Protein Glycosylation in Campylobacter jejuni: Partial Suppression of pglF by Mutation of pseC

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Received 24 April 2007/Accepted 6 July 2007

Campylobacter jejuni has systems for N- and O-linked protein glycosylation. Although biochemical evidence demonstrated that a pseC mutant in the O-linked pathway accumulated the product of pglF in the N-linked pathway, analyses of transformation frequencies and glycosylation statuses of N-glycosylated proteins indicated a partial suppression of pglF by pseC.

Campylobacter jejuni has two protein glycosylation systems (18). Campylobacter flagellins, like those of many other polar flagellates, are decorated with O-linked glycans (12). One such sugar is pseudaminic acid (5,7-diacetamido-3,5,7,9-tetradeoxy-\(\alpha\)-L-glycero-L-manno-nonulosonic acid; Pse5Ac7Ac) (13). Genetic analyses have identified the genes responsible for the production of Pse5Ac7Ac (4, 5, 20), and recently, the biosynthetic pathway of Pse5Ac7Ac within Helicobacter pylori and C. jejuni was elucidated (16). Glycosylation of flagellin subunits is required for filament biogenesis in C. jejuni (4), and this O-linked system appears to specifically glycosylate flagellin (12). The N-linked glycosylation system modifies numerous periplasmic proteins with a heptasaccharide containing 2,4-diacetamido-2,4,6-trideoxy-\(\alpha\)-D-glucopyranose (2,4-diacetamido-Bac) at the reducing end of the glycan (23). The N-linked system includes an oligosaccharide transferase that resembles that of eukaryotes, and it has been characterized biochemically (2, 3, 7, 11, 15, 21). The phenotype of C. jejuni mutants defective in the N-linked system is pleiotropic, likely reflecting the variety of proteins glycosylated by this system. Although fully motile (19), mutants defective in N-linked glycosylation have a reduced ability to invade intestinal epithelial cells in vitro (19), reduced levels of colonization in animals (6, 19), and a significant reduction in natural transformability (9).

Synthesis of Pse5Ac7Ac and 2,4-diacetamido-Bac begins with the modification of UDP-GlcNAc by distinct pairs of enzymes and biosynthetic intermediates of the initial steps in each pathway, as determined by Schoenhofen et al. (16, 17), are indicated.

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Published ahead of print on 13 July 2007.

FIG. 1. CMP-pseudaminic acid and UDP-2,4-diacetamido-Bac pathways in C. jejuni. The enzymes and biosynthetic intermediates of the initial steps in each pathway, as determined by Schoenhofen et al. (16, 17), are indicated.
The latter sugar is also the product of PglF, a UDP-amido-2,6-dideoxy-D-arabino-ketone product, it was dem-
higher than that of the pglF mutant but did not reach the level seen with the wild type. PglF and PseB belong to a family of dehydratases that can be divided into two subfamilies. The first subfamily, which includes PglF and WbpM, consists of large proteins associated with the inner membrane of the cell (1). Thus, it is likely that the biosynthesis of the 2,4-diacetamido-Bac is closely associated with the cytoplasmic face of the inner membrane. Additionally, 2,4-diacetamido-Bac is transferred onto a membrane-associated lipid carrier by PglC (2). In contrast, PseB belongs to the second subfamily of dehydratase enzymes, whose members are smaller than and lack the membrane-anchoring domain associated with the first subfamily. This difference in cellular localization may contribute in part to the inability of PseB to supply sufficient precursor to the pgl system to fully glycosylate N-linked proteins in C. jejuni.

This work was supported by National Institute of Allergy and Infectious Disease grant RO1 AI43559 (to P.G.) and Navy Work Unit no. 6000.RAD1.DA3.A0308 from the Military Infectious Diseases Program.

We thank David Rockabrand for the CmeC antisera.

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