Thiol additive-free native chemical ligation

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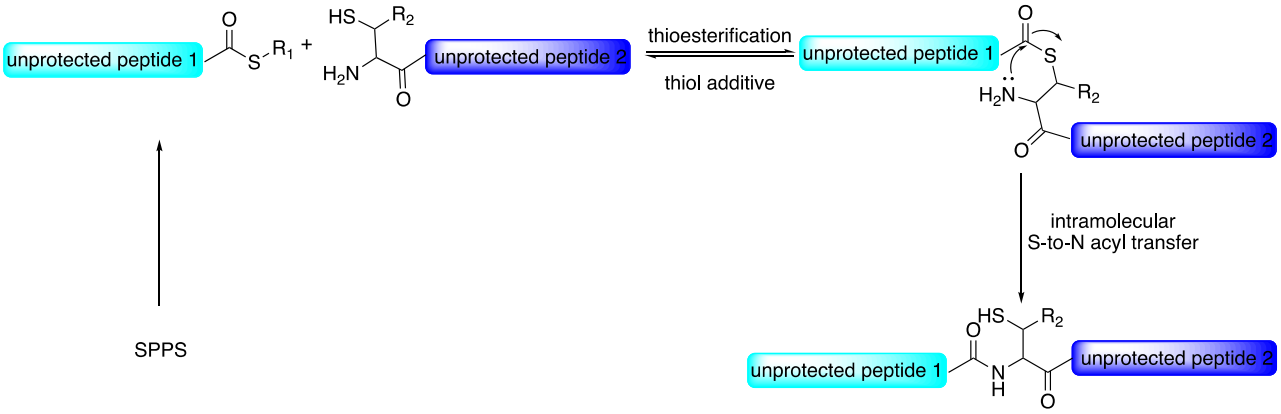
NATIVE CHEMICAL LIGATION

In general, native chemical ligation (NCL) utilizes a peptide thioester reacted with a peptide bearing an N-terminal ß-thiol to construct the amide bonds, which has been developed by S. Kent et al in 1994[1]. Mechanistically, NCL proceeds with an intermolecular thioesterification, followed by a rapid intramolecular S-to-N acyl transfer *via* a five-membered ring to yield the native amide bond (Scheme 1)[2, 3]. To date, various peptide thioester preparation strategies have been developed, including N-acyl-benzimidazolinone (Nbz)[4], hydrazide[5], *N*,*N*-Bis(2-mercaptoethyl)amide (BMEA)[6], Weinreb amide[7], cysteinylprolyl ester (CPE) and N-alkylcysteine (NAC)[8]. In order to generate a more active thioester, it normally requires an excess amount of thiol additive. However, the exogenous thiol additives, including widely used mercaptophenylacetic acid MPAA, have shown several limitations. Because of the bulky side chains from the peptide, MPAA could compete for the S-to-N peptidyl acyl transfer to form side products as a tethered peptide. Moreover, MPAA needs to be removed after ligation for the following desulfurization. Several research groups are working on the development of the thiol additive-free NCL. Herein, this short report will focus on two recent publications of the development and application of thiol additive-free NCL strategy[9, 10].

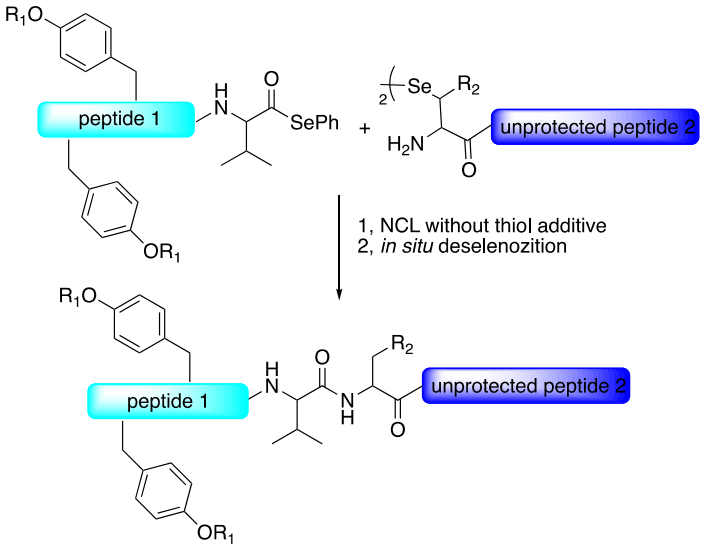


Scheme 1. The approach of Native chemical ligation.

SELENOESTER MEDIATED THIOL ADDITIVE-FREE NCL

Due to the higher nucleophilic property of selenolate (RSe^-) than thiolate (RS^-), L-selenocysteine (Sec/U) has been used to efficiently react with thioester at lower pH for NCL and expressed protein ligation (EPL)[11-13]. On the other hand, peptidyl prolyl selenoester has been used as a more effective acyl donor than the corresponding thioester to react with Cys-peptide in NCL reactions[14]. To further address the limitations of general thioesters, Payne group has developed a rapid additive-free Sec-selenoester peptide ligation[15]. Therefore, I will briefly describe the first selected paper published by Payne group in *Angewandte Chemie* (Wang et al, 2017, 56, 8490). In this paper, the authors applied a novel β-selenoleucine to prepare a sulfated chemokine-binding protein, UL22A, by one-pot Sec-selenoester ligation and *in situ* deselenization[9].

UL22A is a chemokine-binding protein (CKBP) involving infection or injury in human cytomegalovirus and shows a selectively binding affinity with the inflammatory chemokine, RANTES. In this paper, they utilized a thiol additive-free Sec-selenoester NCL to prepare a library of homogenously posttranslational modified UL22A, including unsulfated, monosulfated on Tyr65, monosulfated on Tyr69 and disulfated Tyr modifications. During the ligation process, an additional water-soluble phosphine reductant TCEP was used to avoid thiol-selenylsulfide formation. In the ligation mixture, diphenyl diselenide, a radical scavenger, was also added to prevent the TCEP-mediated deselenization. Afterwards, the generated intermediates from the ligation mixture were further applied *in situ* deselenization in the presence of TCEP and DTT.



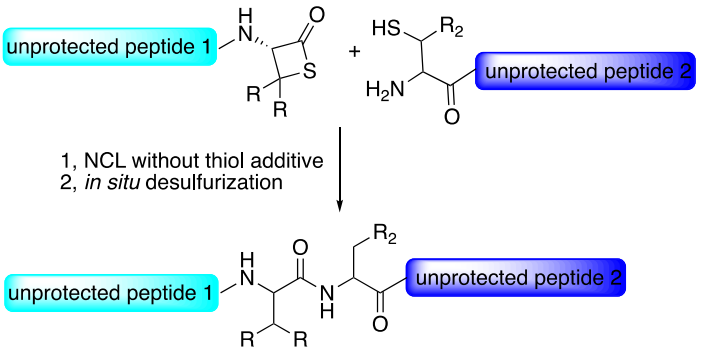
Scheme 2. Sec-selenoester thiol additive-free NCL with sulfated modifications.

Furthermore, with an optimized ligation condition, Wang et al obtained a series of homogeneous sulfated UL22A. Then, these sulfated UL22A analogues were tested with RANTES for their binding affinity. Comparing with the expressed UL22A, the binding affinity results implied that all the synthetic mono-sulfated and disulfated analogues can enhance their binding affinity. In summary, the authors demonstrated an efficient Sec-selenoester ligation with a novel β-selenoleucine motif to prepare the homogeneously sulfated chemokine binding protein, which will decipher the effects of site-specific sulfation on protein level.

THIOLACTONE-MEDIATED THIOL ADDITIVE-FREE NCL

β-Thiolactone has been used as thioester surrogate by Crich et al, in which a dipeptide β-thiolactone can be activated by the exogenous thiol[16]. Furthermore, the γ-thiolactone mediated crosslinking strategy has been applied to prepare a series of biocompatible hybrid polypeptide hygrogels[17]. Interestingly, Nishiuchi et al proposed that the macrothiolactone could be considered as the thioester precursor to form NCL product[18]. Therefore, I will summarise a very recent publication in *Chemical Science* (Chen et al, 2018, 9, 1982). This report has applied β-thiolactone as thioester surrogate for the additive-free NCL on the notorious sterically peptidyl sites like Val-Val and Val-Pro[10].

Firstly, Chen et al optimised the β-thiolactone mediated NCL condition with small amino acid or dipeptide in the absence of thiol additive, MPAA. The optimised condition showed an efficient ligation with *in situ* desulfurization to generate the final products. With the optimum condition, they further investigated the reaction scope with different ligation sites, which turned out this ligation method with a great compatibility on sterical hinder sites like Val-Val and Val-Pro. In their experimental examples, the β-thiolactone mediated additive-free NCL has shown a wide functional group tolerance on the ligated peptide sequence, including Asp, Lys, Arg, His, Thr, Glu, Gln and Tyr.



Scheme 3. ß-Thiolactone-mediated additive-free NCL with in situ thiol removal.

Comparing with the current thioester and selenoester, Chen et al further investigated the mechanism and reaction rate of this β-thiolactone-mediated NCL. Using a cross-over experiment, the aminolysis mechanism of thietan-mediated peptide ligation has been excluded in this NCL. Moreover, the reaction rate of the analogous peptide esters with Cys-peptide further supported the high efficiency of β-thiolactone-mediated NCL. Finally, Chen et al applied this β-thiolactone mediated additive-free NCL to prepare a bioactive cyclic peptide, axinastatin.

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