Reaction of a ^{10}B Cluster (BSH) with a Chloroacetyl-modified Peptide Is Facilitated by Basic Amino Acid Residues in the Peptide

Sota Watanabe,*[a] Ken Inoue,[a] Hiroyuki Michiue,[b] Mizuki Kitamatsu,[a]

[a] *Department of Applied Chemistry, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan* [b] *Neutron Therapy Research Center, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan.*
E-mail: kitamatu@apch.kindai.ac.jp

Boron neutron capture therapy (BNCT) is a treatment method that accumulates ^{10}B in target cancers and kills them through the nuclear reaction by epithermal neutrons. Currently, 4-bromo-L-phenylalanine (BPA) and boron cluster BSH are representative drugs used in BNCT. The BPA is introduced into cells but the boron occupancy rate in the molecule is low and a large amount of drug must be administered. In contrast, BSH has a very high boron occupancy rate but does not be introduced into cells. Therefore, we have improved this problem by conjugating of cell-penetrating peptide (CPP) with BSH. Recently, in the conjugation, we have utilized a reaction between the SH group on BSH and a chloroacetyl (ClAc) group modified with CPP. In this reaction, we noticed that the reaction was promoted when Arg was included in the peptide. Therefore, we decided to investigate in detail the relationship between various amino acid sequences and the conversion of ClAc-modified peptide to BSH derivatives. To the purpose, we first synthesized tripeptides **Cl-3X**, **Cl-2R**, **Cl-1R** and **Cl-Cn-3R** (Fig. 1). **Cl-3X** and BSH were reacted to evaluate the relationship between amino acid units and the conversion. As a result, the basic amino acids R, K, and H successfully reacted with BSH (data is not shown). This result suggests that the progress of this reaction is affected by the basic amino acid unit. Next, we investigated the relationship between the number of Arg units and the conversion (Fig. 2A). Consequently, increasing the number of Arg units gave a boost to the reactivity. This result supports the above result. Furthermore, we assessed the relationship of the distance between the ClAc group and amino acid units and the conversion (Fig. 2B). As a result, an increase in the distance decreased its reactivity. These results showed that an electrostatic interaction between the negative charge of BSH and the positive charge of Arg, affects the reactivity of ClAc-modified peptides and BSH.

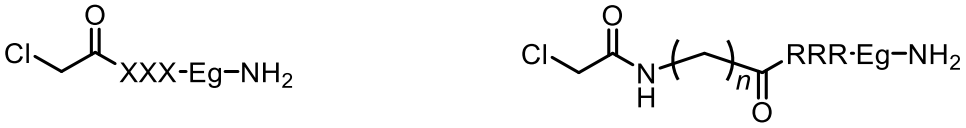

Figure 1. Chemical structures of ClAc-modified tripeptides (**Cl-3X**, **Cl-2R**, **Cl-1R**) and ClAc-modified tripeptides with alkyl linkers (**Cl-Cn-3R**).

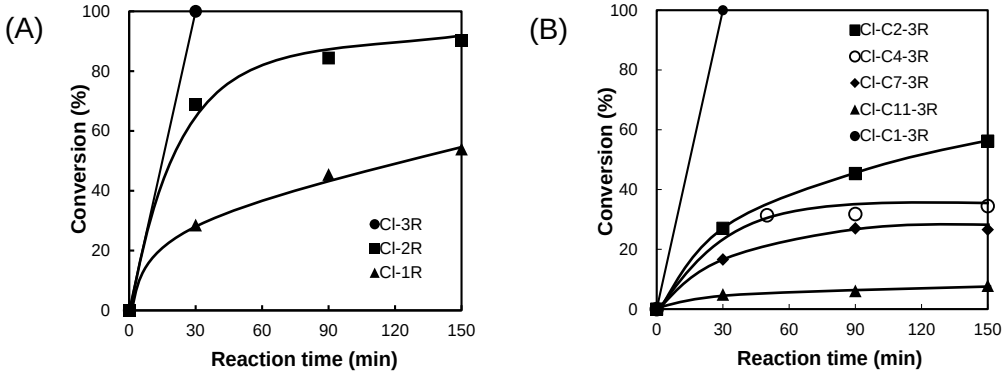

Figure 2. (A) Plots of conversion of **Cl-3R**, **Cl-2R** and **Cl-1R** to BSH-modified tripeptides versus reaction time. (B) Plots of conversion of **Cl-C*n*-3R** to **BS-C*n*-3R** versus reaction.

Reference

[1] Yoshiya Iguchi, *Hiroyuki Michiue, Mizuki Kitamatsu, Yuri Hayashi, Fumiaki Takenaka, Tei-ichi Nishiki, Hideki Matsui., *Biomaterials* **2015**, *56*, 10-17.