A Novel Metal Catalyzed Peptide Ligation Method

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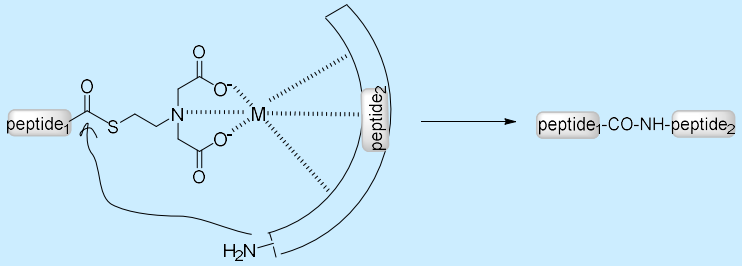
Introduction.

Native Chemical Ligation (NCL[1]) is a widely used chemoselective peptide ligation method for the semi-synthesis or total chemical synthesis of proteins. NCL involves the condensation of a peptide thioester with a cysteinyl (Cys) peptide, forming a larger peptide linked via a native amide bond at the cysteine residue. However, the low natural abundance of Cys in proteins significantly limits the applicability of NCL.[2]

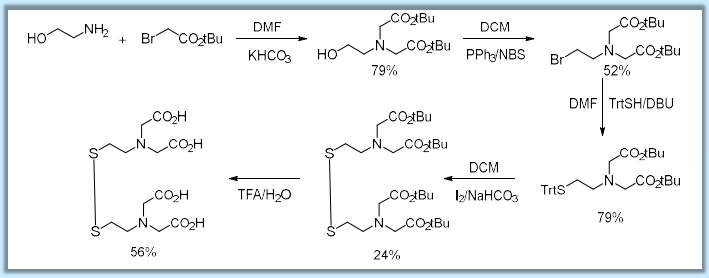
Peptide thioesters are valuable acyl donors for developing novel ligation strategies, as they can be readily synthesized through chemical, biochemical, or biological methods. Motivated by their unique reactivity, we aimed to design new peptide ligation methods that expand beyond the constraints of NCL.

Here we present our preliminary investigations of the use of metal catalysis for promoting the condensation of peptide thioesters derived from iminodiacetic acid (IDA) with peptide amine nucleophiles.

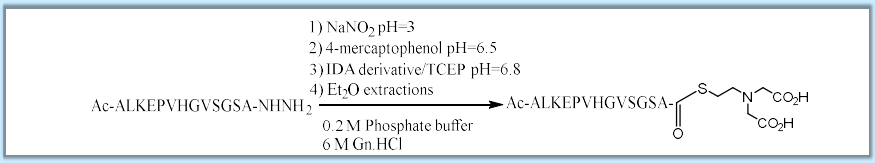
Principle of the metal catalyzed peptide ligation method



Synthesis of IDA derivative (n=2)

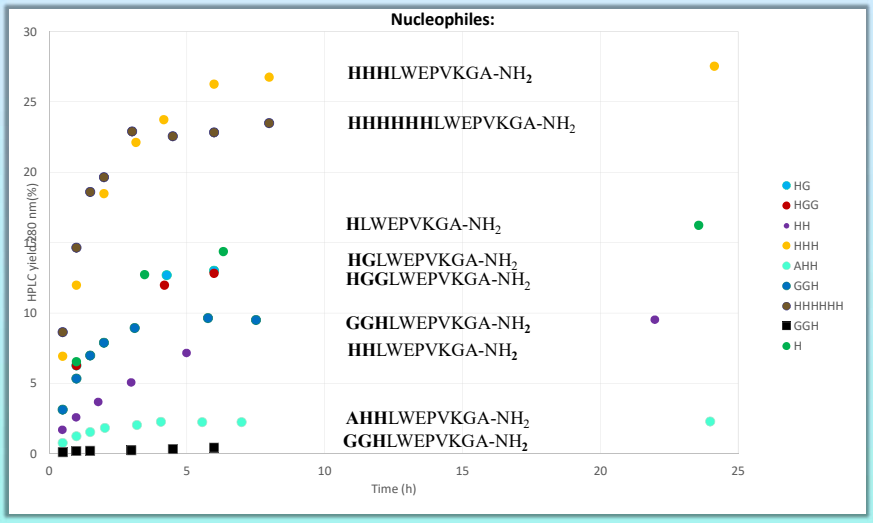


Synthesis of IDA thioester peptide (n=2)

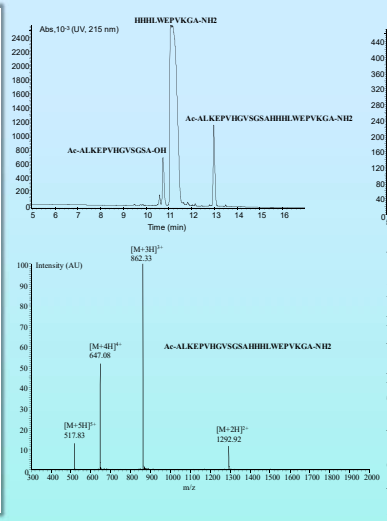


We used here an adapted procedure that significantly enhances thioester production with just a minimal thiol excess, facilitating the use of advanced thiol nucleophiles.[3]

Cu^{2+} catalyzed peptide ligation between IDA thioester peptide (n = 2 or n = 3) and various nucleophiles at pH = 5.5



Histidyl peptide (5 mM) was dissolved in 50 mM acetate buffer pH = 5.5 and added to IDA thioester (n = 2 circle, n = 3 square, 1 mM). pH was adjusted to 5.5. A solution of copper sulfate (1 mM) was then added to the reaction mixture, which was then placed at 25 °C.



UPLC-MS profiles (at 215 or 280 nm) of the final reaction mixture between IDA (n = 2) thioester peptide and HHH nucleophile peptide at pH = 5.5. The presence of tryptophan in the sequence enables quantification of the progress of the reaction by monitoring at 280 nm.

Conclusion.

Several parameters were systematically investigated. Two IDA thioesters were synthesized, with the variant containing n = 2 yielding the best results. Chemoselective ligation was achieved at pH 5.5, whereas no selectivity was observed at pH 7.

A range of metal ions (Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, and Mg^{2+}) were screened as potential catalysts. Among them, Cu^{2+} provided the highest efficiency. Various histidine-containing peptides were evaluated, and the best ligation yield was obtained with the peptide bearing three histidine residues at the N-terminus.

Although the crude product profile was satisfactory, the overall yield was limited by competing hydrolysis of the thioester.

References.

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