Sugar and Spice Make Bacteria Not Nice: Protein Glycosylation and Its Influence in Pathogenesis

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Abstract

Protein glycosylation is a post-translational modification that occurs across the whole tree of life. In the recent years, multiple *N*- and *O*-glycosylation mechanisms have been identified and characterized in diverse bacterial species, including human pathogens. This review focuses on bacterial protein glycosylation and its impact in pathogenesis. Bacteria carry *N*- and *O*-glycosylation systems that are mediated by an oligosaccharyltransferase (OTase). In OTase-dependent glycosylation mechanisms, an oligosaccharide is synthesized on a lipid carrier and subsequently transferred to proteins *en bloc* by an OTase. Multiple proteins are glycosylated using this mechanism. OTase-independent glycosylation refers to the pathway in which Protein *N*- and *O*-glycosyltransferases (PGTases) sequentially add monosaccharides onto the target proteins. This pathway is employed for glycosylation of flagella and autotransporters. By exploiting glycosylation machineries, it is now possible to generate tailor-made glycoconjugates by attaching polysaccharides derived from lipopolysaccharide or capsule biosynthesis onto a protein of choice. These glycoproteins can be used in developing vaccines and diagnostics of bacterial infections. Furthermore, both *N*- and *O*-glycosylation systems are promising targets for antibiotic development. Recently, the discovery of GTase toxins produced by bacterial pathogens and secreted into the host cells has greatly expanded. These proteins are a key factor in host–pathogen interactions and are required by certain pathogenic bacteria to establish a successful infection. The exact functions of bacterial glycoproteins in pathogenesis are just starting to emerge. Understanding these roles is key for new opportunities in the prevention of bacterial infections, which is crucial in times when antibiotic resistance continues to increase.

Introduction

Protein glycosylation, the covalent attachment of a carbohydrate to an amino acid residue, was formally demonstrated in 1938 and is the most abundant post-translational modification in nature [1,2]. The first identified prokaryotic glycoproteins were the cell surface proteins of the archaeon *Halobacterium* and the bacterial species *Clostridium*, showing that this phenomenon occurs in all the kingdoms of life [3,4]. This review focuses on bacterial protein glycosylation and its impact in pathogenesis.

Protein glycosylation occurs in two main forms, *N*-linked, which is the attachment of the carbohydrate on to the nitrogen of an asparagine residue, or *O*-linked, where the glycan is attached to the oxygen of the hydroxyl group of serine or threonine residues in most cases, although a few species present proteins glycosylated on tyrosine residues [5–7]. Beyond the linkage definition, protein glycosylation occurs mainly by two different pathways that differ in the way the sugars are assembled and transferred to the acceptor protein. These are known as oligosaccharyltransferase (OTase) dependent and OTase independent. OTases are a family of glycosyltransferases (GTases) that catalyze the *en bloc* transfer of an oligosaccharide from a lipid donor to an acceptor molecule, usually a protein. In OTase-dependent systems, as shown in Fig. 1, an initiating GTase transfers a monosaccharide from its nucleotide-activated sugar onto the lipid

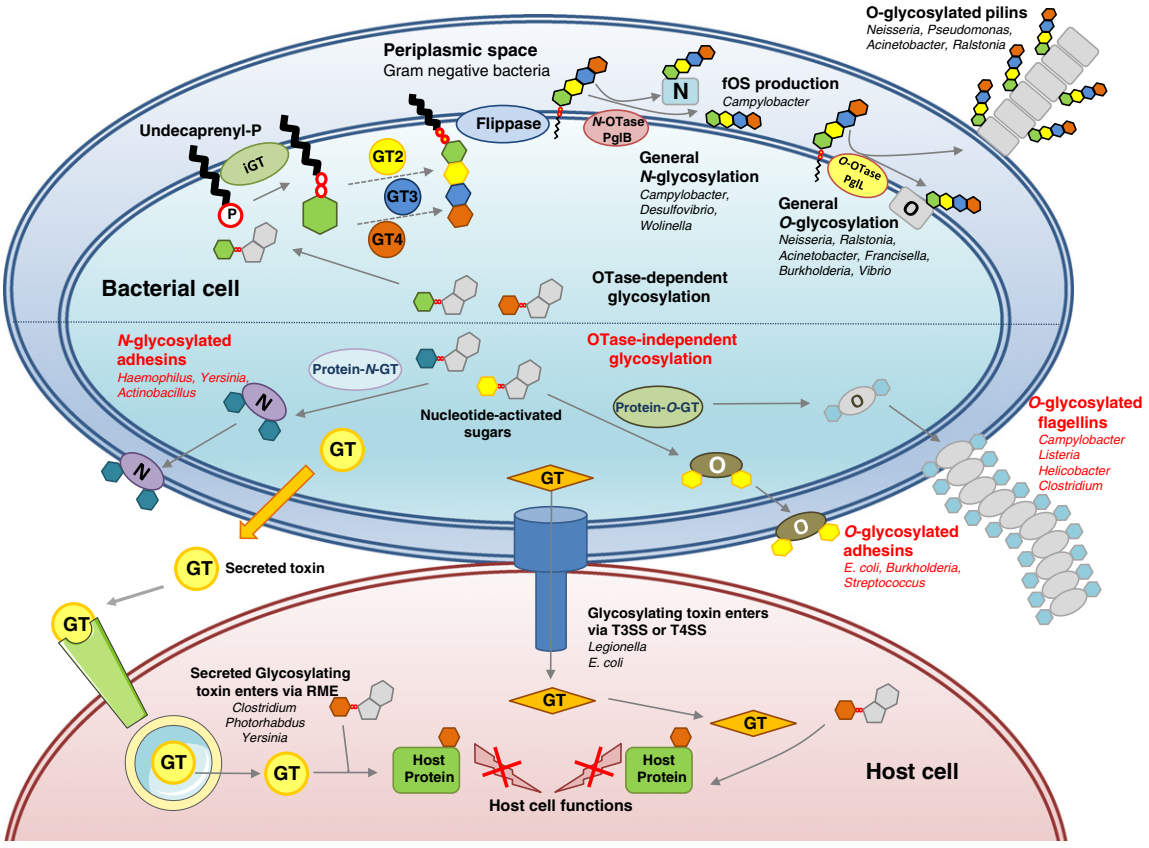

Fig. 1. The general landscape of bacterial protein glycosylation. The bacterial intracellular pool of nucleotide-activated sugars feeds all protein glycosylation pathways. In OTase-dependent protein glycosylation (upper half of bacterial cell), a monosaccharide is attached to an Und-P molecule in the cytoplasmic face of the inner membrane by an initiating GTase. The rest of the oligosaccharide is synthesized by the consecutive action of cytoplasmic GTases (i.e., GT2, GT3, and GT4). Then, a flippase turns the lipid-linked oligosaccharide from the cytoplasmic face to the periplasmic face of the inner membrane. The oligosaccharide is covalently attached to either an asparagine residue by an *N*-OTase (i.e., PglB) or to a serine/threonine residue by an *O*-OTase (i.e., PglL). In the case of *N*-glycosylation, fOS) are generated in a PglB-dependent manner, as an end product of the hydrolase activity in PglB. Glycosylated proteins either remain as membrane-associated, periplasmic or are secreted to the cell surface. In OTase-independent glycosylation (lower half of the bacterial cell), cytoplasmic GTases known as Protein *N*-GTases or Protein *O*-GTases transfer mono- or di-hexoses onto target proteins. Most of the targets from Protein *N*-GTases are adhesins, while Protein *O*-GTases modifiy both adhesins and flagellins. Glycosylated adhesins and flagellins are secreted to the cell surface. Bacterial GTase toxins are depicted as orange diamonds and yellow circles in the lower part of the bacterial cell. Clostridial and clostridial-like toxins (yellow circles) are secreted to the extracellular media where they bind to receptors on the host cell membrane. Following receptor-mediated endocytosis, at least part of the toxin is exposed into the cytoplasm of the host cell. The toxin then modifies target proteins, specifically proteins from the Rho family, affecting central processes in the host cell. Differently, toxins from *Legionella* and *E. coli* (orange diamonds) are injected to the host cell cytoplasm by Type 4 and Type 3 secretion systems, respectively. Toxins from *Legionella* modify elongation factor eEF1A, blocking translation of the host cell. Toxins from *E. coli* glycosylate GAPDH and Death Domain-containing proteins, blocking adequate immune responses from the host.

carrier undecaprenyl phosphate (Und-P) at the cytoplasmic face of the inner membrane [8]. Cytoplasmic GTases consecutively add monosaccharides to the growing lipid-linked carbohydrate. The complete Und-PP-linked glycan is then flipped to the periplasmic side of the inner membrane by a flippase, where the OTase transfers the glycan to specific residues on the target protein. This mechanism resembles eukaryotic *N*-linked glycosylation occurring in the endoplasmic reticulum and shares similarities with the synthesis of lipopolysaccharide (LPS) in Gram-negative bacteria [8–11]. In contrast, OTase-independent glycosylation occurs in the cytoplasm, where monosaccharides are transferred one at a time onto the acceptor protein by the successive action of GTases (Fig. 1). These glycoproteins are subsequently transported to the outer membrane or, in the case of flagellin, are secreted, forming the flagella filament [12–14]. It is