Protein Glycosylation in *Campylobacter jejuni*: Partial Suppression of \textit{pglF} by Mutation of \textit{pseC}\textsuperscript{\textcopyright}

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*C. 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*.

*C. 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-D-glycero-\alpha-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

\[ \text{UDP-GlcNAC} \xrightarrow{\text{PglF}} \text{PseB} \xrightarrow{\text{PseB}} \text{UDP-2-acetamido-2,6-dideoxy-\alpha-D-xylo-hexos-4-ulse} \xrightarrow{\text{PseC}} \text{UDP-2,4-diacetamido-Bac} \]

\[ \text{UDP-2-acetamido-2,6-dideoxy-\beta-L-arabinono-hexos-4-ulse} \xrightarrow{\text{PseC}} \text{UDP-4-amino-4,6-dideoxy-\beta-L-AltNAc} \xrightarrow{\text{PseH, G, I, and F}} \text{CMP-Pse5Ac7Ac} \]

\[ \text{CMP-Pse5Ac7Ac} \xrightarrow{\text{PseB}} \text{UDP-2-acetamido-2,6-dideoxy-\alpha-D-xylo-hexos-4-ulse} \xrightarrow{\text{PseC}} \text{UDP-2,4-diacetamido-Bac} \]

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.
latter sugar is also the product of PglF, a UDP-H9251-D-amido-2,6-dideoxy-epimerization, which results in the production of UDP-2-acetamide gels and immunoblotted with SBA lectin as described by Kelly et al. (7). Lane 1, 81-176; lane 2, pglF mutant; lane 3, pglF pseC mutant; lane 4, pseC mutant. The positions of molecular mass markers (in kilodaltons) are shown on the left. wt, wild type.

Dehydratase/aminotransferase enzymes (17). These are Cj1293 (PseB) and Cj1294 (PseC) for the Pse5Ac7Ac pathway and Cj1120c (PglF) and Cj1121c (PglE) for the 2,4-diacetamido-Bac pathway (17). PseB has C4,6 dehydratase/C5 epimerase activity that results in the production of UDP-2-acetamido-2,6-dideoxy-β-L-arabino-hexos-4-ulose, which is the substrate for the second of the enzyme pair, PseC, an aminotransferase which produces UDP-4-amino-4,6-dideoxy-β-L-AltNAc. Upon accumulation of the UDP-arabinose-ketone product, it was demonstrated that the PseB enzyme can also perform a second epimerization, which results in the production of UDP-2-acetamido-2,6-dideoxy-α-D-xylo-hexos-4-ulose (17) (Fig. 1). The latter sugar is also the product of PglF, a UDP-α-D-GlcNAc C6 dehydratase (17; Fig. 1). A recent metabolomic study confirmed the accumulation of UDP-2,4-diacetamido-Bac in a pseC mutant in vivo (14). The accumulation of this intermediate led us to determine whether a pseC mutation could suppress pglF by supplying the missing intermediate in the pgl pathway (see Fig. 1).

A double mutant of 81-176 was constructed by transformation of the pglF:aph3 (19) gene into 81-176 pseC (5). The construction was confirmed by PCR analysis using primers that bracketed the insertion point of aph3 into pglF (data not shown). The original pglF:aph3 mutant was fully motile (19), but the double mutant, like the pseC::cat parent (4), was nonmotile (data not shown). Figure 2 shows that loss of glycosylation in the pglF mutant resulted in reduced reactivity with soybean agglutinin (SBA), which binds to terminal GalNAc residues (7), compared to those for wild-type 81-176 and the pseC mutant. Lectin reactivity appeared to be restored to the wild-type pattern in the pglF pseC double mutant (lane 3), consistent with a restoration of N-linked glycosylation (7, 19).

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In order to study this apparent suppression in more detail, we examined glycosylation of two unrelated proteins directly. VirB10, a periplasmic component of a plasmid-encoded type IV secretion system in 81-176, has been shown experimentally to contain two sites of N-linked glycosylation (9). In wild-type 81-176 and the pseC mutant, VirB10 migrates as a doublet that represents glycosylation at one or two sites (9) (Fig. 3). In pglF, VirB10 runs at the predicted mass of the unglycosylated protein; in a pglF pseC double mutant, VirB10 migrates at a position consistent with either no glycosylation or glycosylation at a single site (Fig. 3). The consensus site for N-linked glycosylation has recently been defined (8), and CmeC (Cj0365c), an outer membrane component of an efflux pump (10), is predicted to have two sites of N-linked glycosylation. As shown in Fig. 3, in wild-type 81-176 and the pseC mutant, a single band is visible in immunoblots with anti-CmeC antiserum. However, in the pglF mutant, the CmeC band migrates more rapidly, consistent with the loss of glycosylation. In the double mutant, there are three bands. One corresponds to the band seen in pglF (unglycosylated), one is comparable to that of the wild type (fully glycosylated), and one is intermediate in size (one glycosylation site). Thus, in the wild type, CmeC appears to be glycosylated at two sites; in the double mutant, there appears to be a mixture, with one, two, or no sites glycosylated.

Mutants in the pgl glycosylation system are defective in natural transformation (9). Natural transformation of C. jejuni is dependent on a type II secretion system (22), and multiple components of this secretion system have potential sites for N-linked glycans (8). Table 1 compares the natural transformation frequencies of wild-type 81-176 with those of the mutants. There was a significant difference between results for the wild type and for the pglF mutant (P < 0.05), as previously reported for other pgl mutants (9), and there was no difference between results for the wild type and the pseC mutant. The transformation frequency of the pglF pseC double mutant was

TABLE 1. Natural transformation frequencies

<table>
<thead>
<tr>
<th>Strain</th>
<th>Transformation frequency</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>81-176</td>
<td>1.2 × 10&lt;sup&gt;−2&lt;/sup&gt; ± 0</td>
<td>NA</td>
</tr>
<tr>
<td>pglF mutant</td>
<td>4.75 × 10&lt;sup&gt;−6&lt;/sup&gt; ± 0.000004 &lt;0.05</td>
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</tr>
<tr>
<td>pseC mutant</td>
<td>1.2 × 10&lt;sup&gt;−2&lt;/sup&gt; ± 0.005 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>pglF pseC mutant</td>
<td>3 × 10&lt;sup&gt;−5&lt;/sup&gt; ± 0.000002 &lt;0.05</td>
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<sup>a</sup> DNA from a Str<sup>r</sup> mutant of 81-176 (9) was used to transform the C. jejuni strains listed. Transformation frequency is expressed as the number of Str<sup>r</sup> transformants/cell/μg. The means and standard deviations from two separate experiments are shown. 

<sup>b</sup> P value relative to the transformation frequency of 81-176 was calculated by t test using Prism GraphPad Software. NA, not applicable.
higher than that of the pgf 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 pgf system to fully glycosylate N-linked proteins in C. jejuni.

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REFERENCES


