J|A|C|S
COMMUNICATIONS

Published on Web 01/27/2005

Escherichia coli O86 O-Antigen Biosynthetic Gene Cluster and Stepwise Enzymatic Synthesis of Human Blood Group B Antigen Tetrasaccharide

Wen Yi,† Jun Shao,† Lizhi Zhu,† Mei Li,† Mamata Singh,† Yuquan Lu,† Steven Lin,† Hanfen Li,† Kang Ryu,† Jie Shen,† Hongjie Guo,† Qingjia Yao,† C. Allen Bush,‡ and Peng G. Wang*,†

Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, and Department of Chemistry, University of Maryland, Baltimore County, Maryland 21250

Received August 18, 2004; Revised Manuscript Received December 5, 2004; E-mail: pgwang@chemistry.ohio-state.edu

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 stereoselective.[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 the strong blood group activity of *E. coli* O86 came from LPS that contained the blood group B trisaccharide partial structure. The complete structure (Figure 1) of the O-polysaccharide[12] confirmed that its O-antigen and human blood B antigen share a similar oligosaccharide epitope. In this communication, we sequenced *E. coli* O86 O-antigen biosynthetic gene cluster and identified a new glycosyltransferase as the equivalent of human GTB. The blood group B antigen tetrasaccharide was synthesized in a stepwise manner with three glycosyltransferases from the gene cluster.

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 (wbnI)* belongs to the glycosyltransferase family 6 with 45% similarity to GTA and GTB. Thus, *wbnI* 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

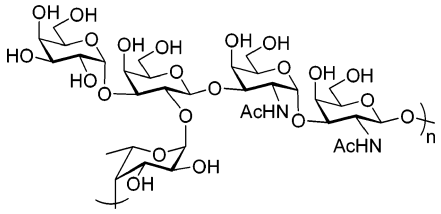


Figure 1. *E. coli* O86 O-antigen repeat unit.

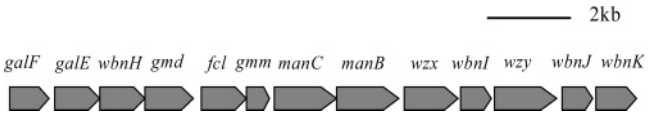


Figure 2. *E. coli* O86 O-antigen gene cluster.

Table 1. Substrate Specificity of WbnI and WbnK

WbnI		WbnK	
acceptor-	R/A[a] (%)	acceptor	R/A[a] (%)
Fucα-1,2Gal*β*-1,3GalNAc-α-OMe	100	Gal*β*-1,3GalNAc-α-OMe	100
Gal*β*-1,3GalNAc-α-OMe	12	Galα-1,3Gal*β*-1,4Glc	N/A
Fucα-1,2Gal*β*-OMe	30	Galα-1,4Gal*β*-1,4Glc	N/A
Gal*β*-1,4Glc*β*-Oph	N/A	Gal*β*-1,4Glc	N/A
Galα-1,3Gal*β*-1,4Glc	N/A	Gal*β*-OMe	15
Galα-1,4Gal*β*-1,4Glc	N/A	GalNAc-α-OMe	N/A

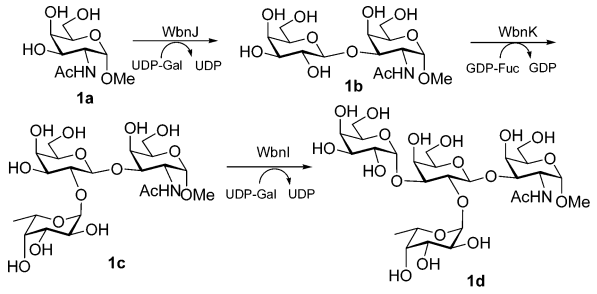
[a] R/A: relative activity.

cloned *wbnI* 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 WbnI was capable of transferring galactose residues at high efficiency, providing evidence that *wbnI* encodes a galactosyltransferase. The acceptor screening showed the narrow specificity of WbnI (Table 1). The activity of WbnI is nearly 7 times higher when the trisaccharide Fuc-α-1,2-Gal-*β*-1,3-GalNAc-α-OMe is used as an acceptor, compared to the disaccharide Gal-*β*-1,3-GalNAc-α-OMe. Thus, WbnI prefers terminal fucosylated sugar structures to nonfucosylated ones. Interestingly, since WbnI 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 WbnI and GTB revealed that Gln148 in WbnI was the equivalent residue of Met266 in GTB. Gln and Met are similar in size, and preliminary modeling studies demonstrated that Gln, in place of Met could fit well in the GTB binding pocket. In the N-terminal of WbnI, we identified a DXD

† The Ohio State University.
‡ University of Maryland.

10.1021/ja045021y CCC: $30.25

Scheme 1 Stepwise Enzymatic Synthesis of Blood Group B Antigen



Entry	Enzyme	Compound	Yield (%)
1	WbnJ	**1b**	85
2	WbnK	**1c**	75
3	WbnI	**1d**	93

motif, which is proposed to interact with phosphate groups of the nucleotide donor through the coordination of a metal cation. The data therefore identify WbnI as a bacterial glycosyltranferase equivalent to human blood group transferase GTB. Future detail studies of WbnI 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 $\beta(1\rightarrow3)$ linkage. The linkage specificity was confirmed by NMR analysis of the synthesized disaccharide Gal-β-1,3-GalNAc-α-OMe (see Supporting Information).

The C-terminal of *orf14* (*wbnK*) has a conserved domain from glycosyltransferase family 11, which mostly consists of α-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-β-1,3-GalNAc (T-antigen) was used as a substrate. The change of linkage from $1\rightarrow3$ to $1\rightarrow4$ or the configuration from β to α will make very poor substrates for WbnK. Thus, the terminal β-1,3-linked galactose residue is critical in WbnK recognition. Since the substrate specificity assay reveals that WbnI has a strong preference for a terminal fucosylated acceptor and WbnK requires a terminal β-1,3-linked Gal sugar sequence, we can identify the biosynthetic pathway 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 α 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-α-1,3-(Fuc-α-1,2)-Gal-β-1,3-GalNAc-α-OMe. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Feizi, T. *Nature* **1985**, *314*, 53−57.
(2) Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc*. **1975**, *97*, 4063−4069.
(3) Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076−4083.
(4) Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc* **1975**, *97*, 4069−4075.
(5) Seto, N. O. L.; Palcic, M. M.; Hindsgaul, O.; Bundle, D. R.; Narang, S. A. *Eur. J. Biochem.* **1995**, *234* (1), 323−328.
(6) Seto, N. O. L.; Palcic, M. M.; Compston, C. A.; Li, H.; Bundle, D. R.; Narang, S. A. *J. Biol. Chem.* **1997**, *272* (22), 14133−14138.
(7) Rasko, D. A.; Wang, G.; Palcic, M. M.; Taylor, D. E. *J. Biol. Chem.* **2000**, *275*, 4988−4994.
(8) Shao, J.; Zhang, J.; Kowal, P.; Lu, Y.; Wang, P. G. *Biochem. Biophys. Res. Commun.* **2002**, *295*, 1−8.
(9) Springer, G. F.; Horton, R. F.; Forbes, M. *J. Exp. Med.* **1959**, *110*, 614.
(10) Springer, G. F.; Horton, R. E. *J. Clin. Invest.* **1969**, *48*, 1280−1291.
(11) Kochibe, N.; Iseki, S. *Jpn. J. Microbiol.* **1968**, *12*, 403−11.
(12) Andersson, M.; Carlin, N.; Leontein, K.; Lindquist, U.; Slettengren, K. *Carohydr. Res.* **1989**, *185*, 211−223.
(13) Larsen, R. D.; Ernst, L. K.; Nair, R. P.; Lowe, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6674−6678.
(14) Yamamoto, F.; Clausen, H.; White, T.; Marken, J.; Hakomori, S. *Nature* **1990**, *345*, 229−233.
(15) Patenaude, S. I.; Seto, N. O. L.; Borisova, S. N.; Szpacenko, A.; Marcus, S. L.; Palcic, M. M.; Evans, S. V. *Nat. Struct. Biol.* **2002**, *9*, 685−690.

JA045021Y