Zhongwu Guo

Profile

Zhongwu Guo received his undergraduate degree from The Second Military Medical University in China, his Ph.D. degree in organic chemistry from the Institute of Organic Chemistry, Polish Academy of Sciences, and his postdoctoral training in glycoscience at Shanghai Institute of Organic Chemistry (SIOC), Chinese Academy of Sciences. He was appointed Assistant and Associate Professor at SIOC (China) from 1994 to 1996, RIKEN Fellow at RIKEN (Japan) from 1996 to 1997, Assistant Research Officer at National Research Council of Canada from 1997 to 1999, and Assistant and Associate Professor at Case Western Reserve University (USA) from 1999 to 2005. He is currently Professor of Chemistry at Wayne State University, USA, and the National Glycoengineering Research Center, Shandong University, China. He is also serving as the Editor-in-Chief of the *Journal of Carbohydrate Chemistry*. His research interests are focused on carbohydrate chemistry, glycobiology, and medicinal chemistry, in particular the development of new methodologies for complex carbohydrate and glycoconjugate synthesis and the application of the synthetic glycoconjugates to the investigation of biological problems and to the development of new therapeutic strategies for various diseases.

Chemical and chemoenzymatic synthesis of GPI anchors and GPI-anchored proteins

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Many surface proteins and glycoproteins are anchored onto the cell membrane through glycosylphosphatidylinositols (GPIs)-a class of complex glycolipids, which share the highly conserved core structure: H_2N-CH_2CH_2-OPO_3-6-α-Man-(1→2)-α-Man-(1→6)-α-Man-(1→4)-α-$GlcNH_2$-(1→6)-*myo*-inositol-1-OPO_3-glycerolipid (Fig.1 A)[1]. Proteins and glycoproteins have their polypeptide C-termini linked invariably to the phosphoethanolamine moiety at the non-reducing end of the GPI core structure. GPI-anchored proteins and glycoproteins play an important role in various biological and pathological processes. To study these processes, it is essential to have access to homogeneous and structurally well-defined GPIs and GPI-anchored proteins and glycoproteins, as well as their derivatives, which is currently a significant challenge. One of the research projects in my laboratory aims at the development of practical methods for the synthesis of natural and unnatural and structurally defined GPI anchors and GPI-anchored peptides, glycopeptides, proteins, and glycoproteins, as well as functionalized GPI derivatives and conjugates, and applications of the synthetic molecules to exploring GPI anchorage to cells and related biological problems.

First, we developed a highly convergent strategy for GPI anchor synthesis[2]. In contrast to the traditional strategy where the phospholipid moiety was introduced at the final stage of the synthesis, we carried out the phospholipidation reaction early with the pseudodisaccharide to obtain a phospholipidated pseudodisaccharide **1** (Figure 1. A), which was used as a common key intermediate in GPI synthesis. Evidently, an advantage of this synthetic strategy was that glycosylation of **1** with oligosaccharides could lead to the backbone of desired GPIs in one step. Another advantage of this new synthetic strategy was that the difficult steps involved in GPI synthesis, such as the installation of the phospholipid and the α-glucosamine moieties, were realized at the initial stage, which helped circumvent some issues met in GPI synthesis and improve the overall synthetic efficiency. This highly convergent synthetic strategy was proved widely applicable and used to prepare a number of GPI anchors and GPI analogs. The synthetic GPI anchors and GPI analogs have been used to study biological problems, such as how some pore-forming bacterial toxins interact with GPI anchors[3].

We also explored the use of *para*-methoxybenzyl group as the permanent protection of hydroxyl groups in GPI synthesis[4], which enabled global deprotection of the target molecules under mild conditions compatible with various functional groups sensitive to hydrogenation. Combined with the convergent synthetic strategy described above, the new protecting tactic was successfully applied to the synthesis of GPI anchors carrying unsaturated lipids and other functionalities such as the azido and alkynyl groups[5][6]. The latter facilitated further specific modifications of GPIs by "click" chemistry. For example, fluorescein and biotin as imaging and affinity probes were effectively coupled to GPIs carrying the azido and alkynyl groups by "click" reactions to generate GPI-fluoro and GPI-biotin conjugates[6]. These functionalized GPI anchors and GPI conjugates are useful tools for biological studies of GPI anchorage.

Since all naturally occurring GPI-anchored proteins and proteins have their polypeptide C-termini linked to the conserved phosphoethanolamine moiety at the GPI non-reducing end, for GPI-anchored peptide, glycopeptide and protein synthesis, a potentially universally useful strategy is to prepare structurally defined GPI anchors, peptides, glycopeptides, and proteins separately and then couple them together site-specifically. In this regard, both chemical and enzymatic coupling reactions were exploited. For the chemical coupling, extensively protected GPIs with a free non-reducing end phosphoethanolamine moiety and peptides/glycopeptides with a free C-terminus were stitched together in a site-specific manner under the conditions for conventional amine and carboxylic acid condensation. Finally, the coupling products were globally deprotected to afford the target molecules[7]. Despite its success, this synthetic method would be difficult to apply to full size proteins because of the difficulty to access extensively protected proteins or to achieve global deprotection of GPIs in the presence of proteins. To deal with the problem, an enzyme-mediated method for coupling GPI anchors with peptides, glycopeptides and proteins was developed (Figure 1 . B).

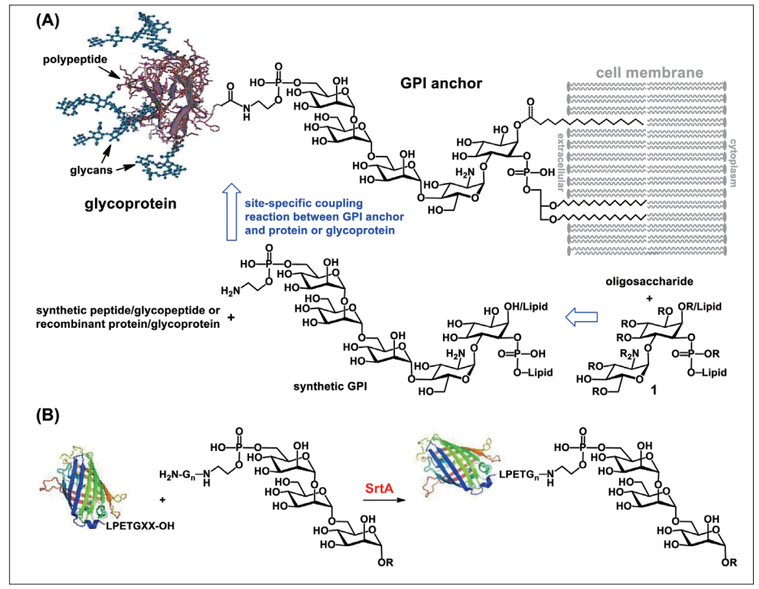

Figure 1. Schematic depiction of GPI anchorage of a glycoprotein to the cell membrane, as well as the strategies developed for the synthesis of GPI anchors and GPI-anchored peptides, glycopeptides, and proteins.
(A) Only the conserved core structure of GPI anchors without additional modifications is shown here. GPI-anchored proteins/glycoproteins always have the polypeptide C-terminus linked to the phosphoethanolamine moiety of the GPI core structure. GPIs anchor proteins and glycoproteins onto the cell surface by inserting the lipid chains into the cell membrane lipid bilayer. A potentially widely applicable method for the synthesis of GPI-linked peptides, glycopeptides, proteins, and glycoproteins is to site-specifically couple the C-terminus of a synthetic peptide/glycopeptide or recombinant protein/glycoprotein to the phosphoethanolamine moiety of a synthetic GPI anchor. The coupling reaction was achieved both chemically and enzymatically. Moreover, a highly convergent strategy was developed for the synthesis of GPI anchors with phospholipidated psudodisaccharide **1** as the common key building block. Glycosylation of this building block with oligosaccharides can lead to the skeleton structures of various GPI anchors in one step.
(B) SrtA-mediated ligation of GPI anchors and proteins for the synthesis of GPI-anchored proteins.

The enzyme employed to ligate GPI anchors with peptides, glycopeptides and proteins was sortase, a bacterial transpeptidase used by bacteria to anchor surface proteins to the cell wall[8]. Sortase recognizes a unique peptide sequence, known as the "sorting signal", near the target protein C-terminus, cleaves a specific peptide bond of the signal, and finally links the target protein to the terminal glycine residue of peptidoglycans on the bacterial cell wall. The specific enzyme utilized in our research was sortase A (SrtA) originated from *Staphylococcus aureus*. It recognizes a pentapeptide LPXTG, where X can be D, E, A, N, Q, or K, cleaves the peptide bond between T and G, and then links the carboxyl group of T to an amino group. We have proved that SrtA would accept GPI anchors with one or multiple glycine residues linked to their non-reducing end phosphoethanolamine moiety as substrates [9] and efficiently couple GPIs to peptides, glycopeptides, and proteins [10][11]. SrtA was also utilized to cyclize peptides and glycopeptides for the preparation of macrocyclic peptides and glycopeptides[12] and to attach proteins to the liposome surface decorated with glycine residues[13]. It is anticipated that SrtA may be generally useful for the synthesis of structurally defined GPI-anchored peptides and glycopeptides, as well as GPI-anchored proteins and glycoproteins that contain complex GPIs and full size proteins.

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