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Sortase A-Mediated Chemoenzymatic Synthesis of Complex Glycosylphosphatidylinositol-Anchored Protein†

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Abstract

Green fluorescent protein and a glycosylphosphatidylinositol (GPI) anchor containing the common core structure and a lipid chain were synthesized and then coupled together under the promotion of bacterial sortase A (SrtA), which was the first example for the synthesis of a full-size GPI-anchored protein by SrtA, demonstrating that this can be a generally useful method for GPI-anchored protein synthesis.

Glycosylphosphatidylinositol (GPI) anchorage of surface proteins to the extracellular membrane is ubiquitous in eukaryotic species,^{1–3} and GPI-anchored proteins play an important role in many biological processes.^{4–9} To gain insight into the biology of GPI-anchored proteins, it is necessary to have access to these molecules in sufficient quantity and purity, which is difficult to achieve through isolation and purification of natural products due to their structural complexity and microheterogeneity. As a result, practical methods for the synthesis of homogenous and structurally well-defined GPI-anchored proteins are highly desirable.

Several strategies have been explored for GPI-anchored peptide or protein synthesis. For instance, our group described a convergent strategy for chemical synthesis of GPI-linked peptides and glycopeptides via regioselective coupling of extensively protected GPIs to peptides and glycopeptides, followed by global deprotection.^{10, 11} Bertozzi group^{12, 13} and Seeberger group¹⁴ reported the synthesis of GPI-linked proteins by native chemical ligation (NCL) of Cys-containing GPI analogs and proteins, such as the green fluorescent protein (GFP)^{12, 13} and prion protein.¹⁴ However, the former strategy may be difficult to apply to large proteins, while the latter requires Cys-modified GPIs and C-terminal thioesters of proteins which are relatively difficult to access. More importantly, the latter resulted in products containing an undesirable Cys residue that may have implications. To deal with the

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issue, our group has recently developed a new strategy that utilized a bacterial enzyme, *Staphylococcus aureus*-derived sortase A (SrtA), to catalyze regiospecific ligation of GPIs and peptides.^{15, 16} As delineated in Scheme 1, SrtA can recognize a pentapeptide sequence, LPXTG (X = any amino acid) known as the sorting signal, react with it to break the peptide bond between T and G, and then transfer the carboxyl group of T to a nucleophile,^{17–20} such as modified GPIs.^{15, 16} This synthetic strategy can be widely useful, but in our previous studies, only simple GPI analogs and peptides and glycopeptides were examined. It is still unclear whether large proteins and complex GPI anchors, especially lipidated GPI anchors, can be accepted by SrtA for the ligation. To answer this question and explore the scope and limitations of the new enzymatic synthetic strategy, SrtA-catalyzed ligation of large protein and complex GPI anchor was investigated.

GPI **1**, which contained the common core structure of all GPI anchors and a lipid, was used in this research to examine GPI anchor-protein ligation. The synthesis of **1** is outlined in Scheme 2. Glycosylation of an inositol derivative **2**²¹ by **3** afforded an inseparable mixture **4** of α,β -anomers (2.5:1). Its deacetylation with sodium methoxide yielded a mixture that was relatively easily separated by column chromatography to afford pure α -isomer **5**. Subsequently, α -glycosylation of **5** by trimannosyl donor **6**, prepared according to a reported procedure,²² led to pseudopentasaccharide **7** that was deacetylated to give **8** in a 76% yield over two steps. All of the mannosyl residues in **8** were proved α -linked, judging from their J_{H1-C1} coupling constants derived from the coupled DEPT NMR spectrum. Phosphorylation of **8** was achieved by the one-pot two-step *H*-phosphonate method,²³ including coupling **8** to **9** under the influence of pivaloyl chloride and then oxidation of the resultant intermediate by iodine. Subsequently, **10** was treated with 5% trifluoroacetic acid in dichloromethane to remove the *para*-methoxybenzyl (PMB) protecting group, so as to expose the inositol 1-*O*-position. Again, the *H*-phosphonate method was employed to install phospholipid to **11**, which proceeded smoothly to offer **13** in a good yield. Finally, global deprotection of **13** under a H₂ atmosphere using 10% Pd(OH)₂/C as the catalyst provided the desired GPI anchor **1**.

A GFP derivative **14** carrying a LPATGGLEGRHHHHHH sequence at the C-terminus (Scheme 2), in which the His₆ tag was located beyond the sorting signal, was utilized to explore SrtA-catalyzed GPI-protein ligation. This design had the advantage that, after serving as an affinity tag to facilitate the purification of recombinant **14**, the His₆ tag would be removed during the ligation reaction, because SrtA breaks the peptide bond between T and G and links only the residual *N*-terminal sequence to the GPI anchor. GFP derivative **14** was expressed and purified according to a previously described procedure.²⁴

To examine SrtA-catalyzed GPI-protein ligation outlined in Scheme 2, GPI **1**, GFP **14**, and SrtA were incubated in the Tris-HCl buffer (0.3 M, pH 7.5) containing 150 mM NaCl, 5 mM CaCl₂, and 0.5 mM mercaptoethanol, while the reaction was monitored by HPLC. The HPLC results depicted in Figure 1 revealed that the reaction gave a new protein product (in 60% yield) that had longer retention time (*R*_t: 25.8 min) than the substrate protein **14** (*R*_t: 18.8 min). Actually, the product could only be eluted out of the column at higher concentrations of acetonitrile. This was a property anticipated from the desired GPI-GFP

conjugate **15** that contained a long lipid chain in the structure and should be more lipophilic than **14**. The product was purified by HPLC to get a green fluorescent protein, which was another piece of evidence to verify that the product was indeed GFP-GPI conjugate **15**. It should be mentioned that the reaction shown in Scheme 3 gave peptide GRHHHHHH as a side product while the resulting GFP-GPI conjugate **15** contained the sorting signal LPATG. As a result, they could act as SrtA substrates to accomplish a reverse reaction. Using a large excess (25 eq.) of the substrate **14**, which can be recovered from the reaction mixture, was found to improve the reaction yield.

The reaction product was also purified and characterized by additional methods. One of the major differences between the desired conjugate **15** and the enzyme SrtA or substrate protein **14** used in the reaction was that SrtA and **14** contained the His₆ tag but not **15**, thus **15** should be readily separable from SrtA and **14** by Ni-resin. Accordingly, after the reaction was completed, it was incubated with Ni-resin that was thereafter removed by centrifugation. The resultant supernatant was treated with acetone to precipitate proteins that were analyzed by HPLC. It was confirmed that the isolated product was the same as that obtained above by HPLC purification. To further characterize the product, it was then applied to SDS PAGE analysis. As shown in Figure 2, the molecular weight of this product was very similar to that of the substrate GFP – a result consistent with expectation, as the projected molecular weights for **14** and **15**, 28,672 and 28,658 Da respectively, were indeed very similar. Thus, the isolated product was once more verified to be GPI-GFP conjugate **15** that contained the GPI anchor and GFP but not the affinity tag. Unfortunately, attempts to obtain the accurate molecular weight of **15** by ESI or MALDI MS was unsuccessful, probably due to the presence of a long lipid and a couple of phosphate groups in **15**, as both functionalities make MS analysis difficult.²⁵

In summary, catalyzed by SrtA, a GFP derivative carrying a sorting signal was successfully coupled to a GPI anchor containing the intact GPI core and a lipid chain. This work has demonstrated for the first time that SrtA could accept complex GPI anchor and link it to large protein, thereby verifying that SrtA-catalyzed GPI-protein ligation can be a generally useful method for the synthesis of large and complex GPI-anchored proteins. In the meantime, the resultant GPI-GFP conjugate **15** and similar products with a fluorescent tag or other molecular handles can be powerful tools for various biological studies of GPI anchors and GPI-anchored proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Ferguson MAJ, Williams AF. *Ann Rev Biochem.* 1988; 57:285–320. [PubMed: 3052274]

2. Thomas JR, Dwek RA, Rademacher TW. *Biochemistry*. 1990; 29:5413–5422. [PubMed: 2143679]
3. Englund PT. *Ann Rev Biochem*. 1993; 62:121–138. [PubMed: 8352586]
4. Robinson PJ. *Cell Biol Intern Rep*. 1991; 15:761–767.
5. Gaulton GN, Pratt JC. *Seminars Immunol*. 1994; 6:97–104.
6. Ferguson MAJ. *J Cell Sci*. 1999; 112:2799–2809. [PubMed: 10444375]
7. Menon, AK.; Baumann, NA.; Rancour, DM. *Carbohydrates in Chemistry and Biology*. Hart, GW.; Hart, G.; Sinaÿ, P.; Ernst, B.; Sinaikin, P., editors. Vol. 4. Wiley-VCH; New York: 2000. p. 757-769.
8. Hwa K. *Adv Exp Med Biol*. 2001; 491:207–214. [PubMed: 14533800]
9. Loertscher R, Lavery P. *Transpl Immun*. 2002; 9:93–96.
10. Xue J, Shao N, Guo Z. *J Org Chem*. 2003; 68:4020–4029. [PubMed: 12737586]
11. Shao N, Xue J, Guo Z. *Angew Chem Int Ed*. 2004; 43:1569–1573.
12. Groves JT, Grogan MJ, Kaizuka Y, Conrad RM, Bertozzi CR. *J Am Chem Soc*. 2005; 127:14383–14387. [PubMed: 16218633]
13. Bertozzi CR, Paulick MG, Wise AR, Forstner MB, Groves JT. *J Am Chem Soc*. 2007; 129:11543–11550. [PubMed: 17715922]
14. Becker CFW, Liu X, Olschewski D, Castelli R, Seidel R, Seeberger PH. *Angew Che, Int Ed*. 2008; 47:8215–8219.
15. Guo X, Wang Q, Swarts BM, Guo Z. *J Am Chem Soc*. 2009; 131:9878–9879. [PubMed: 19583255]
16. Wu Z, Guo X, Wang Q, Swarts BM, Guo Z. *J Am Chem Soc*. 2010; 132:1567–1571. [PubMed: 20078124]
17. Ton-That H, Mazmanian SK, Faull KF, Schneewind O. *J Biol Chem*. 2000; 275:9876–9881. [PubMed: 10734144]
18. Popp MW, Antos JM, Grotenbreg GM, Spooner E, Ploegh HL. *Nat Chem Biol*. 2007:707–708. [PubMed: 17891153]
19. Marraffini LA, DeDent AC, Schneewind O. *Microbiol Mol Biol Rev*. 2006; 70:192–221. [PubMed: 16524923]
20. Wu Z, Guo Z. *J Carbohydr Chem*. 2012; 31:48–66. [PubMed: 22468018]
21. Pekari K, Tailler D, Weingart R, Schmidt RR. *J Org Chem*. 2001; 66:7432–7442. [PubMed: 11681958]
22. Wu X, Guo Z. *Org Lett*. 2007; 9:4311–4313. [PubMed: 17877362]
23. Lindh I, Stawinski J. *J Org Chem*. 1989; 54:1338–1342.
24. Guo X, Wu Z, Guo Z. *Bioconjugate Chem*. 2012; 23:650–655.
25. Harvey DJ. *Int J Mass Spectr*. 2003; 226:1–35.

Figure 1.
HPLC diagram of SrtA-mediated reaction of GPI anchor **1** and GFP **14**. HPLC conditions:
C-8 column (5 mm x 15 cm); flow rate: 1 mL/min; eluent: 10% to 60% aq. CH₃CN in 30
min; UV-Vis monitor at 220 nm.

Figure 2.

SDS PAGE results of: molecular markers (Lane 1), the reaction mixture (Lane 2), isolated product **15** (Lane 3), GFP **14** (Lane 4), and SrtA (Lane 5).

Scheme 1.

A general design for SrtA-catalyzed synthesis of GPI-anchored proteins

Scheme 2.

Chemical synthesis of GPI anchor **1**. Reaction conditions: (a) TMSOTf, toluene, 1,4-dioxane, -40°C ; (b) NaOMe, CH₂Cl₂, MeOH, 46% (2 steps); (c) TMSOTf, Et₂O, -40°C ; (d) NaOMe, CH₂Cl₂, MeOH, 76% (2 steps); (e) PivCl, pyridine, r.t.; then I₂, pyridine, H₂O, 74%; (f) 10% TFA in CH₂Cl₂, 69%; (g) (1) PivCl, pyridine, r.t.; (2) I₂, pyridine, H₂O, 72%; (h) H₂, 10% Pd(OH)₂/C, 88%.

Scheme 3.
SrtA-mediated synthesis of GPI-GFP conjugate