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Development of Chemistry-Based Protocol for Sequence-Dependent Thioesterification

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Introduction

One of the useful methods for exploration of the function of proteins is utilization of the synthesized proteins which include artificial units such as fluorescence dye. For the synthesis of such proteins, a peptide thioester and an *N*-terminal cysteinyl peptide prepared by Solid Phase Peptide Synthesis (SPPS) are coupled to each other using Native Chemical Ligation (NCL) [1]. However, due to the limitation of the number of amino acid residues applicable to SPPS, multistep NCLs are required for chemical synthesis of large proteins. On the other hand, in semi-synthesis of proteins, only single NCL of protein thioester prepared from expressed protein with chemically synthesized *N*-terminal cysteinyl peptide enables to incorporate functional moieties to large proteins. However, there are only a few methods for preparation of protein thioesters applicable to naturally occurring sequences [2]. Therefore, new protocols for preparation of protein thioesters has been required.

Results and Discussion

We attempted to develop a chemistry-based novel protocol for sequence-dependent thioesterification of protein using sequential quadruple acyl transfer consisting of *N–O*, *O–O*, *O–N*, and *N–S* acyl transfer. We named this system SQAT system (Figure 1) [3]. At first, Ni(II)-mediated *N–O* acyl transfer at Ser residue in Ser-Xaa-His-Zaa sequence containing peptide resulted in the formation of *O*-acyl intermediate reported by Bal's group [4]. Secondly, this intermediate was converted to the corresponding methyl ester by *O–O* acyl transfer with methanol. Thirdly, by addition of hydrazine, resulting peptide methyl ester was transformed to acyl hydrazide (*O–N* acyl transfer). In the last conversion, peptide hydrazide was converted to thioester *via* peptidyl azide by *N–S* acyl transfer reported by Liu and co-workers [5].

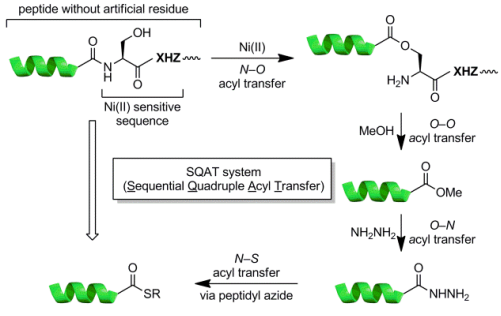

Fig. 1. Sequential quadruple acyl transfer (SQAT) system.

Table 1. Evaluation of Ni(II)-mediated methanolysis.

Ac-**LYRAA**-**SRHWKFL**-NH_2 (**1**) —0.2 M HEPES, Ni(II), MeOH, pH, 37 °C, 12 h→ Ac-**LYRAA**-OMe (**2**) + Ac-**LYRAA**-OH (**3**) + H-**SRHWKFL**-NH_2 (**4**)

Entry	Ni(II) [mM]	MeOH [% (v/v)]	pH	Fraction converted[a]
1	10	10	8.2	0.44
2	10	30	8.2	0.70
3	10	50	8.2	0.72
4	1	30	8.2	0.69
5	20	30	8.2	0.68
6	10	30	7.8	0.61
7	10	30	8.6	0.53
8[b]	10	30	8.2	0.43

[a] The fraction converted was determined by HPLC separation and integration of **2** as a fraction of the sum of the integration of unreacted **1** + hydrolysed **3** + **2**. [b] In the presence of 6 M Gn·HCl.

We examined Ni(II)-mediated methanolysis using model peptide **1** (Table 1). Fraction converted was depended on the concentration of methanol (entries 1-3). Concentration of Ni(II) had no influence on the fraction converted (entries 2, 4 and 5). Appropriate pH of the reaction mixture was found to be 8.2 (entries 2, 6 and 7). Addition of guanidine hydrochloride as a denaturating agent decreased the fraction converted (entries 2 and 8). Then, Ni(II)-mediated methanolysis of peptide **1** followed by addition of

$NH_2NH_2{\cdot}H_2O$ (final concentration: 5% (v/v) NH_2NH_2) into the reaction mixture yielded the corresponding peptide hydrazide (data not shown).

In order to examine the applicability of the SQAT system, we attempted the chemical synthesis of CNP 53 peptide by using this system. For the preparation of 36-residue thioester peptide, initially 43-residue SRHW-Ni(II)-sensitive sequence-incorporated peptide **5** was synthesized chemically. Ni(II)-mediated methanolysis of peptide **5** yielded the desired peptide methyl ester **6** as major product. Then addition of hydrazine into the reaction mixture gave 36-residue peptide hydrazide **7**. After conversion of peptide hydrazide **7** to peptide thioester via peptidyl azide according to Liu's protocol, NCL of the resulting thioester with *N*-terminal cysteinyl peptide **8** followed by folding of the resulting peptide yielded the desired CNP 53.

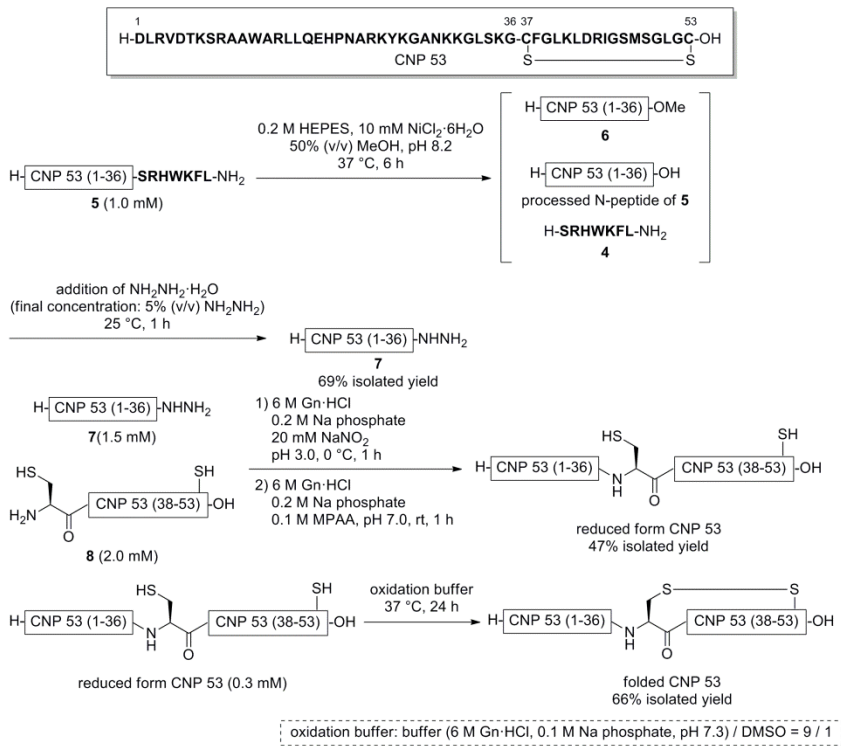

Fig. 2. Synthesis of CNP 53 using SQAT system.

Acknowledgments

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