

Click peptide: Chemical Biology-oriented analogues of Alzheimer's amyloid β peptide 1–42

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Introduction

The use of intact peptides in a biological experiment to investigate their functions sometimes causes considerable discrepancies in the biological data because of the difficulties in handling and controlling the properties of the peptides such as low water-solubility and aggregative nature. For example, recent Alzheimer's amyloid β peptide ($A\beta$)-related studies have often encountered such problems because of the uncontrolled aggregative feature of $A\beta$. A clearer understanding of the pathological mechanism of $A\beta$ would be of great value for the discovery of novel drug targets against Alzheimer's disease (AD).

A "click peptide" is a chemically-modified peptide analogue used to understand the biological function of peptides [1–4]. The "click peptide" 1) does not exhibit the inherent biological activity of the original peptide due to a chemical modification of the peptide backbone and; 2) by adding an exogenous action ("click") such as pH-change or photo-irradiation, easily affords the native peptide *in situ* with a quick and easy one-way conversion via a native amide bond-forming reaction.

In 2003, we discovered that the presence of an *O*-acyl instead of *N*-acyl hydroxyamino acid residue within the peptide backbone significantly changed the secondary structure of the native peptide and masked the activity of the native peptide. Additionally, the target peptide was generated via an *O*→*N* intramolecular acyl migration reaction. These findings led to the development of an "*O*-acyl isopeptide method" (Fig. 1) [5, 6]. The method began to be utilized by several other groups [7–9],

indicating that the *O*-acyl isopeptide method is widely advantageous for peptide preparation. Herein, we designed and synthesized click peptides of $A\beta$ 1–42 based on the *O*-acyl isopeptide method (Fig. 1) [1–4].

Results and Discussion

An application of the *O*-acyl isopeptide method to Alzheimer's $A\beta$ 1–42 (**1**) revealed that 26-*O*-acyl iso $A\beta$ 1–42 (**2**), in which the Gly²⁵-Ser²⁶ sequence of $A\beta$ 1–42 was isomerized to a β -ester bond, could be effectively synthesized and stored without spontaneous self-assembly [1, 3, 4]. The water solubility of the TFA salt of **2** was 100-fold higher than that of $A\beta$ 1–42 (**1**) (0.14 mg mL⁻¹). Purified **2** could be quantitatively converted to $A\beta$ 1–42 (**1**) via the *O*→*N* intramolecular acyl migration in phosphate buffered saline (PBS, pH 7.4) at 37 °C with a half-life of 1 min. Since this pH-dependent *O*→*N* intramolecular acyl migration enabled pH-triggered "click" for controlled *in situ* production of intact $A\beta$ 1–42 (**1**) from **2**, we gave **2** the name, pH-triggered "click peptide".

Furthermore, we have synthesized a photo-triggered "click peptide" (26-*N*-Nvoc-26-*O*-acyl iso $A\beta$ 1–42, **3**) of $A\beta$ 1–42, in which a photocleavable 6-nitroveratryloxycarbonyl (Nvoc) group was introduced at the α -amino group of Ser²⁶ in **2** (Fig. 2) [2–4]. In size-exclusion chromatography and thioflavin-T assay, photo-triggered click peptide **3** was non-aggregative under physiological conditions (pH 7.4, 37 °C) for at least 24 h, while $A\beta$ 1–42 (**1**) formed the oligomer and aggregates. The *O*-acyl isopeptide structure in **3** resulted in complete

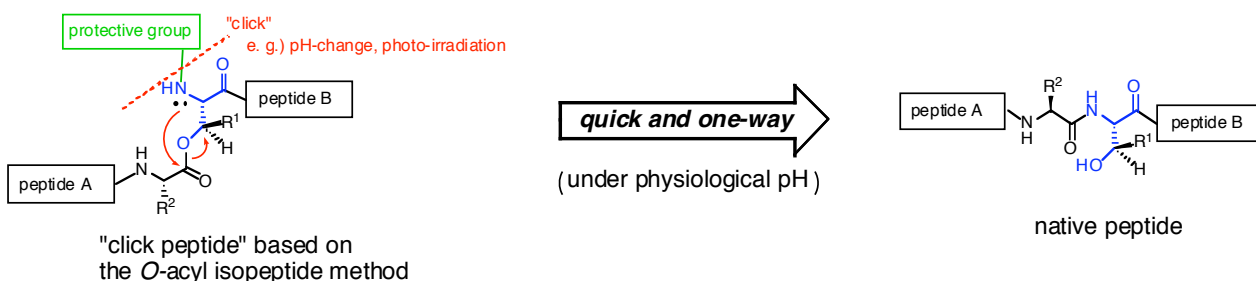


Fig. 1. "Click peptide" based on the *O*-acyl isopeptide method affords the native peptide with a quick and one-way conversion via an *O*→*N* intramolecular acyl migration under physiological conditions.

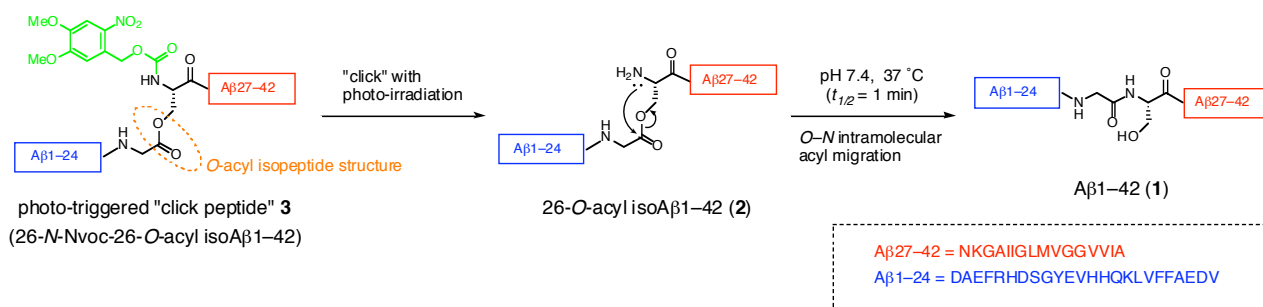


Fig. 2. Photo-triggered "click peptide" **3** (26-N-Nvoc-26-O-acyl isoAβ1-42): The *in situ* production of Aβ1-42 (**1**) by photo-irradiation (photo-triggered "click") followed by O→N intramolecular acyl migration of 26-O-acyl isoAβ1-42 (**2**).

inhibition of the aggregative nature of Aβ1-42 (**1**). When click peptide **3** in PBS (pH 7.4, 20 μM peptide, 1mM DTT) was photo-irradiated 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 (Fig. 3), indicating that the Nvoc group in **3** was quantitatively removed by photolysis. We also confirmed by HPLC analysis that a newly produced peak, which was identical to Aβ1-42 (**1**) by mass spectrometry analysis, was recovered from click peptide **3** after photolysis followed by incubation at 37 °C for 30 min to induce the O→N intramolecular acyl migration. In the absence of light, click peptide **3** was stable during storage. This method may provide a useful system to investigate the biological dynamics of Aβ1-42 (**1**) in AD with high spatial and temporal resolution by photochemically inducible activation of peptide self-assembly.

Because difficulties in handling Aβ1-42 in syntheses and biological experiments would hamper the progress of Aβ1-42-related Alzheimer's disease research, we expect

that the "click peptide" method will aid in clarifying the currently unexplained processes of AD. Moreover, recently, it has been ascertained that the pathological self-assembly nature of inherent peptides or proteins is one of the major events leading to the development of many diseases 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. Thus, we hope that the "click peptide" strategy would widely be applied to these amyloid-related peptides or proteins. The click peptide can serve as a tool to study the functions of peptides in Chemical Biology-oriented research, because the production of the native peptide can be induced *in situ* chemically or photochemically.

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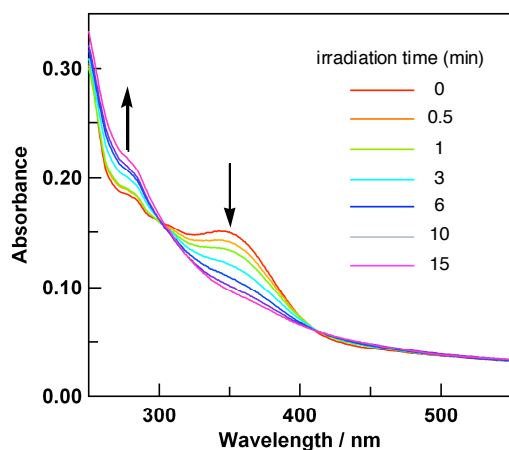


Fig. 3. Absorption spectra in the 250–550 nm region for photo-triggered "click peptide" **3** (26-N-Nvoc-26-O-acyl isoAβ1-42) dissolved in PBS (pH 7.4, 20 μM peptide, 1mM DTT): Spectra at the desired time points after the irradiation of 355 nm laser pulses (10 Hz, 5 mJ) at 4 °C.

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