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Engineering protein glycosylation in prokaryotes

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Abstract
Controlling the specific glycan structure(s) present on a glycoprotein is a key challenge for both understanding its function and for developing effective next generation medical reagents. A decade ago the first engineered glycoproteins were produced in bacteria using the N-glycosylation machinery of *Campylobacter jejuni*. From the extensive development of this system, we learn the requisite features of a successful glycoengineering platform, as well as the factors that limit application in both glycan and protein structural space. From this perspective we review recent developments in the field of prokaryotic protein glycosylation. We highlight the emergence of cytoplasmic glycosylation systems as likely candidates to fuel the next wave of targeted glycoprotein synthesis with applications in research and medicine.

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Introduction

Protein glycosylation is the process of covalently attaching a glycan (mono-, oligo-, or polysaccharide) to an amino acid side chain. Glycosylation pathways can be broadly categorized according to three common characteristics (see Box 1). These parameters largely define the potential applications and limitations of a pathway for glycoengineering.

The functional consequences of protein glycosylation are diverse [1]. Glycans may modulate protein half-life, receptor binding, or enzymatic activity. Alternatively, the glycan may target the glycoprotein to a specific tissue or cell type via glycan-binding receptors. Some glycans are highly immunogenic [2] while others have immune dampening [3] or tolerizing effects [4,5]. Glycoproteins are abundant on the surface of eukaryotic and prokaryotic cells and have emerged as important mediators of host-microbe interactions, especially in the gut [6]. Current applications of protein glycoengineering include half-life extension by addition of N-glycans [7] or polysialic acids [8], the optimization of antibody mediated cytotoxicity [9], and vaccination against bacterial pathogens [10]. Though impressively diverse, these applications exploit only a tiny fraction of the known biological activities of glycans and are sorely limited by the currently available glycoprotein expression systems, which provide access to an equally tiny fraction of the interesting glycan and protein structural space.

For engineering novel glycoprotein structures, bacterial expression systems have distinct advantages over eukaryotic cells. This derives primarily from the fact that bacterial protein glycosylation systems are not essential to viability of the cell. Indeed, the *Escherichia coli* strains commonly used for recombinant protein expression lack protein glycosylation altogether, allowing the rational design and introduction of orthogonal glycosylation pathways. In contrast, eukaryotic cells carry out extensive protein glycosylation via several distinct pathways. Deletion of eukaryotic glycosylation pathways often impacts cell viability dramatically, and modification of the pathways can have unexpected results due to interference by the numerous endogenous glycosyltransferases.

In this review, we outline development of the first bacterial glycoprotein engineering platform, developed from the N-glycosylation system discovered in *Campylobacter jejuni*. We draw attention to the features of bacterial glycosylation systems that lend themselves to successful glycoengineering and those that limit their application. With a view to the next generation of glycoprotein engineering platforms, we highlight some of the diverse protein glycosylation systems that are now known to exist in the bacterial kingdom. Our discussion is limited to protein glycoengineering strategies aimed at total biosynthesis of the target structure; strategies requiring subsequent *in vitro* chemoenzymatic remodeling are beyond the scope of this review.

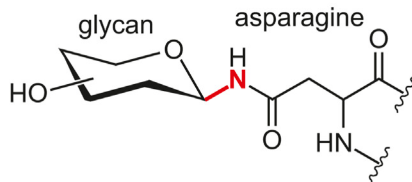
PglB-based protein glycosylation

Functional transfer of the N-linked glycosylation system of *C. jejuni* into the genetically tractable host, *E. coli*, definitively identified the genetic basis of this bacterial glycosylation system in the protein glycosylation (*pgl*) locus [11]. This groundbreaking work facilitated mechanistic studies, and laid the groundwork for development of the first bacterial glycoprotein engineering platform.

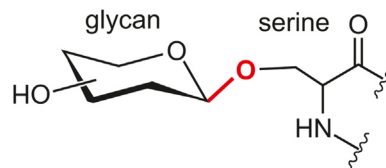
Box 1. Common characteristics of bacterial protein glycosylation pathways

I) Linkage between the protein and glycan chain

N-linked glycosylation - An N-glycosidic bond is formed between the anomeric carbon of the sugar and the amide nitrogen of asparagine. Only the β-anomeric configuration has been observed in nature.

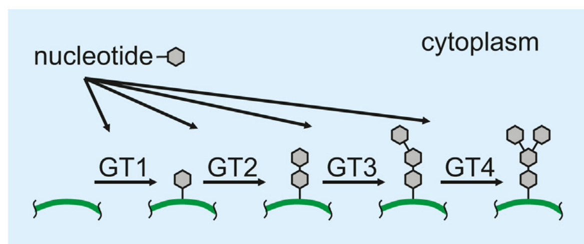


O-linked glycosylation - An O-glycosidic bond is formed between the anomeric carbon and the hydroxyl oxygen of serine or threonine. The bond may be formed with α- or β-anomeric configuration.

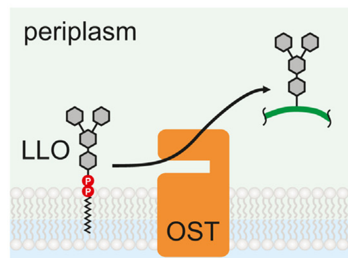


II) Topology of the glycosylation pathway

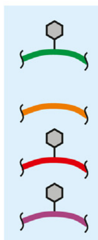
Sequential glycosylation - the complex carbohydrate is built up directly on the protein by successive addition of monosaccharides. This is typically carried out by glycosyltransferases (GT) using nucleotide-sugar substrates in the bacterial cytoplasm. Sequential glycosylation may also take place in the periplasm using lipid-linked monosaccharides.



En bloc glycosylation - a pre-assembled glycan is transferred to the protein from a lipid-linked oligosaccharide (LLO). The reaction is catalyzed by integral membrane proteins, known as oligosaccharyltransferases (OST), and occurs exclusively at the periplasmic side of the inner bacterial membrane. The chemical leaving group is the phospholipid undecaprenylpyrophosphate.

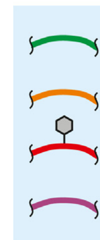


III) Number of proteins targeted by the glycosylation system



General glycosylation - multiple proteins are modified. Recognition of the target proteins may be mediated by a specific sequence motif (sequon) or general structural features of the target glycosylation site.

Dedicated glycosylation - a single protein is targeted for glycosylation.



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