Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.248*

Expanding the Protein Chemical Synthesis Toolbox with *N*- Selenoethyl Cysteine

Vincent Diemer[1], Olga Firstova[1], Vangelis Agouridas[1,2], and Oleg Melnyk[1]
[1]Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL – Center for Infection and Immunity of Lille, F-59000 Lille, France; [2]Centrale Lille, F-59000 Lille, France

Introduction

Assembling peptide segments by native chemical ligation (NCL) is an efficient and powerful strategy for accessing native or modified proteins (Figure 1a) [1-3]. In this field, we recently reported that *N*-selenoethyl cysteine (SetCys) residue behaves as a redox-controlled cysteine (Cys) surrogate, and as such enables to temporally mask the reactivity of key Cys residues involved in peptide assemblies by NCL [4]. The interest of SetCys relies on its capacity to lose spontaneously its selenoethyl appendage after reduction of the cyclic selenosulfide bond (Figure 1b). Although breaking carbon-nitrogen bonds usually requires harsh conditions, we discovered that the SetCys conversion into Cys optimally occurs under mild conditions (pH 6, 37°C) [4]. We show here that the rate of the SetCys conversion into Cys can be controlled by choosing the combination of reductants (phosphine/thiol) used for inducing the ring-opening of the SetCys residue, thereby increasing its scope in protein chemical synthesis.

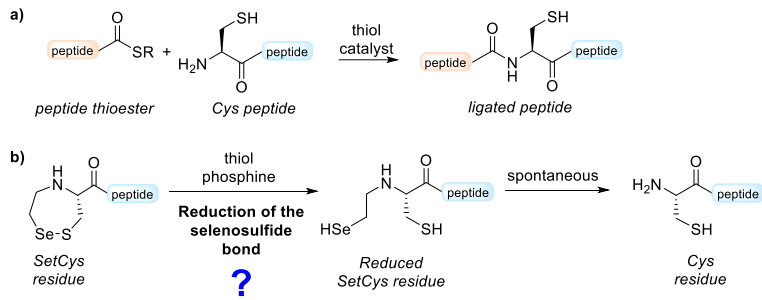

Fig. 1. a) NCL reaction. b) SetCys as a redox controlled Cys surrogate.

Results and Discussion

Peptide **1** was used as a model compound to investigate the impact of thiols and phosphines that are classically employed as additive in NCL reactions on the kinetics and the mechanism of the SetCys conversion into Cys (Figure 2). No reaction was observed in the presence of MPAA (4-mercaptophenylacetic acid), the gold standard catalyst used to accelerate the NCL reaction [4]. In contrast, the addition of water-soluble alkyl or aryl phosphines into the reaction mixture cleanly induced the conversion of SetCys into Cys.

In the presence of TCEP (*tris*(2-carboxyethyl)phosphine), we showed that the rate of the reaction was independent of the phosphine, the thiol additive and the SetCys peptide concentrations [5]. This result points towards a mechanism in which the Se-S bond is directly and quickly reduced by the phosphine (Figure 2b, path a). The ring-opening of SetCys is so fast in these strong reducing conditions that the rate of the whole process is dictated by the subsequent cleavage of the selenoethyl appendage.

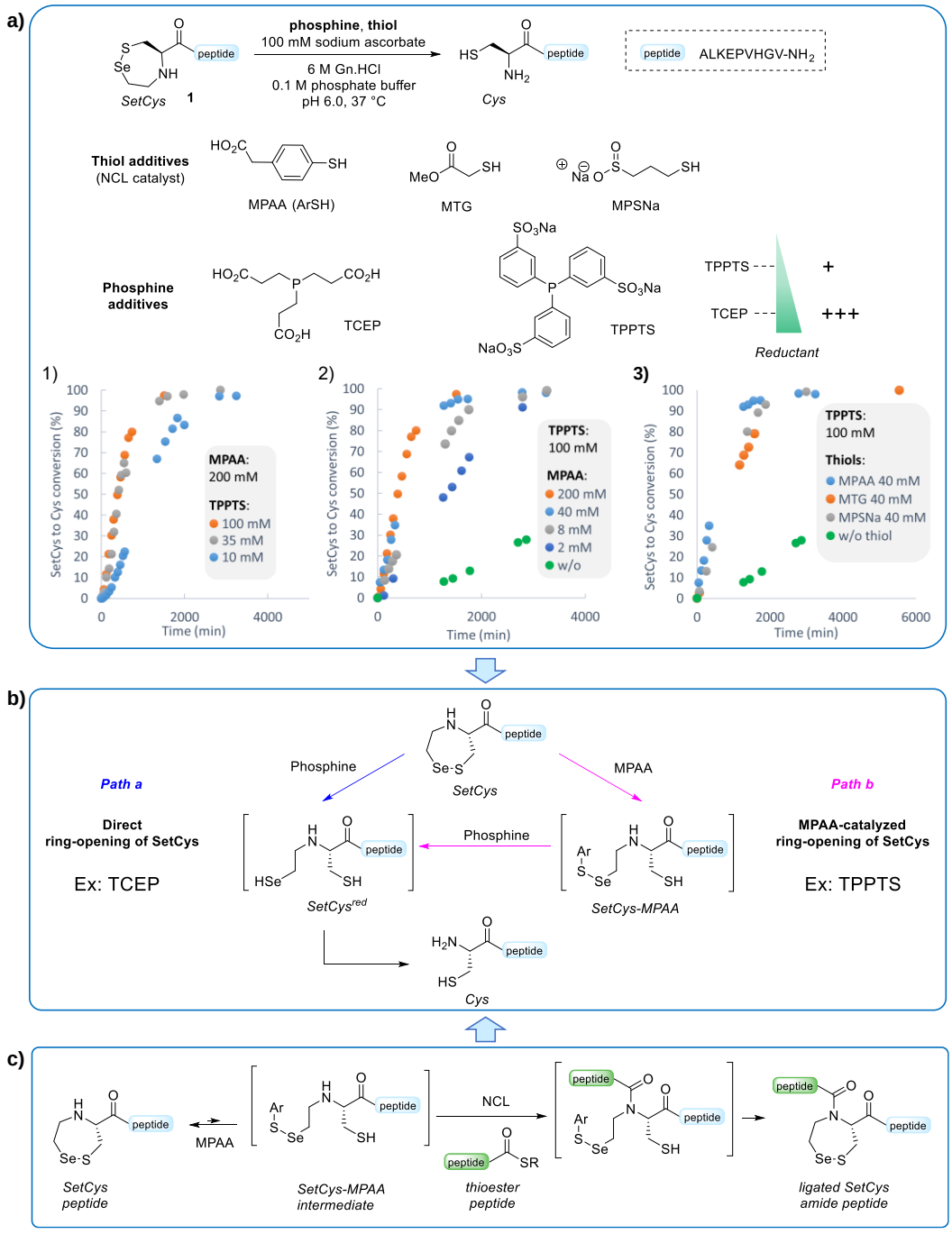

Fig. 2. Study of the SetCys to Cys conversion: effect of the thiol and phosphine additives on the rate of the reaction. a) Model reaction and kinetic data. b) Main mechanisms for the SetCys reduction by phosphines. c) Evidence for SetCys opening by the thiol catalyst.

The situation is clearly different when a less reactive phosphine such as TPPTS (3,3′,3″-phosphanetriyltris(benzenesulfonic acid) trisodium salt) was used as reductant [5]. We found that the

rate of the SetCys to Cys conversion depends in this case on the aryl phosphine concentration (Figure 2a, graph 1), and this up to 35 mM, a concentration above which the reduction process involving TPPTS is no longer rate limiting. Furthermore, we discovered that the SetCys conversion into Cys was efficiently catalysed by MPAA (Figure 2a, graph 2). Very slow in the absence of MPAA (t½ 75.4 h), the reaction is more than 10-fold faster when MPAA is present at 200 mM (t½ 6.5 h). A screening of the reaction conditions showed that the MPAA was the most efficient additive among the tested thiols (Figure 2a, graph 3).

Such an acceleration of the SetCys to Cys conversion in the presence of MPAA led us to postulate a second mechanism for SetCys reduction where the Se-S bond is initially cleaved by the thiol additive in a step which is rate limiting (Figure 2b, path b). The product of SetCys opening by MPAA is postulated to be an alkyl aryl selenosulfide, due to the higher affinity of thiolate nucleophiles for selenium [6-8]. This intermediate is then reduced by TPPTS into a free selenol, a step that triggers the cleavage of the C-N bond and the formation of the target Cys peptide. Although the intermediate alkyl aryl selenosulfide has never been detected by simply incubating SetCys peptides in presence of MPAA, some experiments argue for its formation in situ (Figure 2c). In particular, the formation of SetCys amide peptides by an NCL-like mechanism can occur only if the cyclic structure of the SetCys residue is transiently opened by MPAA during the ligation.

To sum up, the ring-opening of SetCys *via* the reduction of its selenosulfide bond is a fast process in the presence of strong reductant such as TCEP. In these conditions, the rate of SetCys to Cys conversion is dictated by the intramolecular substitution process leading to C-N bond cleavage. In contrast, the SetCys reduction is significantly slower with TPPTS, a milder reductant compared to TCEP, delaying the subsequent conversion of SetCys into Cys. However, we discovered that the lower reducing power of TPPTS can be efficiently balanced by the addition of thiol catalysts. Such a finer tuning of the SetCys reactivity through the combination of phosphine and thiol additives will certainly open the way towards new redox-controlled strategies for concatenating peptide segments by NCL.

Acknowledgments

This project has received financial support from the CNRS through the 80|Prime program (grant to O. F.) and from the French National Research Agency (ANR grant ANR-21-CE06-0006, REPEAT project).

References

1. Diemer, V., Firstova O., Agouridas V., Melnyk O. *Chemistry* **28**, 16, e202104229 (2022), https://doi.org/10.1002/chem.202104229
2. Agouridas, V., El Mahdi, O., Diemer, V., Cargoet, M., Monbaliu, J.-C.M., Melnyk O. *Chem. Rev.* **12**, 7328-7443 (2019), https://doi.org/10.1021/acs.chemrev.8b00712
3. Agouridas, V., El Mahdi, O., Melnyk, O. *J. Med. Chem.* **63**, 15140-15152 (2020), https://doi.org/10.1021/acs.jmedchem.0c01082
4. Diemer, V., Ollivier, N., Leclercq, B., Drobecq, H., Vicogne, J., Agouridas, V., Melnyk, O. *Nat. Commun.* **11**, 2558 (2020), https://doi.org/10.1038/s41467-020-16359-6
5. Firstova, O., Melnyk, O., Diemer, V. *J. Org. Chem.* **87**, 14, 9426-9430 (2022), https://doi.org/10.1021/acs.joc.2c00934
6. Steinmann, D., Nauser, T., Koppenol, W.H. *J. Org. Chem.* **75**, 6696-6699 (2010), https://doi.org/10.1021/jo1011569
7. Lothrop, A.P., Snider, G.W., Ruggles, E.L., Patel, A.S., Lees, W.J., Hondal, R.J. *Biochemistry* **53**, 654-663 (2014), https://doi.org/10.1021/bi400658g
8. Bachrach, S.M., Demoin, D.W., Luk, M., Miller J.V. *J. Phys. Chem. A* **108**, 4040-4046 (2004), https://doi.org/10.1021/jp037972o