Semisynthesis of Homogeneous Intentionally Misfolded Glycoprotein IL-8

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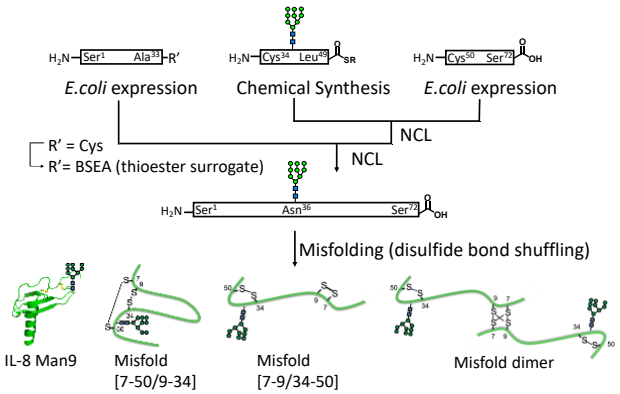
In the endoplasmic reticulum (ER), UDP-glucose:glycoprotein glucosyltransferase (UGGT) is able to differentiate between correctly folded and misfolded glycoproteins, and selectively add a glucose residue to a high-mannose type glycan of undesired misfolded structures as a tag for degradation or refolding. However, it is currently still unclear how UGGT is able to differentiate between correctly folded and misfolded forms.

To better understand the mechanism of UGGT, the detailed structural analyses of homogeneous misfolded glycoproteins are needed. In this study, we examined a new synthetic strategy to prepare homogeneously misfolded glycosylated interleukin-8 (IL-8) by combining chemical synthesis of glycopeptide and *E. coli* expression. Two peptide segments that do not contain a sugar chain were obtained using *E. coli* expression, and the N-terminal peptide was successfully converted into a peptide-thioester from a peptidyl-cysteine by a newly developed thioesterification method by our laboratory.[1] Only the peptide segment having a sugar chain was synthesized chemically. In this way, the benefits of biological expression, which is high yielding and time-efficient, can be combined with the benefits of chemical synthesis, which yields homogeneous glycosylated peptides. After native chemical ligation (NCL) of the prepared peptide segments, we were able to obtain the full length unfolded IL8 polypeptide.[2] Upon oxidative folding of the polypeptide without redox conditions, we were able to successfully obtain homogeneously misfolded glycosylated interleukin-8 (IL-8).[3] Currently, we are working on structural determination of misfolded glycosyl IL-8.



1) Okamoto, et al., *J. Org. Chem.,* **2021**. ASAP. 2) Dawson, et al., *Science,* **1994**, *266*, 776. 3) Izumi, et al., *J. Am. Chem. Soc.,* **2012**, *134,* 7238.