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Development of amino acid derivative with amide cleavage ability and its use for the design of shuttle peptides

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Introduction

Controlling the function of peptides/proteins by a stimulus in spatio-temporally definitive manner has received increasing attention due to its potential in the fields including chemical biology and drug delivery. Recently, photo-induced processing (peptide bond cleavage) has been successfully applied to convert inactive (or active) peptides/proteins into their corresponding active (or inactive) forms at a desired location and time [1], which prompted us to develop an amino acid derivative with amide cleavage ability for the changing the function of peptides. On the basis of the trimethyl lock system developed for pro-drug [2], we designed and synthesized a photo-responsive amino acid which induces cleavage of a peptide bond upon photo-irradiation [3]. Here, we referred this type of amino acids as to Auto Processing Amino Acid Residue: APR. In such APR-containing peptide, photo-induced removal of a protection (PG) on the phenolic hydroxyl group allowed the regenerated hydroxyl group to be involved in nucleophilic cleavage of an adjacent peptide bond, thereby producing a pair of processing peptides (Fig. 1).

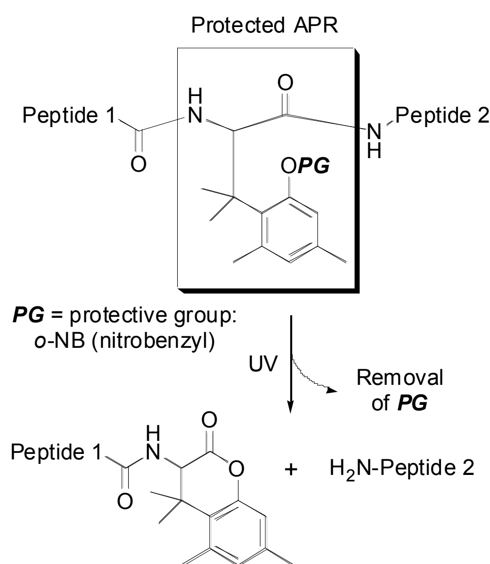
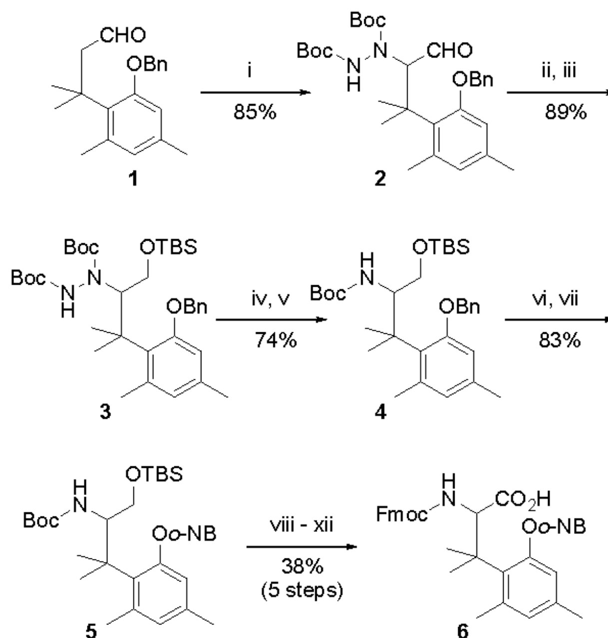


Fig. 1 Concept of auto-processing peptide

Delivery of molecules of biological interest is important in the field of chemical biology [4]. Recently developed peptide-vectors allowed the molecules to be delivered to a target site in one direction; however, controlled-shuttling of the molecules has yet to be achieved. For the accomplishment of the issue, we attempted to develop an unprecedented peptide manipulation methodology consisting of peptide bond processing and *O-N* acyl transfer systems in an *O*-acyl isopeptide [5]. Using its manipulation system, a nucleocytoplasmic shuttle peptide was developed in this study.

Results and Discussion

Synthesis of photo-responsive amino acid is shown in Scheme 1. Starting from aldehyde **1**, reaction with di-*tert*-butyl azodicarboxylate gave nitrogen unit-incorporated material **2**. Reduction of **2** with $NaBH_4$, followed by protection with *tert*-butyldimethylsilyl chloride (TBSCl), afforded silyl ether **3**. Samarium(II) diiodide (SmI_2)-reduction in the presence of HMPA via trifluoroacetylation of **3** gave amino alcohol **4**. The benzyl group on **4** was removed by hydrogenolysis and the resulting phenolic OH was protected with an *o*-nitrobenzyl group to yield **5**. After removal of TBS protection, two-step oxidation, followed by exchange of Boc group to Fmoc, yielded Fmoc-protected photo-responsive amino acid **6**.



Scheme 1. Synthesis of photo-responsive amino acid. i) di-tert-butyl azodicarboxylate, pyrrolidine, CH_2Cl_2; ii) $NaBH_4$, MeOH; iii) TBSOTf, Et_3N, CH_2Cl_2; iv) TFAA, pyridine, CH_2Cl_2; v) SmI_2, HMPA, tert-BuOH, THF; vi) H_2, Pd/C, MeOH; vii) o-nitrobenzyl bromide, K_2CO_3, DMF; viii) AcOH, THF, H_2O; ix) PCC, CH_2Cl_2; x) $NaClO_2$, NaH_2PO_4, tert-BuOH, H_2O, acetone, 2-methyl-2-butene; xi) HCl, AcOEt; xii) Fmoc-OSu, Na_2CO_3, 1,4-dioxane.

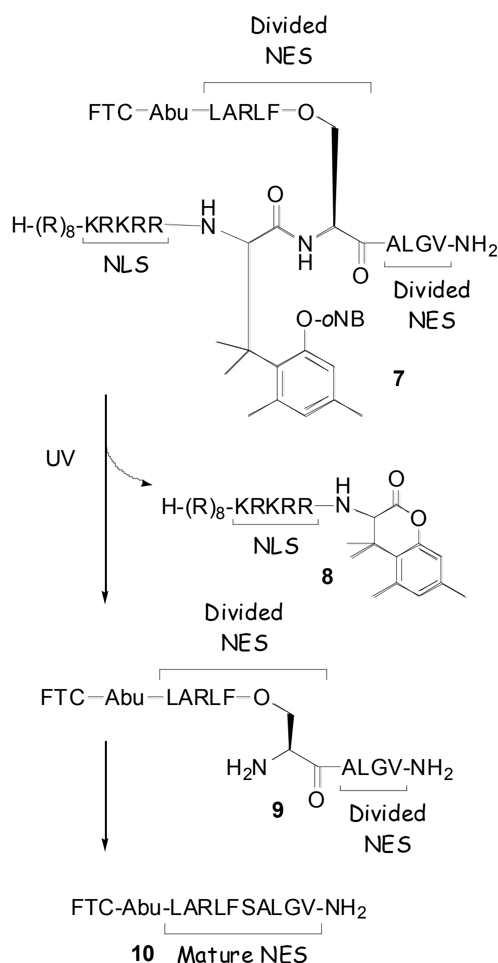


*Fig. 2. Functional change of nucleocytoplasmic shuttle peptide **7**. (Abu: 4-aminobutyryl).*

Having the amino acid **6** with amide cleavage ability upon photo-irradiation, we envisioned the design of a nucleocytoplasmic shuttle peptide. As shown in Fig. 2, designed shuttle peptide **7** possesses a nuclear localization signal (NLS) [6] at the N-terminal position and *O*-acyl isopeptide part as a functionally suppressed nuclear export signal (NES) [7]. Being added to a cell, the shuttle peptide should be concentrated inside a nucleus by the function of NLS. Upon photo-irradiation followed by a removal of NLS, generated *O*-acyl isopeptide **9** can easily undergo *O-N* acyl shift reaction [5] to form another functional peptide **10** with mature NES to return to the cytoplasm. For localization assay in living cells, we planed to introduce a cell penetrating peptide (octa-Arg: $(R)_8$) [4] and 5-fluorescein-5-thiocarbamoyl (FTC) group at N-terminal of main chain and side chain respectively. Nucleocytoplasmic shuttle peptide **7** was synthesized using amino acid **6** by Fmoc SPPS. Then, we conducted *in vitro* experiment to examine a photo-reactivity of shuttle peptide **7**. Shuttle peptide **7** in MeCN/phosphate buffer (pH 7.6) was irradiated by UV light (>365 nm) for 4 min, and then reaction mixture was incubated at 37 °C. The reaction progress was monitored by HPLC (data not shown). After 1.5 h of incubation, shuttle peptide **7** disappeared completely to generate processing products **8** and **10** as the major products.

Next, we evaluated the subcellular localization of the shuttle peptide in living cells. Chinese hamster ovary (CHO)-K1 cells were treated with shuttle peptide **7** (10 μM) at 37 °C for 1 h, then it was irradiated by UV light (>365 nm, 4 min). Before UV irradiation, most of the shuttle peptide was co-localized with Hoechst 33258-stained nuclei. After UV irradiation followed by additional 1 h of incubation, cytoplasmic fluorescence but not a nuclear pattern was observed due to the export of the shuttle peptide (data not shown).

In conclusion, nucleocytoplasmic shuttle peptide **7** was developed. NLS derivative **7** was successfully converted to NES derivative **10** upon UV irradiation. A localization assay in living cells revealed that shuttle peptide **7** was localized inside the nucleus before UV irradiation, but it returned to a cytoplasm after UV irradiation. Application of this shuttle peptide system to chemical biology fields is in progress.

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