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"Click Peptide" Based on the "*O*-Acyl Isopeptide Method": Control of Aβ1−42 Production from a Photo-Triggered Aβ1−42 Analogue

Atsuhiko Taniguchi,[†] Youhei Sohma,[†] Maiko Kimura,[†] Takuma Okada,[#] Keisuke Ikeda,[#] Yoshio Hayashi,*[,†] Tooru Kimura,[†] Shun Hirota,[‡,§] Katsumi Matsuzaki,[#] and Yoshiaki Kiso*[,†]

Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science and Physical Chemistry, 21st Century COE Program, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan, PRESTO, JST, Kawaguchi, Saitama 332-0012, Japan, and Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

Received October 18, 2005; E-mail: kiso@mb.kyoto-phu.ac.jp

A clear understanding of the currently unexplained processes of pathological folding, self-assembly, and aggregation of amyloid β peptide (Aβ) 1−42 would be of great significance in Alzheimer's disease (AD) research.[1] However, elucidation of these Aβ1−42 dynamic events is a difficult issue due to uncontrolled polymerization, which also poses a significant obstacle to establish an experimental system that clarifies the pathological function of Aβ1−42.[2]

A "caged" compound, a synthetic molecule whose biological activity is masked by a covalently attached photocleavable protecting group, is generally considered to be advantageous for studying the dynamic processes of peptides or proteins because, upon photoactivation, only a short duration of time is required to control the spatiotemporal dynamics of the native compounds.[3,4] However, the attachment of a small photocleavable group would not be able to mask the spontaneous self-assemble potency of aggregative peptides since this sort of potency is generally extremely strong and is attributed to the large sections of the peptide structure. To overcome this issue, Imperiali et al. introduced an additional cationic fibril inhibitory unit, which was covalently attached through a photocleavable linker to an aggregative peptide derived from prion protein.[5] This analogue suppressed the self-assembling nature of the original aggregative peptide. However, the density of fibrils formed from the original peptide released by photolysis was insufficient due to a side effect of the co-released fibril inhibitory unit.

Using a different approach to develop a photo-triggered Aβ1−42 analogue with effective inactivation of self-assembling nature, a strategy based on an *O*-acyl isopeptide protected by a photocleavable group was planned. The *O*-acyl isopeptide was expected to be nonaggregative and to be able to convert to the inherent aggregative peptide via a quick and one-way reaction (so-called "click") by photoirradiation without any additional fibril inhibitory unit. Consequently, in this study, we designed and synthesized a photo-triggered "click peptide" of Aβ1−42 (**1**), that is, 26-*N*-Nvoc-26-AIAβ42 (**2**), to establish a novel biological evaluation system in which the activation of the self-assembly process can be readily controlled (Figure 1). This system is crucial in current AD-related research. Recently, we have disclosed an *O*-acyl isopeptide method[6a,b] for the synthesis of difficult sequence-containing peptides and successfully synthesized Aβ1−42 isopeptide, 26-*O*-acyl isoAβ1−42 (26-AIAβ42, **3**, Figure 1),[7] in which a native Gly[25]−Ser[26] amide bond in **1** was isomerized to the β-ester bond. Isopeptide **3** was synthesized efficiently and migrated to aggregative **1** quickly via a pH-dependent O−N intramolecular acyl migration reaction ($t_{1/2}$ = 1 min, pH 7.4, 37 °C) at a hydroxyamino acid residue, such as Ser[26]. Mutter et al.[6c] and Carpino et al.[6d] have also proved the efficacy of the *O*-acyl isopeptide method.

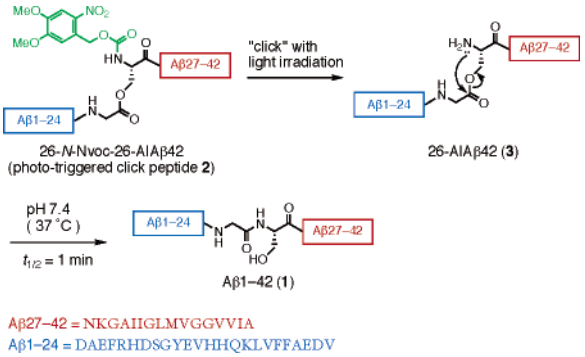

Figure 1. 26-*N*-Nvoc-26-AIAβ42 (click peptide **2**): The production of Aβ1−42 (**1**) by photo-triggered click followed by O−N intramolecular acyl migration reaction of 26-AIAβ42 (**3**).

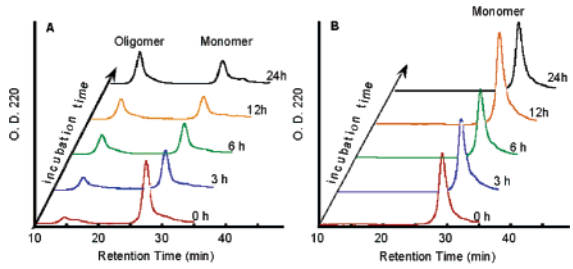

Figure 2. Peptide aggregation determined by size-exclusion chromatography: (A) Aβ1−42 (**1**); (B) 26-*N*-Nvoc-26-AIAβ42 (click peptide **2**). Incubation time in PBS (pH 7.4) at 37 °C is indicated.

To create the photo-triggered click peptide **2**, a photocleavable 6-nitroveratryloxycarbonyl (Nvoc) group[8] was introduced at the α-amino group of Ser[26] in **3**, as depicted in Scheme S1.[9] A solution of synthetic peptide (**1** or **2**) in phosphate buffered saline (PBS, pH 7.4, 15 μM peptide) was incubated at 37 °C, and the self-assembly kinetics of each peptide was analyzed using both size-exclusion chromatography (SEC) and thioflavin-T (Th-T) assay.[10] In SEC, a peak corresponding to an oligomer (~8-mer) of **1** (RT = 15 min) increased with incubation time at the expense of the monomer peak (RT = 27 min), while, in the click peptide **2**, the monomeric form was clearly retained even after 24 h incubation (Figure 2). Similarly, Th-T fluorescence intensity, which corresponds to the extent of fibril formation, increased with time in **1**, while fluorescence intensity unchanged in **2** during 24 h incubation (Figure S1).[9] These results clearly indicate that click peptide **2** is nonaggregative, and that isomerization of the peptide backbone at

[†] Department of Medicinal Chemistry, Kyoto Pharmaceutical University.
[‡] Department of Physical Chemistry, Kyoto Pharmaceutical University.
[§] JST.
[#] Kyoto University.

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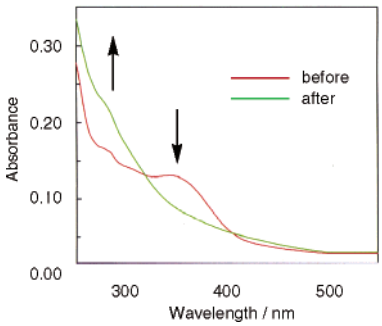

Figure 3. Absorption spectra in the 250−550 nm region for 26-*N*-Nvoc-26-AIAβ42 (click peptide **2**) dissolved in PBS (pH 7.4, 20 μM peptide, 1 mM DTT): spectra before (red line) and after (green line) irradiation of 355 nm laser pulses (10 Hz, 5 mJ) for 15 min at 4 °C.

only one position of the whole peptide sequence, for example, formation of a branched ester structure in **2** significantly changed the secondary structure of **1**, resulted in the complete masking of the aggregative nature of **1**.

Under nonphotolytic conditions, click peptide **2** demonstrated only slight hydrolysis (<2%) at the ester bond between Gly^{25} and Ser^{26} after 6 h incubation in PBS (pH 7.4, 20 μM peptide) at 37 °C. This suggests that click peptide **2** is sufficiently stable to be used for biological assays in ambient light.[4] Peptide **2** was stable for at least 24 h in buffer (pH 7.4) solution at −20 °C and 3 months in either a solid state or a DMSO solution at −20 °C. On the other hand, when a solution of **2** in PBS (pH 7.4, 20 μM peptide, 1 mM DTT) was photoirradiated with UV pulses (355 nm, 10 Hz, 5 mJ) for 15 min at 4 °C, the Nvoc group-derived absorption band at around 355 nm completely disappeared (Figure 3), indicating that the Nvoc group in **2** was quantitatively removed by photolysis. We also confirmed by HPLC analysis that a newly produced peak, which was identical to **1** (M_{calcd} 4514.0; M + H_{found} 4515.4) by mass spectrometry analysis, was quantitatively recovered from **2** after photolysis followed by incubation at 37 °C for 30 min to induce migration. No byproducts arising from 4,5-dimethoxy-2-nitrosobenzaldehyde co-released with **1** by photolysis were observed in either HPLC or mass spectrometric analysis.

These results suggest that (1) the click peptide **2** did not exhibit the self-assembling nature under physiological conditions, (2) photoirradiation of **2** and subsequent O−N intramolecular acyl migration rapidly afforded the intact **1** in situ, while **2** was stable under nonphotolytic or storage conditions, and (3) no additional fibril inhibitory auxiliaries were required. This method provides a novel system that is useful for investigating the biological dynamics of Aβ1−42 in AD by inducible activation of Aβ1−42 self-assembly.

Recently, it has been ascertained that the pathological self-assembly of inherent peptides or proteins is one of the major events leading to the development of many diseases,[11] such as prion protein in Prion disease, α-synuclein in Parkinson's disease, and islet amyloid polypeptide in Type 2 diabetes, as well as Aβ1−42 in AD. Our click peptide strategy could widely be applied to elucidate the pathological mechanisms of these amyloid-related diseases. Moreover, a fundamental drawback of the caged strategy toward large peptides or proteins is that a small photocleavable group is not always possible to mask their biological activities. This drawback would be overcome by our click peptide strategy, in which the inherent property can be masked by simple isomerization of the backbone structure from *N*-acyl peptide to *O*-acyl isopeptide at the hydroxyamino acid residues, such as Ser and Thr. This method would open doors for the development of novel and useful photo-triggered tools in chemical biology and medical science.

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Supporting Information Available: Scheme S1, Figure S1, experimental procedures, and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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