Elucidation of glycan functions by a new semi-synthetic method of glycoproteins

Yasuhiro Kajihara (Department of Chemistry, Osaka University)

To understand the biological function of glycoform, the homogeneous glycoproteins are essential for extensive biological experiments. We found a new method of glycoprotein synthesis. Glycosylated asparagine thioacid was designed to chemoselectively be inserted between two peptide segments where was the peptide-Asn(N-glycan)-Cys-Ser/Thr-peptide junction by two coupling reactions which consist of diacyl disulfide coupling (DDC) and thioacid capture ligation (TCL). This insertion strategy successfully afforded the full-length polypeptide of target glycoproteins within two steps from glycosylated asparagine thioacid (*J. Am. Chem. Soc.* 2021, https://doi.org/10.1021/jacs.1c02601). This method enabled us to synthesize Interleukin 3, chemokine-CCL1 and Serine protease inhibitor Kazal type 13 (SPINK13). DDC concept was also found to use highly steric hindrance amino acids between proline and valine (*Chem. Eur. J.* 2023, accepted).

In terms of semisynthesis of glycoproteins, we found two chemical approaches for transformation of the C-terminus of recombinant polypeptides to thioester surrogates that use for peptide coupling. The first approach relies on the substitution of the C-terminal Cys residue with bis(2-sulfanylethyl)amine to yield peptide-thioester surrogates. The second approach employs a native tripeptide, cysteinyl-glycyl-cysteine, to yield peptide-thioesters. Both chemical transformation methods employ native peptide sequences and were thereby successfully applied to recombinant polypeptides. Therefore, we succeeded in the semisynthesis of glycosyl inducible T-cell costimulator (*J. Org. Chem.* 2022, https://doi.org/10.1021/acs.joc.1c02031)

Antifreeze glycoprotein (AFGP) is highly O-glycosylated with a disaccharide D-Galβ1-3-D-GalNAcα and inhibits freezing of water. Sugar residues of AFGP play an essential role in the antifreeze activity, however, the mechanism have not been fully understood. We found that the stereochemistry of sugar residues on AFGPs precisely correlates with the antifreeze activity. These results indicate that sugar residues on AFGP form unique dynamic water phases that disturb the absorbance of water molecules onto the ice surface, resulting in the inhibition of freezing. In addition to the AFGP synthesis, we also use glycoprotein synthesis for the understanding glycan function (*Chem. Eur. J.* 2023, https://doi.org/10.1002/chem.202203553). We could discuss that glycans on the protein surface regulate water behavior to enhance protein-protein binding (*J. Am. Chem. Soc.* 2021, https://doi.org/10.1021/jacs.1c02601)