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Fast-SEA: Modifying Proteins in the Nanomolar Concentration Range with an NCL Inspired Ligation

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Introduction

Chemical Protein Synthesis has greatly benefitted from the properties of the thioester functional group, in particular with the development of the Native Chemical Ligation (NCL) reaction (Figure 1a) [1,2]. However, NCL-based modification of proteins suffers from the moderate reactivity of classical alkyl and aryl thioesters, especially in highly dilute media [3]. Seeking for fast reactive thioesters surrogates, we have discovered that oxalyl derivatives of bis(2-sulfanylethyl)amides (^{oxo}SEA) in the form of a latent thioester precursor could act as powerful and chemoselective acylating agents of β-aminothiols upon activation by reductants (Figure 1b) [4].

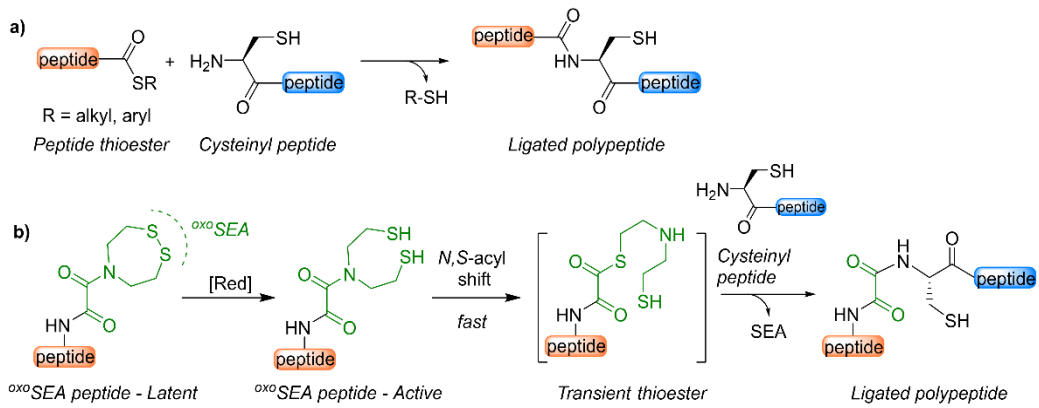

Fig. 1. a) Principle of the Native Chemical Ligation reaction; b) Principle of the ^{oxo}SEA-mediated ligation.

Results and Discussion

The SPPS-compatible lysine derivative **1** featuring an ^{oxo}SEA moiety on its side chain was prepared at the gram scale with 17% overall yield and its structure was validated through X-ray diffraction (Figure 2a,b).

The residue was incorporated into short model peptides of type **2**, which served as reactants to assess the reactivity of the ^{oxo}SEA group in the presence of a cysteinyl peptide **3** (Figure 2c). The study enabled to establish that the formation of ligation product **4a** through the ^{oxo}SEA-mediated ligation reaction of peptides **2a** and **3a** was: *i)* highly efficient even at peptide concentrations in the nanomolar concentration range, *ii)* rapid (~30 M^{-1} s^{-1} as compared to 0.3-4 M^{-1} s^{-1} for classical thioesters [5]), *iii)* triggered on-demand by the addition of a reductant (Figure 2d).

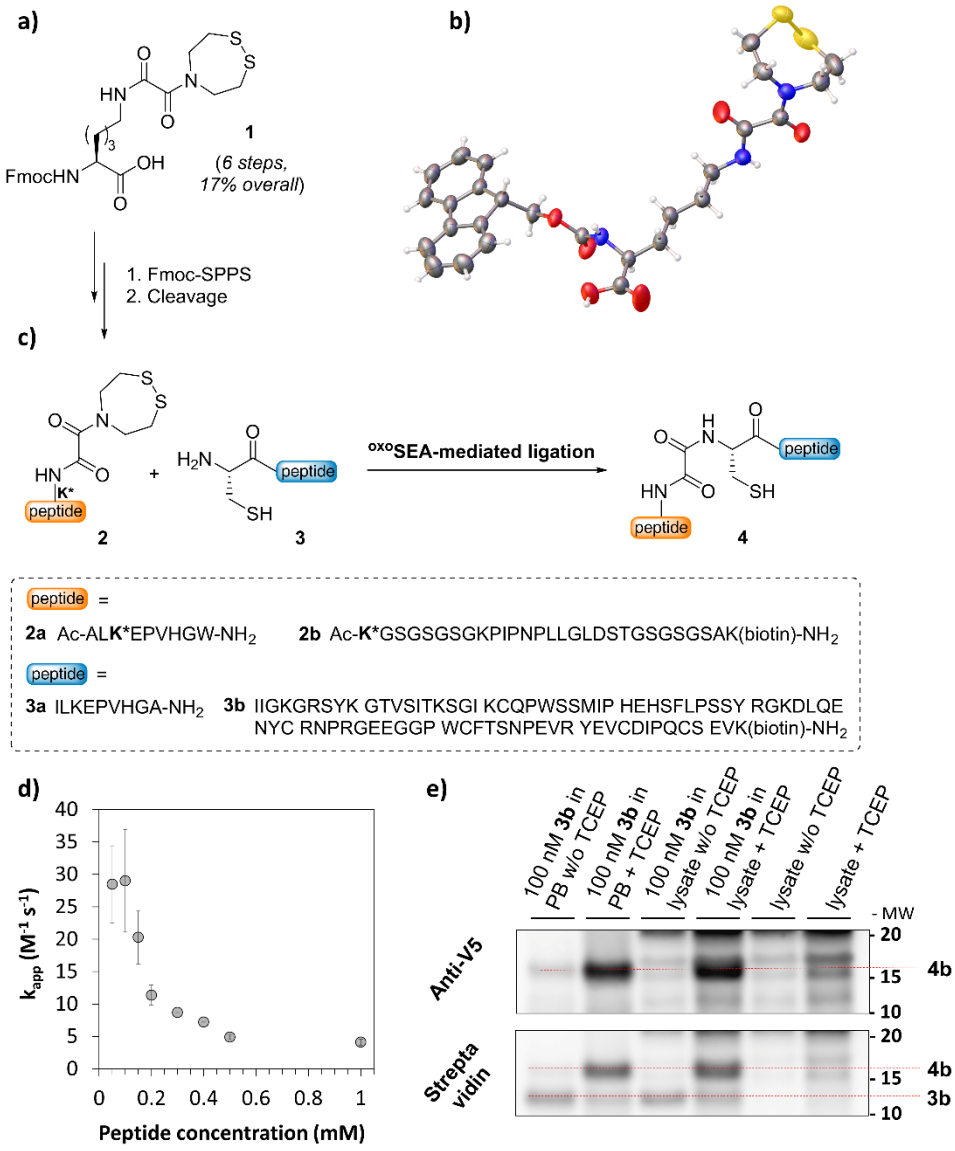

*Fig. 2. a) Structure of Fmoc-Lys(oxoSEA)-OH synthetic residue **1**; b) Crystal structure of compound **1** with ellipsoids shown at the 50% probability level (ccdb 2164270); c) Sequence of the peptides used for the study of the oxoSEA-mediated ligation; d) Apparent second order rate constant of peptide **4a** formation determined by nonlinear regression fitting. The data correspond to the mean ± standard error (95% confidence limit interval) determined from three independent replicates. Conversion to ligated peptide **4a** from peptides **2a** and **3a** was performed using the following experimental conditions: 1 equiv. **2a**, 1.2 equiv. **3a**, 200 mM 4-mercaptophenylacetic acid (MPAA), 100 mM TCEP, pH 5.5, 37 °C in 6 M guanidinium chloride in 100 mM phosphate buffer; e) Western-blot analysis of the formation of **4b** in a crude THP-1 whole cell protein extract (1.6 µg µL-1) supplemented with 100 nM **3b** (i.e. 93 ng µL^{-1}) in the presence of a 15-fold excess of **2b**.*

To challenge the performance of the ^{oxo}SEA-mediated ligation at the protein level, an ^{oxo}SEA peptide derived from V5 peptidic tag **3a** and a *C*-terminally biotinylated kringle 1 domain of the hepatocyte growth factor (K1/HGF) equipped with an *N*-terminal cysteine **3b** were prepared (Figure 2c,e). Those were ligated in a crude protein extract of a THP-1 whole cell lysate supplemented with 100 nM of **3b** in the presence of a 15-fold excess of **3a**. Monitoring of the reaction was achieved by SDS-PAGE separation followed by Western Blot analysis and specific detection by anti-V5 monoclonal antibody labelled with horseradish peroxidase (HRP) and streptavidin-HRP. Formation of ligated product **4b** was successfully observed in the presence of TCEP, thereby demonstrating the chemoselectivity and the efficiency of the ^{oxo}SEA-mediated process.

Conclusion

The spectacular reactivity of this activatable oxalyl thioester surrogate allows to perform peptide and protein modification through a chemoselective ligation reaction. The latter proceeds extremely fast in aqueous media, under mild conditions and in highly dilute medium which can be as complex as a cell lysate. Introduced within peptide segments in the form of a lysine derivative, oxalyl thioesters offer an interesting extension to NCL as a conjugation method and might be useful for the development of novel crosslinking strategies or the polycondensation of water-soluble (bio)molecules.

Acknowledgments

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