Thiazolidine ring-opening by 2-aminobenzamide-based formaldehyde scavengers for one-pot multiple peptide ligation

([1]*Graduate School of Engineering, Nagoya University,* [2]*Institute of Nano-Life-Systems, Nagoya University,* [3]*Graduate School of Engineering, The University of Tokyo,* [4]*Research Center for Advanced Science and Technology, The University of Tokyo*) ○Koki Nakatsu[1], Hiroshi Murakami[1,2], Gosuke Hayashi[1], Akimitsu Okamoto[3,4]

Keywords: Chemical Protein Synthesis; Native Chemical Ligation; Thiazolidine; 2-aminobenzamide; One-pot peptide ligation

In chemical protein synthesis, native chemical ligation (NCL)[1] is a promising strategy to ligate two unprotected peptide segments between an N-terminal cysteinyl peptide and a C-terminal peptide thioester. For multiple peptide segment condensation, the reactivity of the N- or C-terminus of internal segments should be controlled appropriately to avoid self-ligation and cyclization. For this purpose, 1,3-thiazolidine-4-carbonyl (Thz) was employed as a precursor of reactive Cys residue in many previous reports. Thz ring opens when methoxyamine is added at acidic condition.[2] However, due to its high nucleophilicity, methoxyamine attacks thioester moieties and produces a methoxyamine adduct during NCL (Figure 1a).

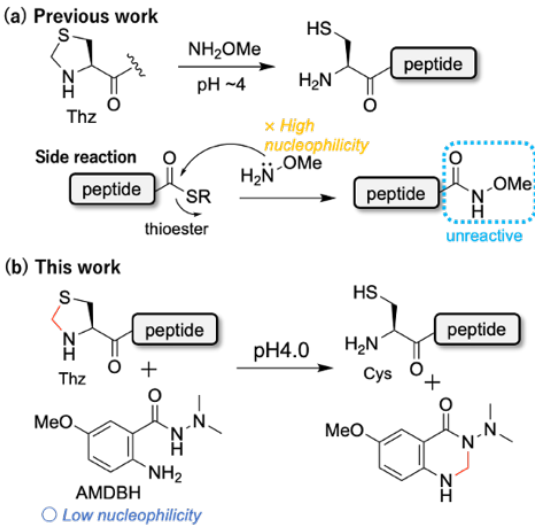

Figure 1. (a) Thz deprotection by methoxyamine. (b) Thz deprotection by AMDBH.

In this presentation, we will report a new Thz ring-opening method using 2-aminobenzamide-type formaldehyde scavengers. These scavengers have a weak nucleophile moiety at the *o*-position of the amine group, and after imine formation with aldehyde, a ring-closing reaction occurs and stable products is produced.[3] After screening some derivatives, we found that 2-amino-5-methoxy-*N*′,*N*′-dimethylbenzohydrazide (AMDBH) efficiently scavenged formaldehyde and converted Thz to Cys in NCL buffer (pH4.0) (Figure 1b). Due to the lower nucleophilicity of AMDBH, this scavenger did not affect thioester and ligation reaction at pH~7. Using ADMBH-mediated Thz ring-opening, we developed a one-pot four segments peptide ligation method. We applied this method to the chemical synthesis of monoubiquitinated histone H2A.Z (209 amino acids, 23 kD).

1) P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. Kent, *Science* **1994**, *266*, 776–779. 2) D. Bang, S. B. H. Kent, *Angew. Chem. Int. Ed Engl.* **2004**, *43*, 2534–2538. 3) P. I. Kitov, D. F. Vinals, S. Ng, K. F. Tjhung, R. Derda, *J. Am. Chem. Soc.* **2014**, *136*, 8149–8152.