The aggregation of peptides/proteins is intimately related to a number of human diseases. More than 20 have been identified which aggregate into fibrils containing extensive β-sheet structures, and species generated in the aggregation processes (i.e., oligomers and fibrils) contribute to disease development. Amyloid-β peptide (designated Aβ), related to Alzheimer’s disease (AD), is the representative example. The intensive aggregation property of Aβ also leads to difficulty in its synthesis. To improve the synthetic problem, we developed an O-acyl isopeptide of Aβ1–42, in which the N-acyl linkage (amide bond) of Ser26 was replaced with an O-acyl linkage (ester bond) at the side chain. The O-acyl isopeptide demonstrated markedly higher watersolubility than that of Aβ1–42, while it quickly converted to intact monomer Aβ1–42 via an O-to-N acyl rearrangement under physiological conditions. Inhibition of the pathogenic aggregation of Aβ1–42 might be a therapeutic strategy for curing AD. We succeeded in the rational design and identification of a small molecule aggregation inhibitor based on a pharmacophore motif obtained from cyclo[-Lys-Leu-Val-Phe-Phe-]. Moreover, the inhibition of Aβ aggregation was achieved via oxygenation (i.e., incorporation of oxygen atoms to Aβ) using an artificial catalyst. We identified a selective, cell-compatible photo-oxygenation catalyst of Aβ, a flavin catalyst attached to an Aβ-binding peptide, which markedly decreased the aggregation potency and neurotoxicity of Aβ.

Key words amyloid; peptide; aggregation; Alzheimer’s disease; inhibitor; catalyst

1. Introduction

Amyloid-β (Aβ), comprising mainly 40- and 42-residue peptides (designated Aβ1–40 and Aβ1–42, respectively, for the amino acid sequences; see Fig. 1), is the primary pathogenesis underlying Alzheimer’s disease (AD). AD is an age-related neurodegenerative disorder, and affected individuals exhibit progressive memory loss and cognitive impairment. Thirty million people worldwide are estimated to have the disease, and the number is predicted to increase to 106 million by 2050. Senile plaques in the brain, one of the pathological hallmarks of AD, are formed by the accumulation of aggregated Aβ with an extensive β-sheet structure. Aβ is produced from amyloid precursor protein through sequential cleavages by β- and γ-secretases. Although the precise etiology of AD remains unclear, the aggregation process of monomer Aβ to oligomers and successively to fibrils is associated with the onset of neurotoxicity. Although Aβ1–40 is the predominant product, Aβ1–42 is far more aggregative, neurotoxic, and pathogenic.

Therapeutic approaches targeting the aggregative, pathogenic Aβ are thus one promising direction to overcome AD. Non-Aβ targets, such as tau-derived neurofibrillary tangles and neuroinflammation, are concomitantly considered important targets. An antibody to Aβ would be the leading candidate such that several monoclonal antibodies have been tested in clinical trials, although their ultimate efficacy in treating AD remains unclear. Inhibitors of the secretases, Aβ-producing enzymes, have been also extensively studied. It is, however, essential to explore new concepts continuously to attenuate the pathogenicity of Aβ, to increase the probability of success of anti-AD therapy.

2. Construction of Aβ1–42

The intensive aggregation property of Aβ1–42 constitutes barriers to chemical synthesis of the peptide. Insufficient peptide coupling–deprotection reactions during solid phase peptide synthesis (SPPS) occurs owing to the aggregation of the on-resin constructing Aβ chain. Upon purification using HPLC after SPPS, the aggregation of Aβ1–42 obstructs the elution of the peptide from the reverse-phase column, resulting in a broad, inseparable peak. In order to produce Aβ1–42 efficiently, therefore, we set out to develop a synthetic method suited for the peptide.

2.1. O-Acyl Isopeptide Method

When the N-acyl linkage on a hydroxyamino acid residue (e.g., Ser and Thr) was replaced with an O-acyl linkage at the side chain, synthesis of the resulting species (referred to as an O-acyl isopeptide, the structure taken by Aβ, for example; see Fig. 1) was found to suppress the formation of by-products derived from aggregation during SPPS. The O-acyl moiety interfered with the

Review

Medicinal Chemistry Focusing on Aggregation of Amyloid-β

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formation of the β-sheet structure that leads to aggregation. In addition, due to the presence of the protonated amino group, the O-acyl isopeptide was water-soluble. Thus, it allows for efficient purification by reverse-phase HPLC, because the O-acyl isopeptide has favorable solubility in polar solvents and elutes as a sharp peak. Finally, the synthesized O-acyl isopeptide can be converted to the target peptide via an O-to-N acyl migration reaction under neutral-to-slightly basic pH conditions (Fig. 1).

The synthetic efficacy of the O-acyl isopeptide method has been demonstrated with various peptides/proteins, including human insulin, of which synthesis in the conventional manner was difficult. Moreover, coupling of the O-acyl isopeptide as a building block by the segment condensation method or chemical ligation yielded the long-chain polypeptide as the O-acyl form, which enabled synthesis of hydrophobic, and thus synthetically challenging, proteins. For example, amylin, transmembrane domain peptide, saposin-C, Tim-3 immunoglobulin domain, S100A4 calcium-binding protein, and interleukin-2 were successfully synthesized in this manner. In addition, numerous applications of the O-acyl isopeptide as a precursor of the target peptide, in a similar manner to those of Aβ and amylin (see below), have been reported. Thus, the O-acyl isopeptide can serve as a means to mask the function of the parent peptide temporarily, which can then be regained by triggering the O-to-N acyl migration. Molecular mechanisms of amyloid formation, modulator protein of DNA-binding (Id) proteins, have been received several awards, including the Dr. Bert L. Schram Award (2004), the Distinguished Young Scientist Award of The Japanese Peptide Society (2006), the Young Scientist’s Research Award in Natural Product Chemistry (2011), and the Pharmaceutical Society of Japan Award for Young Scientists (2015). Dr. Sohma’s research interests are the synthesis of functional peptides and proteins oriented to medicinal chemistry.

Youhei Sohma was born in 1979 in Osaka, Japan, and received his bachelor’s degree from Kyoto Pharmaceutical University in 2001. He obtained a Ph.D. from Kyoto Pharmaceutical University in 2005 under the direction of Professor Yoshiaki Kiso. He then moved to the University of Chicago, U.S.A., as a research associate in the group of Professor Stephen B. H. Kent. In 2009, he returned to Japan and joined Professor Yoshiaki Kiso’s group at Kyoto Pharmaceutical University as an assistant professor. In 2012, Dr. Sohma joined Professor Motomu Kanai’s group at The University of Tokyo as a lecturer and group leader of the Kanai Life Science Catalysis Project, ERATO, JST. He has received several awards, including the Dr. Bert L. Schram Award (2004), the Distinguished Young Scientist Award of The Japanese Peptide Society (2006), the Young Scientist’s Research Award in Natural Product Chemistry (2011), and the Pharmaceutical Society of Japan Award for Young Scientists (2015). Dr. Sohma’s research interests are the synthesis of functional peptides and proteins oriented to medicinal chemistry.
and stromal cell-derived factor-1α were studied using the corresponding O-acyl precursors. Moreover, the O-acyl isopeptide unit was incorporated as a part of functional peptides, which can control the intracellular localization of a target molecule and protein splicing to regulate protein catalysis. A unique application of the O-acyl isopeptide involves the efficient preparation of robust nanofibers, an important issue in the field of material science.

2.2. O-Acyl Isopeptide of Aβ1–42 The O-acyl isopeptide of Aβ1–42, which has an ester bond between Gly and Ser (Fig. 1), was efficiently constructed using SPPS and easily purified by reverse-phase HPLC thanks to the sharp elution profile with reasonable water solubility. Carpio et al. also reported a positive result with the synthesis of Aβ1–42. This suggests that only one insertion of the isopeptide structure into the 42-residue peptide can suppress the aggregation nature of the peptide. On a relevant note, the O-acyl isopeptide possessed inhibitory activity against the aggregation of native Aβ1–42. The O-acyl isopeptide could convert intact Aβ1–42 quantitatively with a half-life ($t_{1/2}$) of ca. 10 s, via the O-to-N acyl rearrangement, under physiological conditions (Fig. 1).

We considered that the O-acyl isopeptide of Aβ1–42 possessed important features as a water-soluble precursor of native Aβ in regard to biological study. The intense aggregative nature of Aβ1–42 causes difficulties in preparing monomeric Aβ1–42, which result in irreproducible or discrepant study outcomes. An in situ system that prepares intact, monomeric Aβ1–42 under physiological conditions, while suppressing the aggregation under storage conditions would therefore be a useful experimental tool. The water-soluble O-acyl isopeptide of Aβ1–42 adopted and retained a monomer random coil state under acidic conditions. As a result of the rapid O-to-N conversion, monomer Aβ1–42 with a random coil structure could thus be produced in situ.

A photo-triggered production system of Aβ1–42 via the O-acyl isopeptide was also prepared. In addition, the O-acyl isopeptides of various Aβ derivatives, i.e., E22A-type and [Pyr11]Aβ1–42, were utilized to identify new functions of the Aβ species. Other research groups have also utilized the O-acyl isopeptide for a wide range of Aβ studies. Moreover, the O-acyl isopeptide of amylin, a 37-residue amyloid peptide associated with type II diabetes mellitus, was reported. Thus, the effective preparation of Aβ1–42 via the O-acyl isopeptide allowed us to conduct medicinal chemistry studies focusing on the aggregation of Aβ1–42.

3. Aggregation Inhibitors

Inhibition of pathogenic Aβ aggregation may be an effective, straightforward therapeutic strategy. The molecules that can inhibit the aggregation of Aβ are therefore candidate drugs. Most of the small-molecules possessing inhibitory activities against Aβ aggregation are dyes or polyphenol derivatives and, because these compounds bind non-selectively to a wide range of biomolecules, there is high potential for various side effects. Logical structural optimization to enhance Aβ specificity is difficult, however, due both to the unknown three-dimensional structures of Aβ aggregates (especially oligomers) and the transient, unstable Aβ conformation during aggregation.

An approach based on the primary structure of Aβ would be advantageous in logical potentiation of aggregation inhibitors. We focused attention on a report that a Lys–Leu–Val–Phe–Phe (KLVFF) peptide corresponding to the Aβ16–20 fragment, a region that plays a critical role in generating Aβ fibrils by forming a core β-strand structure, can bind to full-length Aβ and inhibit its aggregation. The fragment peptide is thought to interfere with Aβ aggregation by binding to the specific self-recognition region of Aβ via hydrogen bonding and hydrophobic interactions.

We derivatized KLVFF to a macrocyclic structure, designated cyclo[KLVFF] (1) to facilitate the identification of pharmacophores by stabilizing the active conformation and conducted extensive structure–activity relationship studies on the cyclic peptide (2). The aggregation inhibitory activity of 1 was significantly higher than that of linear KLVFF. Interestingly, the inhibitory activity of the enantiomer, cyclo-α-[KLVFF] (3), was similar to that of 1. Side chains of the Leu, Val, and two Phe residues could be overlaid between 1 and 2 by reversing the backbone amide bonds. Thus, we hypothesized that these side chains contributed substantially to the activity of the cyclic peptides. A retro-inverso derivative, i.e., the chirality of Ca and the order of the amino acid sequence are reversed of 2, cyclo[FFVLK] (3), completely retained the inhibitory activity. All five side chains of 2 can be overlaid with those of 3 despite reversing the direction of the amide bonds. Substitutions of the d-amino acids of 2 with l-Ala and the corresponding l-amino acids, respectively, provided useful insights into the contributions of side chain to the structure. Finally, a pharmacophore motif that comprises the side chains of Leu, Val, and two Phe residues to exert potent inhibitory activity was identified from the cyclic KLVFF peptides.

This finding allowed us to design the non-peptidic, small-molecule aggregation inhibitor 4 (Fig. 2). Compound 4 contains an isopentyl carboxamide group (Leu side chain mimic), a benzyl group (Phe side chain mimic), and a phenoxyl (Phe side chain mimic) group arranged at the 2-, 4-, and 6-positions of a pyridine ring core, respectively. Compound 4 exhibited dose-dependent inhibitory activity against Aβ aggregation. Molecular modeling suggested that the isopentyl group and the two phenyl groups of 4 can be overlaid with those of the cyclic peptide 1. We further identified 5 (pyridine−pyrimidine and isopentyl−2-adamantyl) with increased inhibitory activity to an extent similar to that of the original cyclic KLVFF peptide. To the best of our knowledge, this is the first rational design of non-peptidic, small-sized molecule aggregation inhibitors of Aβ starting from a peptidic structure.

A further increase in the inhibitory activity is necessary. The high specificity for Aβ, which minimizes the possibility of side effects, and favorable pharmacokinetic profiles in vivo are also required for the inhibitors. Studies to address these issues are ongoing by our group.

4. Catalytic Photo-oxygenation of Aβ1–42

Because the aggregation property of Aβ depends on thermodynamics in its interaction with aqueous medium, covalent installation of hydrophilic oxygen atoms to Aβ would alter the pathogenic aggregation potency. We therefore embarked on the transformation using aerobic oxygen and visible light as oxygen atom and energy sources, respectively, in the presence of a catalyst (i.e., catalytic photo-oxygenation).
We selected riboflavin (vitamin B$_2$, Fig. 3)-catalyzed photo-oxygenation$^{75,76}$ of Aβ. Aβ1–42 was efficiently oxygenated under physiological conditions (pH 7.4, 37°C) using the riboflavin catalyst and visible light irradiation, with modifications at the Tyr$^{10}$, His$^{13,14}$, and Met$^{35}$ residues (Fig. 4A). The oxygenated Aβ1–42 exhibited considerably lower aggregation potency and neurotoxicity compared with native Aβ1–42 (Figs. 4B, C). Interestingly, the oxygenated Aβ also exhibited inhibitory activity against the aggregation and cytotoxicity of native Aβ. Thus, the catalytic oxygenation not only attenuated the original toxicity of native Aβ but also switched the native Aβ to the aggregation inhibitor (i.e., oxygenated Aβ).

When the riboflavin catalyst was used in the presence of cells with photoirradiation, however, most cells died owing to nonselective oxidative damage to them (Fig. 5). Aβ-selectivity is thus necessary for the application of the catalyst to the multi-component system. The result prompted us to develop a flavin catalyst conjugated with a KLVFF derivative as an Aβ-binding tag (catalyst 6, Fig. 3). Using catalyst 6, more than half of cells were alive after photo-irradiation in the absence of Aβ. Furthermore, when using 6 in the presence of Aβ, the cell viability under photoirradiation was significantly higher than that without photoirradiation (Fig. 5). Thus, a flavin catalyst attached to an Aβ-binding peptide was compatible with the living cells and diminished the toxicity of Aβ even in the presence of the cells.

Thus, artificial chemical transformation of toxic aberrant Aβ to less toxic forms at the disease site might be a new therapeutic candidate. Undesired side reactions with off-target biomolecules would be unavoidable in the application of 6 to an in vivo system, however, because the catalyst is always activated during light irradiation. The use of a photocatalyst as a realistic therapeutic approach would demand even higher Aβ selectivity. Another critical point to consider in in vivo application is the light irradiation. If small optic devices were implantable into the brain$^{77}$, a surgical procedure for light irradiation in the brain could be avoidable. In any case, a catalyst that could be excited with more bio-benign, longer wavelength light would be necessary for the application of the catalytic photo-oxygenation approach to patients. We are tackling the development of the catalytic conditions that lead to reactivity with the longer-wavelength light and higher Aβ selectivity suitable for in vivo use.

Fig. 2. Identification of Small-Molecule Aggregation Inhibitor 4 Based on a Pharmacophore Obtained from Cyclic Peptide Derivatives of Aβ16–20

![Fig. 2](image)

Fig. 3. Riboflavin and a Flavin Catalyst Attached to an Aβ-Binding Peptide (6)
5. Conclusion

The aggregation of peptides/proteins is intimately related to a number of human diseases. At least 20 have been identified which aggregate into fibrils containing extensive $\beta$-sheet structures, and species generated in the aggregation processes (i.e., oligomers and fibrils) contribute to disease development. A $\beta$ is the representative example. The aggregation of tau protein, which lies downstream of A $\beta$ pathology, is also associated with AD. Parkinson’s disease is characterized by intracellular deposits of Lewy bodies and neurites, both of which are composed of a protein $\alpha$-synuclein. Amyloid aggregates of insulin, $\beta$2-microgloblin, and transthyretin are pathogenic in injection-localized amyloidosis, hemodialysis-related amyloidosis, and senile systemic amyloidosis, respectively. We succeeded in developing an efficient synthetic methodology for A $\beta$1–42, attenuating the pathogenic aggregation of A $\beta$1–42 by rationally designed small molecules, and covalently introducing oxygen atoms using an artificial catalyst. The basic concepts described in this review may find extensive applications in the treatment of amyloid peptide/protein-associated diseases.

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**Conflict of Interest** The author declares no conflict of interest.

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