https://doi.org/10.17952/38EPS.2026.P563

EDMAN DEGRADATION BEYOND STANDARD

Toni Kühl,[1] Alexander Terekhov,[1] and Diana Imhof[1]*

[1]*Pharmaceutical Biochemistry and Bioanalytics, Pharmaceutical Institute, University of Bonn, Bonn, Germany*

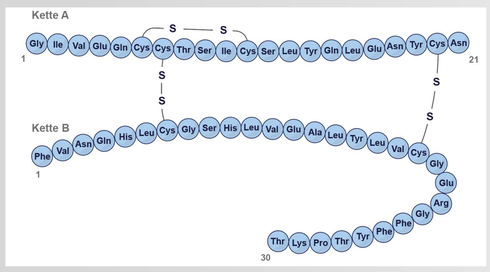
→ BACKGROUND

This study has been published recently in the *Journal of Peptide Science* [1]. Detection and annotation of disulfide bridges in multiply bridged peptides and proteins is complicated and requires careful evaluation of multiple data sets. We studied the elucidation of the cysteine connectivity by relaunching Edman sequencing to solve this specific structural feature [1,2]. Here, we demonstrate the identification of **diPTH-cystine** and **PTH-cysteine** for comparison with **PTH-Cys(Cam)** [2] during Edman sequencing when a disulfide bond is detected in a peptide. In addition, Edman sequencing was applied to selected peptides containing two or three disulfide bridges [1]. The study provides further insight into the differentiation of cysteines that form a disulfide bridge for half-cystines both in the same cycle and in separated cycles enabling correct disulfide bond assignment of cysteine-rich peptides.

→ AIM OF STUDY

To solve the problem of cysteine and disulfide bridge detection in automated Edman sequencing of multiple disulfide-bonded peptides and proteins.

Insulin

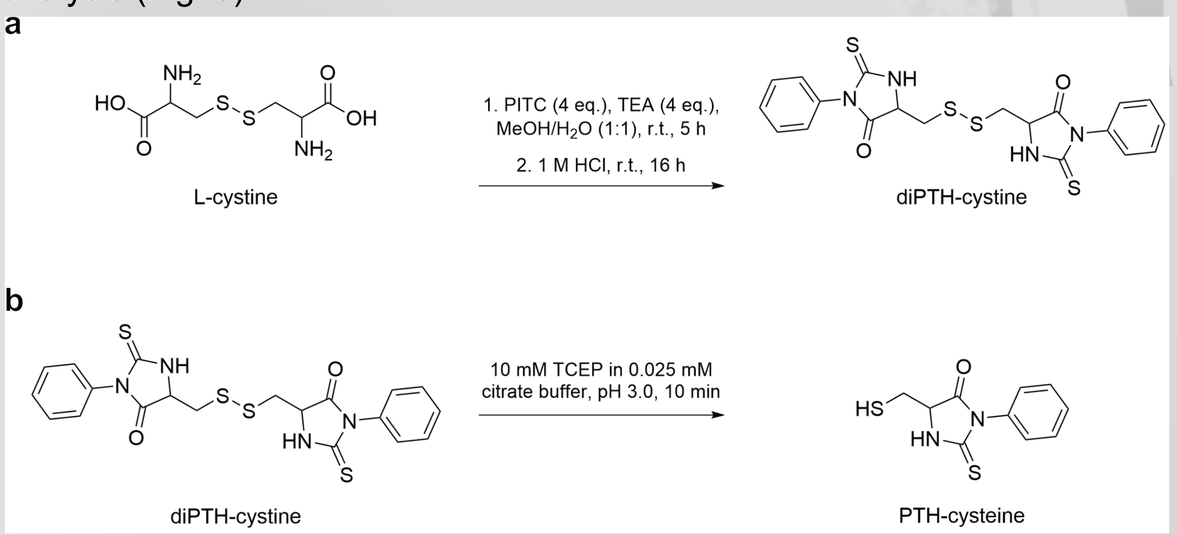


→ MATERIALS AND METHODS

Synthesis of diPTH-cystine:
L-Cystine was reacted with triethylamine, methanol, and PITC under argon. DiPTH-cystine was obtained after acidification and drying (Fig. 1a). Crude product: 95%; purified: 31%. Purification was done via C18-HPLC (Shimadzu) and identity confirmed by LC-ESI MS and $^{1}H/^{13}C$-NMR. MS: m/z 475.04 $[M+H]^+$; HPLC retention time: 28.2 min.

Synthesis of PTH-cysteine:
DiPTH-cystine was reduced with 10 mM TCEP in citrate buffer (pH 3) for 10 min (Fig. 1b). Conversion was >95%. MS: m/z 239.03 $[M+H]^+$; retention time: 22.2 min.

Automated Edman sequencing was performed using a Shimadzu PPSQ-53A. The new PTH-amino acids were separated chromatographically and identified using standards (Fig. 2). DiPTH-cystine and PTH-cysteine served as references for Cys-rich peptide analysis (Fig. 3).



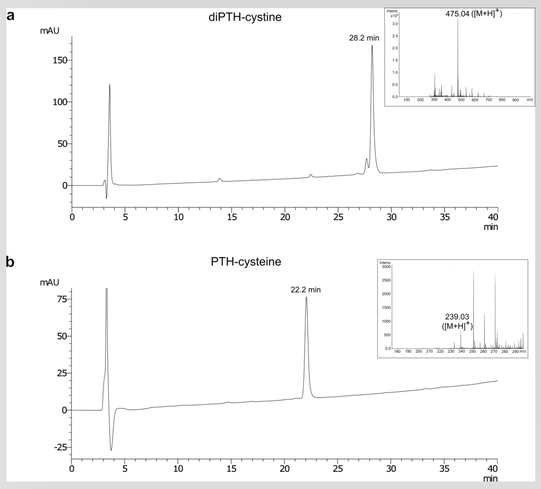


Fig. 1 (above)
Synthesis scheme for the generation of (a) diPTH-cystine and (b) PTH-cysteine by solution synthesis.

Fig. 1 (left)
Analytical characterization by RP-HPLC and mass spectrometry (inset) of diPTH-cystine (gradient: 20-60% acetonitrile containing 0.1% TFA (eluent B) in water containing 0.1% TFA (eluent A) in 40 min; calculated molecular weight: 474.03 g/mol) (a) and PTH-cysteine (gradient: 10-50% eluent B in eluent A in 40 min; calculated molecular weight: 238.02 g/mol) (b).

→ RESULTS

Edman sequencing was performed on human insulin, conotoxin μ-KIIIA, and the tridegin analogue $B_{[C19S,C25S]}$ under the manufacturer's recommended reducing conditions. In the case of the insulin C7(A)–C7(B) disulfide bridge, where both half-cystines are released in the same cycle, a distinct peak was observed in cycle 7. Based on retention time and DTT-mediated reduction of diPTH-cystine, this peak was unequivocally identified as PTH-cysteine. The results indicate that direct detection of disulfide bridges via Edman degradation is feasible primarily when both half-cystines appear in the same cycle.

„The following quotation reflects on the importance Pehr Edman placed on the discovery of his sequence method. Pehr Edman's mother, … in 1962, tells the following: ‚One day Pehr came home to me and asked me to sit down with him because he had something interesting to tell me. He then told me that **he had discovered a way to analyze proteins which had not been possible before and that this discovery would certainly be of great importance for biochemistry in the future**.'"

Selected Topics in the History of Biochemistry: Personal Recollections VII
Edited by G. Semenza, A.J. Turner, Elsevier, 2003

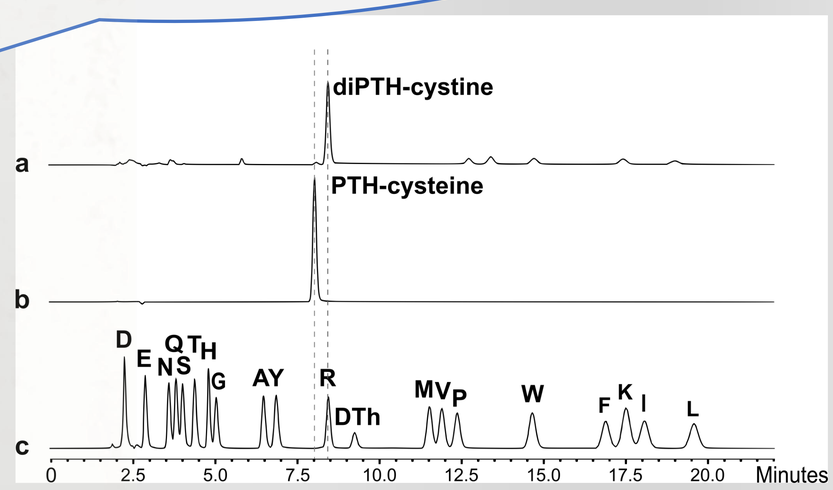


Fig. 2. Direct Edman degradation runs of (a) diPTH-cystine, (b) PTH-cysteine, and (c) PTH-amino acids standard mixture in 37% acetonitrile.[29]

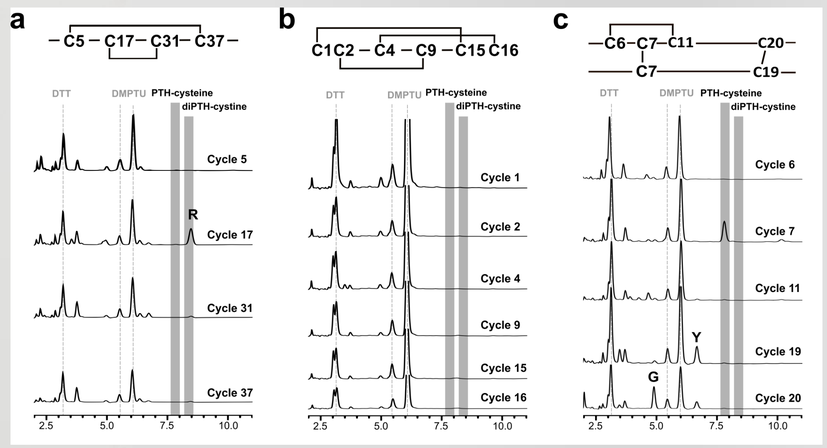


Fig. 3. Chromatograms of cycles covering cysteine positions in (a) tridegin, (b) μ-KIIIA, and (c) insulin. Frames indicate the retention times of PTH-cysteine and diPTH-cystine. DTT, dithiothreitol; DMPTU, (2,6-dimethylphenyl)thiourea.

→ CONCLUSIONS

While Edman sequencing has been used to elucidate disulfide bonds, this approach has largely been replaced by mass spectrometry. However, thorough MS analysis can be time-consuming, and some protein and peptide structures cannot be resolved by MS alone. Combining Edman sequencing with MS/MS enables faster and easier analysis of disulfide connectivity in complex peptides and proteins. It also addresses MS limitations, such as low fragment-ion intensities near disulfide-linked cysteines.

In this study, diPTH-cystine and PTH-cysteine were provided and characterized for Edman sequencing and evaluated as standards for the PPSQ sequencing device. These compounds are particularly valuable when multiple disulfide bridges occur in the same sequencing cycle, as frequently observed in nature, e.g., in antibodies.

We therefore introduce a workflow [1] to elucidate disulfide connectivity: Edman sequencing serves as the starting point for detecting disulfide bridges, followed by complementary MS and Edman analyses, potentially supported by partial reduction. Early identification or exclusion of specific disulfide bridges significantly simplifies the subsequent analysis.

References

1) Kühl T, Elsayed YY, Terekhov A, Imhof D., J. Pept. Sci. 2025, (10):e70053.
2) Elsayed YY, Kühl T, Imhof D., Anal Chem. 2024, 96(10):4057-4066.

Acknowledgments

Financial support by the University of Bonn and the GDCh is gratefully acknowledged. The authors like to thank Yomnah Y. Elsayed for fruitful discussions and Dirk Menche (University of Bonn) for access to the NMR spectrometer.

Check Imhof Lab