Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.245*

Design of Novel Alkylselenol Catalysts Enabling Peptide Thioester and Protein Chemical Synthesis

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Introduction

Thioesters are pivotal intermediates in the native chemical ligation-mediated synthesis or semisynthesis of proteins. Their preparation very often occurs through a thiol-thioester exchange step which involves thiol-based catalysts [1,2]. Although the selenol group also presents interesting properties in this regard, selenol-based catalysts have been largely overlooked. In this study, we designed various selenocysteamine-derived alkyl selenols in the form of the corresponding diselenide precursors **1**-**3** (Figure 1a) and assessed their capacity to promote the formation of thioesters from *bis*(2-sulfanylethyl)amido (SEA) peptides **4** (Figure 1b) [3,4]. The more promising candidate was then used to prepare granulysin (9-GN), a 9 kDa human protein involved in immunity.

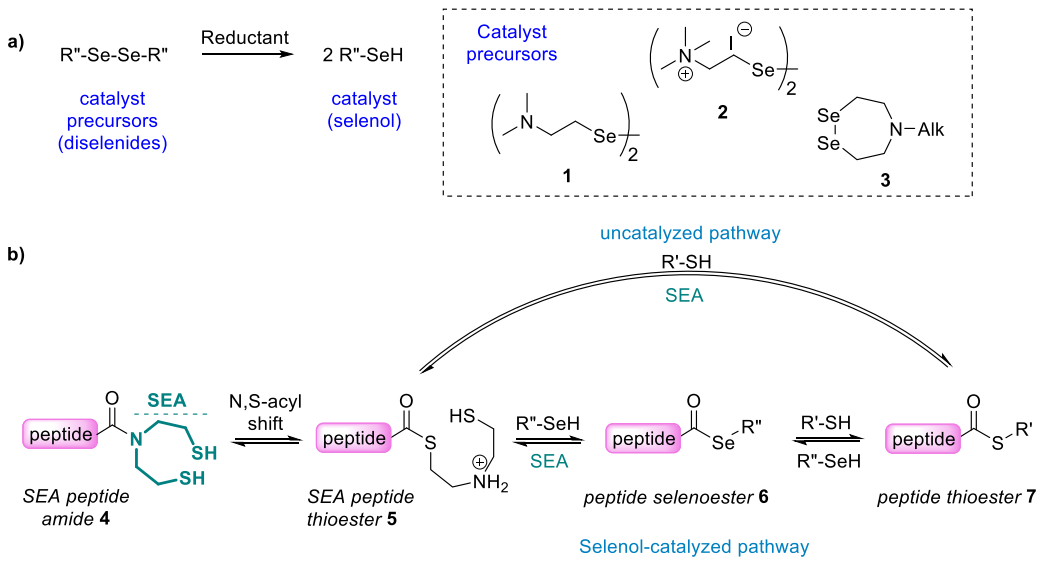

Fig. 1. a) Selenol-based catalysts in the form of their diselenide precursors; b) Principle of the selenol-catalyzed thiol-thioester exchange from SEA-peptides.

Results and Discussion

The exchange reactions were conducted on a 10-mer model peptide in the presence of a selenol-based catalyst introduced at various concentrations and were monitored by HPLC (Figure. 2a,b). The data showed that selenols derived from diselenides **1** and **3** were almost equally efficient for accelerating the formation of MPA-thioester **9** from SEA-peptide **8** when introduced at 25 mM or more (50 mM total selenol concentration) (Figure 2c). Diselenide **2** was found not as potent as **1** or **3**. From a synthetic perspective, catalyst precursors **1** and **2** could be obtained at the gram scale, in fewer chemical steps and in higher yield than compound **3**. Alltogether, these results prompted us to use diselenide **1** as a precatalyst for the total chemical synthesis 9-GN granulysin.

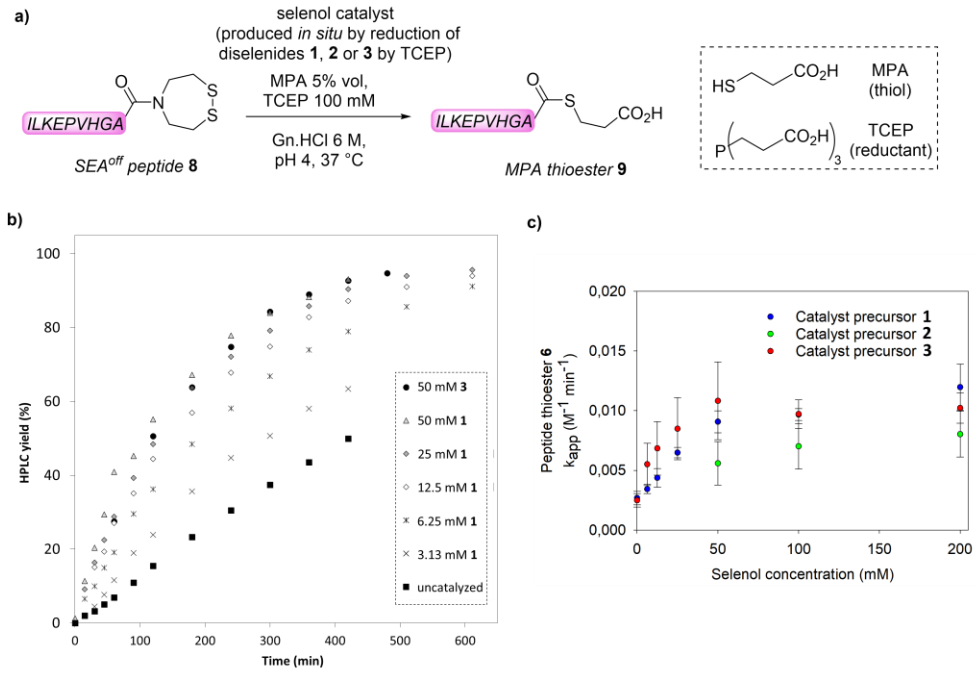

*Fig. 2. a) Model reaction for the SEA/MPA thiol-thioester exchange; b) HPLC monitoring of the reaction (yields calculated on the basis of the UV signal at 215 nm); c) Influence of catalyst's nature and concentration on the rate of the thiol/thioester exchange (apparent second order rate constants of peptide **9** formation determined by nonlinear regression fitting).*

9-GN is a human cytotoxic, chemoattractant and proinflammatory protein secreted by specialized cells from the immune system in response to infections. The design of 9-GN analogues with potential therapeutic interest motivated the development of an efficient and modular synthetic route (Figure 3a). In this approach, diselenide **1** was used as the precatalyst to successfully accelerate the formation of the central segment B in the form of an MPA-thioester from a SEA precursor. The linear polypeptide **9-GN-l** obtained after the concatenation of two additional segments by NCL was folded and the formation of the native pattern of disulfide bonds was determined by trypsic digestion under non-reducing conditions and mass spectrometry. The UPLC-MS analysis of **9-GN** highlights the quality of the protein obtained by the designed synthetic route (Figure 3b).

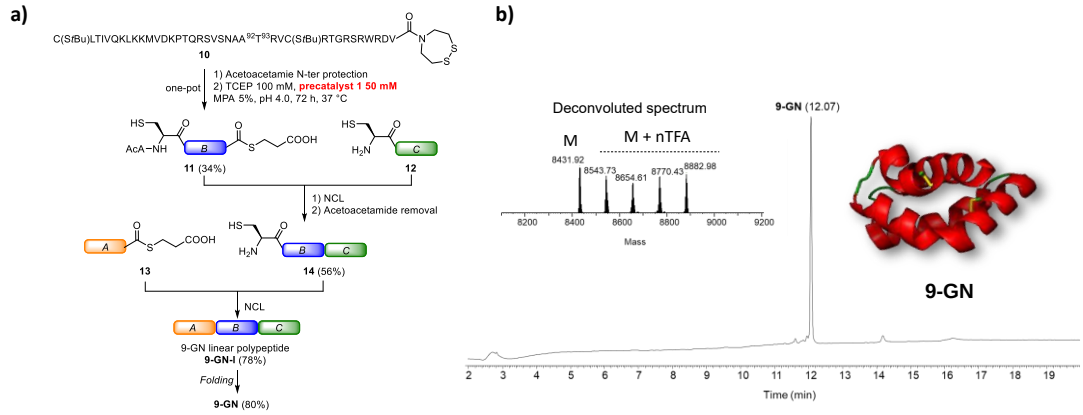

Fig. 3. a) Synthetic approach toward native 9-GN protein; b) UPLC-MS characterization of folded synthetic 9-GN protein.

Conclusion

We evidenced that the *bis*(2-sulfanylethyl)amido (SEA)/thiol exchange process could be efficiently catalyzed by selenocysteamine-derived selenols. These compounds can be easily synthesized at the multigram scale from cheap and commercially available starting materials. As catalysts, they are bench-stable for months in the form of their diselenide precursors and can be used for chemical protein synthesis as illustrated with the production of native 9 kDa granulysin.

References

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