Published in final edited form as:

Chem Commun (Camb). 2010 August 21; 46(31): 5773–5774. doi:10.1039/c0cc00828a.

Chemoenzymatic Synthesis of Glycosylphosphatidylinositol-Anchored Glycopeptides†

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Abstract

MUC1 glycopeptide was efficiently coupled to glycosylphosphatidylinositol (GPI) derivatives by sortase A (SrtA), verifying that SrtA can accept sterically hindered glycopeptide as substrate for ligation with GPIs. This work has established a practical method for the chemoenzymatic synthesis of GPI-linked glycopeptides.

Many surface proteins and glycoproteins are associated to the cell membrane by glycosylphosphatidylinositol (GPI) anchors, a class of naturally occurring glycolipids sharing a conserved core structure Man α 1,2Man α 1,6Man α 1,4GlcNH $_2$ α 1,6Inositol-1-(P)-lipid.¹ The C-terminus of proteins/glycoproteins always links to the 6-O-position of the GPI nonreducing end mannose through a phosphoethanolamine bridge. GPI-anchored proteins/glycoproteins play a pivotal role in many biological events.^{1–5} To study the biological functions of GPI-anchored proteins/glycoproteins in detail, currently a major hindrance is access to these molecules in homogeneous form and sufficient quantity. Cell expression has the microheterogeneity problem, while the chemical synthesis of these very complex molecules represents a significant challenge.^{6–10} To address this issue, we have recently developed a chemoenzymatic strategy for GPI-anchored peptide/protein synthesis, which is based on sortase A (SrtA)-mediated GPI-peptide ligation.^{11,12}

SrtA is a transpeptidase of *Staphylococcus aureus* origin. It recognizes a LPKTG peptide sequence near the C-terminus of substrate proteins, cleaves the peptide bond between Thr and Gly to form a reactive thioester between the acyl group of Thr and SrtA Cys¹⁸⁴ thiol group, and finally transfers the Thr acyl group to the amino group of a Gly residue at the N-terminus of a peptidoglycan, which is the other substrate of SrtA.¹³ SrtA has been disclosed to be substrate-promiscuous and has been broadly used as a ligation tool for a variety of synthetic and biological applications.^{14–18} We have recently shown that SrtA can efficiently catalyze peptide ligation with GPI derivatives with one or two Gly units linked to the phosphoethanolamine group at the glycan nonreducing end of GPI core.^{11,12} Because this construct of GPI-anchored proteins/glycoproteins is highly conserved, the new synthetic strategy can be generally useful. We further envisioned that this strategy may be employed to prepare GPI-anchored glycopeptides/glycoproteins. To verify the hypothesis, we investigated herein the first SrtA-mediated GPI-glycopeptide ligation.

Glycopeptide FmocNH-GGVT(α GalNAc)S(α GalNAc)ADP-T(α GalNAc)LPKTGGS-OH (**1**) containing a partial sequence of MUC1, three O-glycans and the signal peptide LPKTG was used as the model in this research. MUC1 was targeted as it is densely glycosylated.

†Electronic Supplementary Information (ESI) available: Experimental procedures and data; NMR and MS spectra of the intermediates and final products. See DOI: 10.1039/b000000x/

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Moreover, mucins has been found GPI-anchored.¹⁹ The presence of several O-glycans in the structure of **1**, especially the attachment of a glycan to Thr immediately next to the signal peptide, makes **1** sterically hindered. Thus, SrtA acceptance of **1** as a substrate can be well indicative that SrtA may accept larger proteins/glycoproteins. In the structure of **1**, an additional Gly residue was installed at the N-terminus. Therefore, after the GPI-anchored glycopeptide are prepared, they may be used as SrtA substrates for further elongation of the glycopeptide chain to obtain more complex GPI-anchored glycopeptides.

The synthesis of glycopeptide **1** was a main challenge, which was achieved by solid-phase peptide assembly starting from a pre-loaded Wang resin **2** (Scheme 1), using commercial Fmoc amino acids and synthetic Fmoc-Ser(Ac₃-αGalNAc)-OH **3** and Fmoc-Thr(Ac₃-αGalNAc)-OH **4**²⁰ as building blocks. Peptide assembly was essentially performed with an automatic peptide synthesizer by standard Fmoc chemistry, while the coupling reactions of **3** and **4** were carried out manually using HBTU as the condensation reagent. In this case, only 2.5 equivalents of the precious **3** and **4** were utilized to guarantee high coupling efficiencies. After the desired glycopeptide was assembled, it was released from resin with 95% TFA, which deprotected the amino acid side chains concomitantly. Finally, the glycans were deprotected with NaOMe to afford **1**, which was purified by HPLC and characterized with MS.

With **1** in hand, we then explored its reactions with GPIs in the presence of SrtA (Scheme 2). The GPI derivatives used in this research were **6-8** that were formerly employed to explore SrtA-catalyzed GPI-peptide ligation.¹² Reactions were carried out at 37 °C in 0.3 M Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 5 mM CaCl₂, and 0.2 mM mercaptoethanol, and the concentrations of **1**, **6-8**, and SrtA were 0.5 mM, 2.5 mM, and 12.6 μM, respectively. The reaction progress was monitored by HPLC and MALDI-TOF MS. After *ca.* 24 h of incubation, reactions were quenched by adding the same volume of 0.1% TFA, and reaction yields were finally determined with HPLC. The reactions of **1** with **6-8** were found to proceed smoothly to afford ligation products **9** [MS (*m/z*): calcd 2430, found 2431 (*M* + *H*)⁺], **10** [MS (*m/z*): calcd 2845, found 2890 (*M* + 2 Na - *H*)⁺], and **11** [MS (*m/z*): calcd 2902, found 2903 (*M* + *H*)⁺] in 76%, 75%, and 84% yields, respectively. The products were further purified by HPLC and characterized with MS. These results indicated that SrtA could accept **1** as a substrate and effectively couple it to GPI analogues that were modified to carry one or two Gly residues.

Encouraged by the above results, we then studied the SrtA-catalyzed further elongation of the glycopeptide chain of **9** using **1** as the glycopeptide donor (Scheme 3). In this context, **9** was first treated with DBU to disclose its N-terminal Gly residue. Thereafter, the resultant product reacted with **1** in the presence of SrtA under the some conditions described above. HPLC analysis showed the formation of **12** in a 59% yield, as well as the N-terminal deprotected product **13** in a 10% yield, both of which were purified by HPLC and characterized with MALDI-TOF MS.

In summary, this work has verified that SrtA could accept complex glycopeptides as substrates for the ligation with GPI derivatives, and it has established a practical method for GPI-anchored glycopeptide synthesis. Moreover, because SrtA can tolerate very sterically hindered substrates, including both the peptide donor, such as **1**, and the peptide acceptor, such as **7**, **8** and **9**, we anticipate that SrtA will be able to handle more complex protein/glycoprotein and GPI substrates and that the method may be generally useful. Our laboratory is currently studying the application of this synthetic method to full-size GPI-anchored proteins/glycoproteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by Wayne State University and in part by NSF (CHE-0554777). Z. Wu thanks Mr. Benjamin M. Swarts for his help with the automatic peptide synthesizer.

Notes and references

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Scheme 1.
Solid-phase synthesis of glycopeptide **1**

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Scheme 3.
SrtA-catalyzed further elaboration of **9**