent process that can be accelerated dramatically by fibril seeds *in vitro* and *in vivo*.[12,13] Since the nucleation step is faster in Aβ1−42 than in Aβ1−40, Aβ1−42 can aggregate more easily into amyloid fibrils than Aβ1−40. Moreover, Aβ1−42 has a distinct pathway by which it forms heterogeneous mixtures of soluble oligomers (Figure 1).[14,15] Recent studies revealed that these soluble oligomers, including Aβ-derived diffusible ligands (ADDLs)[16] and Aβ*56 (extracellular accumulation of a 56 kDa soluble amyloid-β assembly),[17] have been thought to be the most important features showing higher toxicity to neuronal cells than the monomeric form and mature amyloid fibrils. The structural definition of the soluble oligomers has not been accomplished, because the oligomers are metastable. Circular dichroism (CD) studies indicated that the oligomers are composed of β-sheet structures.[18] On the other hand, structural information about Aβ amyloid fibrils has gradually increased, and the fibrils are thought to adopt cross-β-assembly with well-defined structures. In early stages of structure determination, parts of the Aβ sequence, such as residues 11−25 and 10−35, examined were found to be well-ordered antiparallel β-sheet structures.[19,20] In contrast, structures of Aβ1−40 and Aβ1−42 fibrils were determined to form parallel β-sheet structures.[21,22] Based on the structural features of amyloid fibrils and the mechanisms of the Aβ assembly, several peptides and their analogs have been designed and synthesized toward constructing pharmaceutical compounds. Here, we describe the design of molecules capable of binding to Aβ, thereby inhibiting or accelerating the fibrillization or oligomerization of Aβ using a strategy of peptide and protein mimetics based on the structures of Aβ.

2. Structural Features of the Amyloid Fibrils of Aβ Peptides

Recently, high-resolution structures of amyloid fibrils formed by not only fragmented sequences of Aβ but also Aβ1−40 and Aβ1−42 have been proposed by solid-state NMR techniques.[19−22] The hydrophobic core around residues 16−20 of Aβ is essential to self-assemble into amyloid fibrils, and therefore short peptides containing the core region are synthesized and evaluated for fibril-forming properties. Aβ11−25 peptide can form amyloid fibrils exhibiting a highly

ordered structure, which can be stabilized by electrostatic and hydrophobic interactions with an antiparallel orientation of the β-sheet structures.[19] In contrast to the fragment peptides, residues 1−17 are disordered in full-length Aβ1−42 fibrils, while residues 18−42 form a β-strand−turn−β-strand structure.[22] Parallel and in-register β-sheets are formed by intermolecular interactions. The proposed structural model depicts hydrophobic interactions between Leu17, Phe19, and Ala21 of β-strand 1 (β1; residues 18−26), as well as Leu34, Val36, and Val40 of β-strand 2 (β2; residues 31−42). Of note, the interactions between β1 and β2 are made by intermolecular contacts, which function to stabilize the fibril structures. Aβ1−40 fibrils have also been analyzed using a solid-state NMR technique;[21] however, the structure of Aβ1−40 fibrils differed slightly from that of Aβ1−42. With Aβ1−40 fibrils, residues 1−10 are approximately disordered, and residues 12−24 (β1) and 30−40 (β2) form parallel β-sheet structures with similar topology to Aβ1−42 fibrils. The hydrophobic cluster is created by intermolecular interactions between Leu17 and Phe19 of β1 and Ile32, Leu34, and Val36 of β2. The model structures for both Aβ1−42 and Aβ1−40 fibrils providing a bent structure with an electrostatic interaction between the Asp23 and Lys28 indicate that hydrophobic and electrostatic interactions might increase the stability of the overall amyloid fibril structures. The structural models of Aβ amyloid fibrils are useful in the design of compounds capable of binding to Aβ.

3. Peptide Mimetics for Inhibitor Design of Aβ Fibrillization

Recent progress in elucidating the structural properties of Aβ amyloid fibrils has enabled the design of inhibitors of Aβ amyloid fibril formation.[23−30] The hydrophobic core around residues 11−25, positioned at the β1 region in the structural model of Aβ1−40 fibrils, is crucial for the self-assembly of Aβ into fibrils, and short peptides based on the core sequence were designed to bind to full-length Aβ. Even the pentapeptide sequence involving residues 16−20, Lys-Leu-Val-Phe-Phe (KLVFF), and 17−21, Leu-Val-Phe-Phe-Ala (LVFFA), can bind to the Aβ molecule and therefore be used as "a binding element" to design inhibitors of the fibrillization (Figure 2). Analog peptides of KLVFF having various lengths of the aminoethoxy ethoxy acetic acid (Aeea) unit as a hydrophilic moiety were designed.[24] Increasing the Aeea unit lengths increased the hydrophilicity of the peptide and effectively inhibited the amyloid fibril formation. Moreover, conjugating oligolysine and oligoglutamic acid units with the KLVFF peptide was a useful means of generating binders to Aβ with the

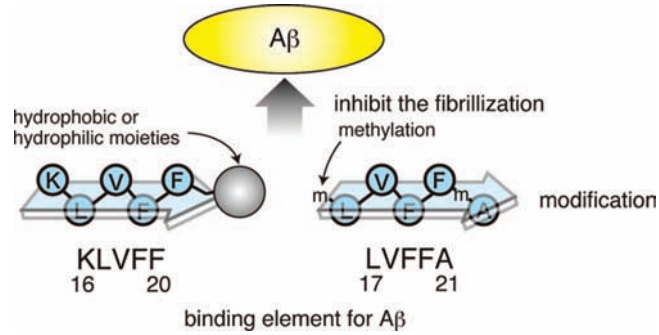

FIGURE 2. Schematic representation of designing the Aβ binding molecules. Two pentapeptides, extracted from the core region for the fibrillogenesis of the Aβ sequence, have been used as binding elements. Several compounds are designed based on these short peptides providing several lead compounds against AD.

resultant formation of large aggregates that might have less cell toxicity.[25−27] The oligolysines as disrupting elements for Aβ toxicity were effective when the length was three or more residues. In contrast, conjugation of a neutral polar amino acid, oligoserine, was ineffective at enhancing or inhibiting Aβ aggregation and toxicity. However, the opposite result with respect to the activity of the KLVFF−KKKKKK peptide was reported: this peptide promoted the protofibril association but the association was ineffective in decreasing cell toxicity of Aβ as determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as a monitoring reagent for the cell viability.[31] On the other hand, a KLVFF tetramer with a dendrimer scaffold was constructed and found to inhibit the transformation of soluble oligomers and protofibrils into fibrils of Aβ1−42 and to promote the disassembly of existing aggregates.[28] A multivalent effect using the dendrimer structure significantly increased the inhibitory activity of the KLVFF binding element.

Conjugation of hydrophobic moieties, not hydrophilic ones such as Aeea and lysine, with Aβ binding elements has been attempted to construct inhibitors of Aβ aggregation. A peptide with a cholic acid as a hydrophobic moiety containing the LVFFA sequence strongly inhibited the fibrillization of Aβ.[29] Moreover, an all-D analog of the cholyl-LVFFA sequence can also bind to Aβ and prevent the fibrillization of Aβ. Based on the LVFFA sequence, a peptide Leu-Pro-Phe-Phe-Asp (LPFFD) containing a proline residue, which is known to be a β-sheet breaker, was designed and observed to inhibit the fibrillization of Aβ1−40 and Aβ1−42.[23] Since proline lacks a proton on nitrogen, hydrogen bonding cannot occur between the β-sheet structures. Thus, once the LPFFD sequence binds to Aβ, the fibril extension ceases. In a similar strategy, methylation of amide groups is also an effective means by which to design inhibitors. A number of N-methylated peptides based on the

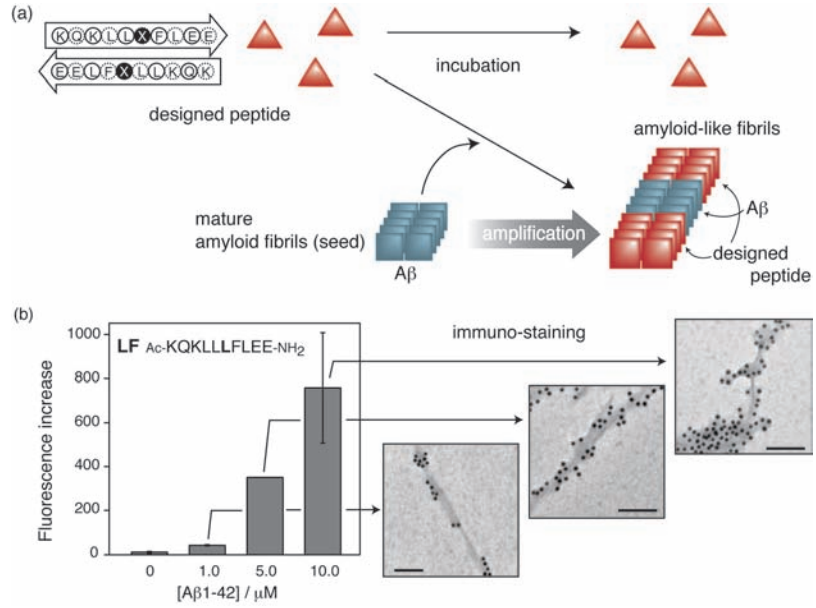

FIGURE 3. (a) Schematic illustration of amplification of the mature amyloid fibrils of Aβ using the designed peptides. Red triangles represent the deigned peptides such as the LF and VF peptides, and red and blue squares represent amyloid-like fibrils. (b) Fluorescent increase of ThT before and after incubation of the LF peptide (100 μM) in the presence and absence of mature Aβ1−42 fibrils at concentrations of 0, 1, 5, and 10 μM Aβ monomer. TEM images (right) show the LF amyloid-like fibrils coassembled with Aβ fibrils stained using 6E10 anti-Aβ antibody and gold nanoparticles. Scale bar = 100 nm.

structure of the KLVFF sequence have been systematically synthesized and analyzed with regard to their ability to function as inhibitors of fibrillization and their effect on the Aβ cell toxicity.[30] The analog peptide D-[chGly-Tyr-chGly-chGly-mLeu]-NH_2 (ch = cyclohexyl, mLeu = *N*-methyl leucine) demonstrates marked inhibitor activity. The *N*-methyl group can increase aqueous solubility of the peptides and reduce inhibitor toxicity. The methylation sites of the peptides also affect the activity of the peptides. These short peptides can be available as a lead compound for and provide valuable insight into the design of inhibitors of Aβ toxicity.

4. Design of Peptides Coassembling with Aβ Fibrils

With regard to the fibrillization of peptides and proteins, it is thought that preformed fibrils promote fibrillization by functioning as nuclei.[12,13] For example, although monomeric Aβ1−40 is difficult to self-assemble into amyloid fibrils, the seed fibrils can accelerate the fibril formation of Aβ1−40.[32,33] On the other hand, thioflavin T (ThT) is a fluorescent reagent that is commonly used to detect amyloid fibrils,[34] but sufficient amounts of fibrils are necessary for the detection. In order to increase the sensitivity of ThT assay, the designed peptides capable of forming amyloid-like fibrils were available (Figure 3).[35] Amplification of Aβ fibrils using peptides designed to associate with Aβ amyloid is an effective means by which to increase the sensitivity of ThT for detection of amyloid fibrils. Binding of the designed peptides to Aβ amyloid fibrils composed of β-sheet structures was anticipated to induce the formation of amyloid-like peptide fibrils from nuclei of the Aβ amyloids.

A 10-residue peptide corresponding to residues 14−23 of Aβ forms amyloid fibrils composed of antiparallel β-sheet structures.[19,36] The electrostatic interaction made by His14 and Asp23 promotes the antiparallel orientation and stabilizes the fibril structure. The insight that provides into the structural basis of Aβ fibrillogenesis creates a foundation for the further design of molecules capable of coassembling with Aβ fibrils. A number of peptide sequences, Ac-Lys-Gln-Lys-Leu-Leu-X-Phe-Leu-Glu-Glu-NH_2 (X = Leu, Val, Phe, Tyr, Ala, and Thr, named as LF, VF, FF, YF, AF, and TF, respectively) were designed by varying the hydrophobicity of each sequence with the expectation that different hydrophobicities might alter the potential for fibrillization and amplification of the Aβ fibrils (Figure 3). The idea is to simplify the amino acid sequences to gain insight into the requirements for self-assembly by maintaining the sequence similarity to Aβ.[37−39] A histidine residue, which is a cationic amino acid, was changed to lysine, and an aspartic acid residue, an anionic amino acid, was changed to glutamic acid. The central hydrophobic amino acids, including residues 17−21 of Aβ14−23 (LVFFA), were changed to leucine with retention of Phe20.

The propensity for fibrillization of the designed peptide alone was evaluated using a ThT fluorescent assay in order to measure the Aβ amyloids in a buffer containing 10% trifluoroethanol as an enhancer of fibrillization.[40] Three hydrophobic peptides containing leucine, valine, and phenylalanine at position X exhibited greater ThT fluorescence corresponding to amyloid-like fibril formation than the peptides having alanine, threonine, and tyrosine at position X. In particular, the LF peptide resulted in greater amyloid-like fibril formation than the other designed peptides. VF peptide had a reduced ability to promote fibril formation. The different hydrophobicities of the designed peptides resulted in a variety of fibrillization abilities. Two peptides, LF and VF, with strong and weak fibrillization abilities, respectively, were used to amplify small amounts of mature Aβ1−42 fibrils. When large amounts (i.e., 100 μM) of LF and VF peptides in the presence of small amounts (i.e., 1−10 μM/monomer) of Aβ1−42 fibrils were incubated in a buffer at 37 °C for several hours, ThT fluorescence increased dramatically. In contrast, the ThT fluorescence increased only slightly in the absence of the mature Aβ1−42 fibrils. On the other hand, the FF peptide, capable of fibril formation as indicated by enhanced ThT fluorescence, did not effectively amplify the amyloid fibrils. These findings indicate that the LF and VF peptides, but not FF, form amyloid-like fibrils using Aβ1−42 fibrils as nuclei. Moreover, the use of mature Aβ1−42 fibrils as nuclei clearly enhanced the formation of amyloid-like fibrils by the designed peptides in a concentration-dependent manner (Figure 3).

To confirm that mature Aβ1−42 fibrils existed as nuclei within LF and VF amyloid-like fibrils, an immunostaining assay was successfully performed.[35] The amyloid-like fibrils of LF and VF peptides produced using Aβ1−42 fibrils as nuclei were stained with biotinylated 6E10 anti-Aβ antibody, which can recognize residues 1−16 of Aβ,[41] and gold nanoparticle−streptavidin conjugate. Gold dots on the transmission electron microscope (TEM) grid were clearly observed along the peptide fibrils (Figure 3). The observed gold dots were well-localized rather than evenly distributed along the fibrils, presumably due to seeding of previously aggregated Aβ1−42 fibrils as nuclei. Moreover, the fibril structures of the designed peptides differed markedly depending on whether they formed in the presence or absence of Aβ1−42 fibrils. The peptide fibrils of LF or VF alone, which were unable to enhance the ThT fluorescence, had flat tape-like morphologies with short lengths (0.2−1.5 μm in lengths), while the amyloid-like fibrils of LF and VF peptides formed in the presence of Aβ1−42 fibrils had long tangled fibrils, similar to the amyloid fibrils of Aβ itself. LF and VF peptides, designed to share structural similarities with Aβ, were observed to bind to Aβ fibrils by forming β-sheet structures. Once the peptide binds to the Aβ fibrils, bound peptide on the Aβ1−42 fibrils also initiates fibrillization by functioning as a nucleus after which amyloid-like fibrils of the designed peptides can dramatically increase depending on the amount of Aβ1−42 fibrils available. Thus, the designed simple peptides can be available as an indicator for existing mature Aβ1−42 fibrils. This strategy for detecting the small amounts of Aβ1−42 fibrils might also be used to screen for peptide-based compounds capable of binding to Aβ1−42 fibrils.

5. Designed Peptides Capturing Soluble Oligomers of Aβ1−42

The Aβ molecule self-assembles into amyloid fibrils via a variety of oligomers of Aβ,[42−46] which are thought to be toxic, resulting in the development of AD.[47−50] ADDLs are toxic to rat pheochromacytoma PC12 cells examined by MTT assay[47] and induce neuronal oxidative stress through a mechanism requiring *N*-methyl-D-aspartate receptor (NMDA-R) activation.[51] The soluble oligomers of both Aβ1−40 and Aβ1−42 also showed toxicity to human neuroblastoma SH-SY5Y cells, while the fibril forms of the Aβ peptides exhibited lower toxicity.[52] Aβ*56, a 56 kDa soluble amyloid-β assembly purified from the brain in impaired *Tg2576* transgenic mice, disrupts memory when administered to young rats.[17] Moreover, high expression of Aβ1−42 in transgenic mice generates synaptotoxicity without significant amyloid plaque formation, indicating that the soluble oligomers, not fibrils, cause the neuronal cell death.[53] Therefore, much effort has been devoted to suppressing the oligomer formation of Aβ. In contrast to this inhibition strategy for Aβ oligomerization, acceleration of the fibrillogenesis of Aβ has also been shown to reduce toxicity. If toxic oligomers can be quickly converted to mature fibrils, the toxicity of Aβ might be reduced. The LF peptide can be used as a trapping reagent, thereby converting Aβ1−42 oligomers into amyloid-like fibrils (Figure 4).[35] Incubation of large amounts (i.e., 100 μM) of the LF peptide with small amounts of Aβ1−42 oligomers (i.e., 1−10 μM/monomer) in a buffer produced amyloid-like fibrils immediately, which mirrors the result using mature Aβ1−42 fibrils as nuclei. Thus, the soluble oligomers can function as an inducer of the fibrillization. LF peptide captures the Aβ1−42 oligomers and accelerates the formation of amyloid-like fibrils. Size-exclusion chromatography (SEC) analysis has revealed that Aβ1−42 oligomers disappear after 2 h of incubation of LF with Aβ after centrifugation to remove mature amyloid-like fibrils. An

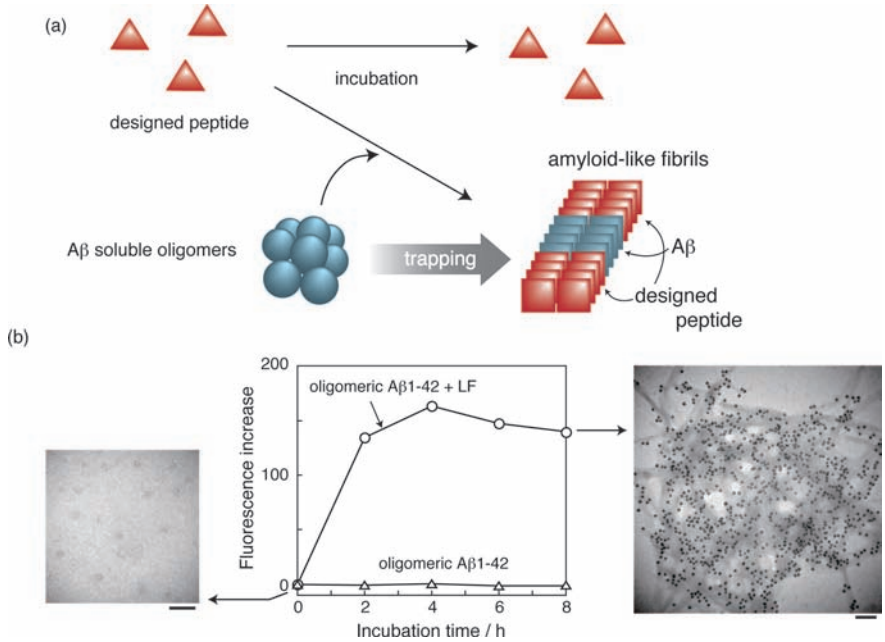

FIGURE 4. (a) Schematic illustration of amplification of the soluble oligomers of Aβ using the designed peptides. Red triangles represent the designed peptides LF, and blue spheres represent Aβ oligomers. (b) Time course of ThT fluorescence. Aβ1−42 soluble oligomers (10 μM/monomer) were incubated with (○) and without (△) LF (100 μM). TEM images show the soluble Aβ oligomers alone (left) and incubated with LF (right) stained by 6E10 anti-Aβ antibody and gold nanoparticles. Scale bar = 100 nm.

enzyme-linked immunosorbent assay (ELISA) using 6E10 anti-Aβ antibody demonstrated that Aβ1−42 oligomers completely disappear in the supernatant. Since the soluble oligomers of Aβ1−42 are metastable, while the mature fibrils of Aβ are a final product of the aggregation reaction, the reactivity of metastable Aβ1−42 oligomers toward the LF peptide exceeds that of mature amyloid fibrils. The presence of Aβ1−42 molecules within amyloid-like fibrils was confirmed by immunostaining with gold nanoparticles, as shown in Figure 4. Nanoparticles staining the Aβ oligomers−peptide fibrils were more uniformly distributed than on LF and mature Aβ1−42 fibrils as described above. Since the size of the gold particles (10 nm diameter) was similar to that of large Aβ1−42 oligomers, one gold particle was capable of binding to one Aβ oligomer. Therefore, the particles might appear well-dispersed on the assembled LF fibrils. By this strategy, the LF peptide is also used to transfer toxic Aβ oligomers into amyloid-like fibrils of the designed peptide, thereby reducing toxicity in a PC12 cell assay system.[54]

6. Protein Mimetics of Inhibition of Aβ Oligomerization

Molecules capable of binding to Aβ may not only prevent Aβ aggregation but also neutralize the toxicity of Aβ to neuronal cells. This would require binding of molecules to Aβ with high affinity and inhibition of oligomerization in the early stages of aggregation. Therefore, antibodies against Aβ are potential drug candidates against AD. Some anti-Aβ antibodies can inhibit Aβ oligomerization and neutralize Aβ toxicity *in vivo*.[55,56] Thus, the construction of proteins capable of binding to Aβ with high affinity similar to anti-Aβ antibodies is a valid research approach in the treatment of AD.

Residues 14−23 of Aβ represent an important region for amyloid fibril formation of the Aβ molecule and participate in the formation of amyloid β-sheet structures.[19] In addition, the region is thought to represent "a binding element" of Aβ as described above. During aggregation, Aβ recognizes itself, probably using surface residues on parallel β-sheets. Proteins that share part of the Aβ-like β-sheet structure may bind to Aβ in a similar manner as to Aβ itself. In the case of simple short peptide sequences, such as the pentapeptide sequence described above, the peptides can form only one β-strand, and it may be difficult to bind tightly to Aβ. In contrast, protein surface engineering might generate high-affinity binding sites for Aβ by enabling two or more binding elements for the Aβ molecule to be incorporated. On the other hand, green fluorescent protein (GFP), which is commonly used as a fluorescent tool in bioengineering and molecular biology, folds into a β-barrel structure composed of eleven β-strands, ten of which are aligned in an antiparallel arrangement and one that is oriented in a parallel fashion.[57] Since GFP has a stable structure, it can be engineered on its surface residues. The first and

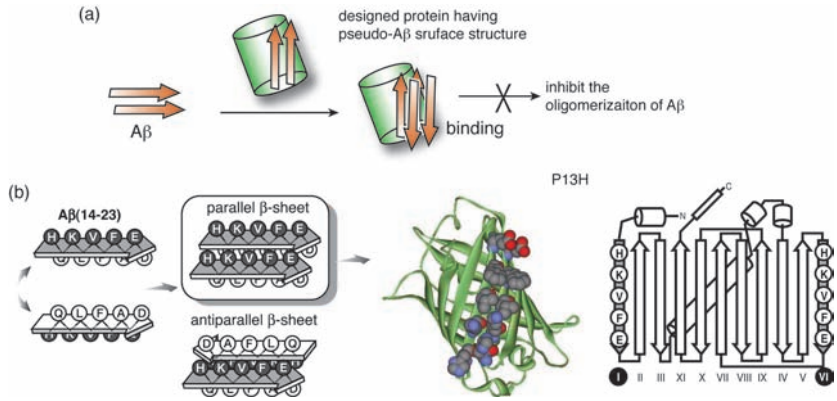

FIGURE 5. (a) Schematic representation of designed proteins embedding Aβ binding structure into its surface capable of binding to Aβ and inhibiting the oligomerization of Aβ in early stage. (b) Model image of the protein P13H designed using GFP as a structural scaffold.

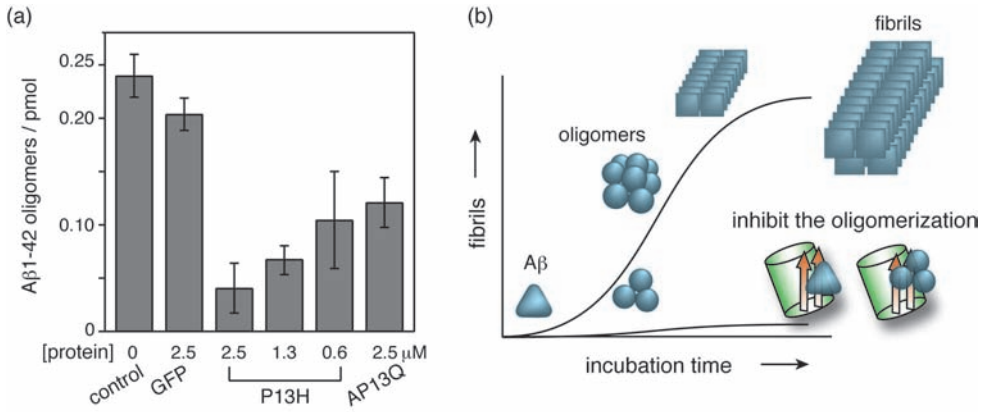

FIGURE 6. (a) Inhibition of Aβ1−42 oligomerization by GFP and P13H. The amounts of Aβ1−42 oligomers were calculated from the data of ELISA. Aβ1−42 (10 μM) was incubated with or without GFP and P13H. (b) Schematic illustration of the inhibition for Aβ oligoemerization using P13H.

sixth strands of GFP are parallel and were therefore chosen to function as a parallel β-sheet model of the Aβ structure (Figure 5).[58] Two β-strands corresponding to the β1 regions of the amyloid fibril models[21] were incorporated into GFP. Ten surface amino acids on the first and sixth strands of GFP (Pro13, Leu15, Glu17, Asp19, Asp21, Thr118, Val120, Arg122, Glu124, and Lys126) were substituted for amino acids corresponding to residues 14−23 of Aβ (His14, Lys16, Val18, Phe20, and Glu22) in the GFP variant referred to as P13H. Short peptide binders for Aβ designed based on the structure of Aβ were capable of interacting with Aβ via side-chain contacts and hydrogen bonds between the backbone amides of the peptides and Aβ. Thus, the short peptides can also self-assemble by forming β-sheet structures. With P13H, however, the β-barrel structure of P13H protects backbone amides at the engineered mutation sites from self-association. This ability to create a highly structured protein is a great advantage in the design of inhibitors of early Aβ oligomerization.

CD and fluorescence studies of P13H revealed that the GFP variant has a native-like structure and fluorescence, and thus substitution of the surface amino acids on GFP did not affect its structural properties. This indicates that P13H contains pseudo-Aβ β-sheets on its surface capable of recognizing the Aβ molecule. Moreover, SEC analysis was performed to evaluate the association properties of P13H. P13H protein demonstrated almost a peak corresponding to the monomer state of the protein, even though P13H has a pseudo-Aβ surface. With 6E10 anti-Aβ antibody and its biotinylated derivative, whether P13H can inhibit the Aβ oligomerization in early stage was examined (Figure 6).[59] Although incubation of Aβ (10 μM) increased the oligomers of Aβ, incubation of Aβ mixed with P13H (2.5 μM) clearly repressed the oligomer formation of Aβ to 16% of control levels. Specifically, inhibition of Aβ oligomerization by P13H was observed to occur in a concentration-dependent manner, although even low concentrations of P13H (0.6 μM)

inhibit oligomerization to 40% of control levels. The P13H protein can therefore inhibit oligomerization in a substoichiometric manner. It seems that P13H binds not only to the monomeric state of the Aβ1−42 molecule but also to dimers, trimers, and tetramers, including the smallest undetectable oligomers, thereby effectively preventing further oligomerization. The dissociation constant of P13H with Aβ was calculated as $K_d = 260$ nM using a surface plasmon resonance (SPR) method. A GFP variant in which a pair of antiparallel β-sheets of Aβ were embedded into GFP, referred to as AP13Q, was also designed.[58] However, AP13Q demonstrated less inhibition of Aβ oligomerization and Aβ binding ($K_d = 2.4$ μM), compared with P13H, showing that the parallel β-sheet orientation of a pseudo-Aβ surface on GFP is superior to recognize the Aβ and to prevent its oligomerization.

6. Conclusion

A number of peptide-based compounds have been designed to mimic the Aβ structure, some of which effectively inhibit Aβ aggregation. In particular, two short peptides, KLVFF and LVFFA, known to contain key sequences for Aβ fibril formation, have potential therapeutic benefit. Recent studies suggest that metastable Aβ aggregates including soluble oligomers and protofibrils are the most toxic species in AD. Therefore, the ability to convert toxic metastable oligomers into amyloid fibrils might decrease the toxicity of Aβ to neuronal cells. A designed peptide LF having sequence Ac-KQKLLLFLEE-NH_2 forms amyloid-like fibrils that efficiently coassemble with mature Aβ1−42 fibrils and soluble oligomers of Aβ efficiently, thereby reducing the toxicity of Aβ to a mammalian cell. On the other hand, a designed protein having a pseudo-Aβ β-sheet structure to mimic the Aβ structure of amyloid fibrils inhibits Aβ oligomerization in early stages.

The designed peptides and proteins have not been utilized directly for constructing therapeutic agents against AD. One of the major problems in drug development for AD is the blood−brain barrier (BBB) permeability.[60] Recent studies, however, have revealed that the anti-Aβ antibodies can reduce the amyloid deposits and lead to improvement of cognitive functions of AD patients despite the low permeability of antibodies at the BBB generally. The antibody might promote efflux of Aβ from brain to plasma.[61] Interestingly, polymeric nanoparticles have been constructed for drug delivery materials across the BBB targeting AD.[62] Progress in the delivery agents would promote the availability of designed peptides and proteins to drug development targeting Aβ.[62] Combining with these research developments, peptide and protein mimetics based on Aβ structures during various misfolding states might lead to the identification of compounds with a therapeutic benefit in a range of amyloid disorders, including AD.

BIOGRAPHICAL INFORMATION

Tsuyoshi Takahashi was born in Kanagawa, Japan, in 1972. He received his B.S. degree in 1996, M.S. in 1998, and Dr. Eng. in 2001 at Tokyo Institute of Technology under the supervision of associate professor Hisakazu Mihara. He was a research fellow of the Japan Society for the Promotion of Science (JSPS) for Young Scientist from 2000 to 2002. At present, he is an assistant professor at Tokyo Institute of Technology from 2002. His current research interest is design of proteins capable of reducing Aβ toxicity and detecting Aβ aggregates *in vivo*.

Hisakazu Mihara was born in Ube, Japan, in 1958. He received his Doctor of Science degree in chemistry from Kyushu University under the direction of N. Izumiya in 1986. He was a postdoctoral fellow in E. T. Kaiser's group at the Rockefeller University, New York (1986−1988). He was appointed as an assistant professor in applied chemistry at Kyushu Institute of Technology in 1988 and was promoted to an associate professor in applied chemistry at Nagasaki University in 1993 and in bioengineering at Tokyo Institute of Technology in 1995. Currently, he is a professor in the Graduate School of Bioscience and Biotechnology (since 2005). His research interests include peptide design and synthesis, *de novo* design of artificial proteins, and peptide structure−function relationships, including amyloid fibers and also peptide microarrays.

FOOTNOTES

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