The histo-blood group ABO system is defined by ABO blood group carbohydrate antigens, which play important roles in blood transfusion, cell differentiation, and oncogenesis.1 The blood group A and B antigens are formed by the transfer of two different sugar residues (GalNAc and Gal) to the common H-disaccharide epitope by human blood group transferases GTA and GTB, respectively. Chemical synthesis methodology developed by Lemieux set a milestone in the in vitro synthesis of blood group antigens.2–4 On the other hand, the enzymatic synthesis using recombinant GTA and GTB has proven less laborious and more regio- and stereo-selective.5,6 Recently, microbial glycosyltransferases have become an attractive alternative to the mammalian counterparts, since they are easy to express in active form without complicated gene manipulation and can catalyze similar glycosylation reactions.

Several Gram-negative bacteria express histo-blood group antigens in their lipopolysaccharide (LPS) O-chain.7,8 The sequence of the O-antigen gene cluster facilitates the in vitro expression of microbial glycosyltransferases for the synthesis of human antigens. Escherichia coli O86 was previously shown to possess high blood group B activity. Springer demonstrated that inoculation of germ-free chicken with E. coli O86 resulted in the elevated anti-B antibody production.9 The blood group B activity of E. coli O86 was further confirmed by an immune response on humans, and those individuals with blood group O and A responded with a significant rise of anti-B antibody.10 On the basis of chemical and serological investigations, Springer et al.9 and Kochibe et al.11 showed that E. coli O86 O-antigen and human blood B antigen share a similar overall structure with a continuous region from the O86 O-antigen gene cluster and identified a new O86 O-antigen repeat unit.12 Confirmed that its O-antigen and human blood B antigen share a similar overall structure with a continuous region from the O86 O-antigen gene cluster and identified a new O86 O-antigen repeat unit.12

A base pair sequence of 13 990 bp from E. coli O86:K62:H2 strain was obtained, which covered a continuous region from the galF gene to the start of gnd gene (GenBank accession number AY667408). Thirteen open reading frames were identified (Figure 2), and the respective ORFs were assigned putative functions by sequence comparative analysis.

We biochemically identified three glycosyltransferases involved in the synthesis of blood group B antigen epitope. Orf11 (wbnl) belongs to the glycosyltransferase family 6 with 45% similarity to GTA and GTB. Thus, wbnl most likely encodes an α-1,3-galactosyltransferase that makes the Gal-α-1,3Gal linkage to form the blood group B-like antigen structure. To confirm its function, we cloned wbnl gene into the pET15b plasmid and expressed in E. coli BL21 (DE3) as a His-tagged fusion protein. The sugar donor specificity assays indicated that Wbnl was capable of transferring galactose residues at high efficiency, providing evidence that wbnl encodes a galactosyltransferase. The acceptor screening showed the narrow specificity of Wbnl (Table 1). The activity of Wbnl is nearly 7 times higher when the trisaccharide Fuc-Gal-α-1,3-GalNAc-α-OMe is used as an acceptor, compared to the disaccharide Gal-α-1,4Gal-α-OMe. Interestingly, since Wbnl required α-1,2-linked fucose as a sugar side chain, the biosynthesis of the blood group B antigen in E. coli O86 is essentially the same as that in humans.13 The GTA and GTB are highly homologous except for a difference in only four amino acid residues.14 The change of four residues is sufficient to change the enzyme specificity from using UDP-GalNAc to UDP-Gal as a sugar donor. Crystal structures of GTA and GTB revealed that the Leu/Met 266 residue is critical in differentiating the donors, due to the complementary size between the amino acid residue and the functional group of the sugar donor.15 Amino acid sequence alignment of Wbnl and GTB showed that the Leu/Met 266 residue is critical in differentiating the donors, due to the complementary size between the amino acid residue and the functional group of the sugar donor.15

Table 1. Substrate Specificity of Wbnl and WbnK

<table>
<thead>
<tr>
<th>acceptor</th>
<th>Wbnl</th>
<th>WbnK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuc-Galβ-1,3GalNAc-α-OMe</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Galβ-1,3GalNAc-α-OMe</td>
<td>12</td>
<td>N/A</td>
</tr>
<tr>
<td>Fuc-Galβ-1,4Glc-α-Oph</td>
<td>N/A</td>
<td>100</td>
</tr>
<tr>
<td>Galβ-1,4Galβ-1,4Glc</td>
<td>N/A</td>
<td>100</td>
</tr>
<tr>
<td>Galβ-1,4Galβ-1,4Glc</td>
<td>N/A</td>
<td>100</td>
</tr>
</tbody>
</table>

* R/A: relative activity.
motif, which is proposed to interact with phosphate groups of the nucleotide donor through the coordination of a metal cation. The data therefore identify WbnJ as a bacterial glycosyltransferase equivalent to human blood group transferase GTB. Future detail studies of WbnJ can potentially reveal more insights into its substrate specificity and structure relationships with human GTB.

The orf13 (wbnJ) was assigned as another galactosyltransferase that transfers a Gal residue to GalNAc via a \( \beta(1 \rightarrow 3) \) linkage. The linkage specificity was confirmed by NMR analysis of the synthesized disaccharide Gal-\( \beta \)-1,3-GalNAc-\( \alpha \)-OMe (see Supporting Information).

The C-terminal of orf14 (wbnK) has a conserved domain from glycosyltransferase family 11, which mostly consists of \( \alpha \)-1,2-fucosyltransferases from different organisms. WbnK was expressed in \( E. coli \) as a GST-tagged fusion protein. Notably, WbnK has a strict substrate requirement shown by the acceptor specificity assays (Table 1). The fucosyltransferase activity was identified only when Gal-\( \beta \)-1,3-GalNAc (T-antigen) was used as a substrate. The change of linkage from 1\( \rightarrow 3 \) to 1\( \rightarrow 4 \) or the configuration from \( \beta \) to \( \alpha \) will make very poor substrates for WbnK. Thus, the terminal \( \beta \)-1,3-linked galactose residue is critical in WbnK recognition. Since the substrate specificity assay reveals that WbnJ has a strong preference for a terminal fucosylated acceptor and WbnK requires a terminal \( \beta \)-1,3-linked galactose residue via methyl substitution to facilitate the biosynthesis of blood group B antigen epitope in \( E. coli \) O86. The sequence of enzyme reactions to make blood group B antigens in bacteria is essentially the same as in humans. This new finding provides us not only an economical and attractive way to synthesize human blood group antigens but also clues to elucidate the relationship between human natural antibody production and enteric bacterial stimulation.

On the basis of the O-antigen structure, we carried out the stepwise enzymatic synthesis of blood group B antigen in 10 mg scale by sequentially employing the three glycosyltransferases (Scheme 1). The configuration of the anomeric carbon in starting compound 1a was fixed as \( \alpha \) by methyl substitution to facilitate the NMR analysis. The products from each reaction were purified by gel filtration, and the glycosidic linkage was analyzed by NMR spectroscopy (see Supporting Information). The yield ranging from 75 to 93% was achieved in each single reaction, with the overall yield of 60%. It is a substantial improvement as compared to the chemical synthesis, for which the total yield is normally lower than 40%.

In conclusion, we elucidate the O-antigen biosynthesis in \( E. coli \) O86, an important bacterial strain that possesses strong blood group B activity. More importantly, we identified a new bacterial glycosyltransferase equivalent to human blood group transferase B (GTB). The enzyme substrate specificity and stepwise enzymatic synthesis of blood group B antigen reveal that the biosynthetic pathway of B antigen is essentially the same in \( E. coli \) O86 as in humans, thus providing us an attractive way of synthesizing blood group B antigen with microbial glycosyltransferases.

Acknowledgment. We thank the National Institutes of Health (R01 AI44040) for financial support of this work.

Supporting Information Available: Shotgun sequence of \( E. coli \) O86 O-antigen gene cluster, cloning, expression, purification and enzymatic assay of glycosyltransferases, stepwise enzymatic synthesis and characterization of blood group B antigen Gal-\( \alpha \)1,3-(Fuc-\( \alpha \)-1,2)-Gal-\( \beta \)-1,3-GalNAc-\( \alpha \)-OMe. This material is available free of charge via the Internet at http://pubs.acs.org.

References


JA045021Y