# Bacterial Homologue of Human Blood Group A Transferase

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# Bacterial Homologue of Human Blood Group A Transferase

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The histo-blood group ABH antigens are defined by carbohydrate sequences mainly found on the surface of red blood cells.[1] These antigens are involved in a variety of important biological processes such as blood transfusion, organ transplantation, cell development, and differentiation, and oncogenesis.[2] Additionally, recent research has shown that these antigens are critical for novovirus infections.[3] Moreover, the molecular mimicry of ABH antigens found on the bacterial cell surface has been implicated in bacterial pathogenicity and modulation of human immune responses.[4]

The structures of the ABH carbohydrate epitopes are defined as GalNAc-α1,3(Fuc-α1,2)-Gal$\beta$ (A antigen), Gal-α1,3(Fuc-α1,2)-Gal$\beta$ (B antigen), and Fuc-α1,2-Gal$\beta$ (H antigen). The A and B antigens are formed by the transfer of GalNAc and Gal to the H antigen by human blood group transferases GTA and GTB, respectively.[5] The structures of GTA and GTB uncovered the basis of the donor specificity conferred by these two highly homologous enzymes.[6] A number of bacteria exhibit carbohydrate mimicry of blood group antigens on their cell surfaces as a means to evade host immune clearance.[7] Our group reported a bacterial homologue of GTB from *Escherichia coli* O86 and its use in the synthesis of the blood group B type III structure in vitro.[8] In this study, we report the identification of the first bacterial homologue of GTA from *Helicobacter mustelae*. This enzyme possesses flexible substrate specificity and can be used to synthesize five different types of A antigens with high efficiency. The broad specificity of this enzyme was also demonstrated by redirecting synthesis of B antigen to A antigen on the *E. coli* O86 cell surface.

*H. mustelae*-type strains have been employed as models for *Helicobacter* infection, ulceration, and gastric cancer. Studies showed that anti-blood group A antibody reacted strongly with lipopolysaccharide (LPS) of *H. mustelae*.[9] Structural analysis[9] revealed that the outer core of its LPS expresses the blood group A type I structure, GalNAc-α1,3(Fuc-α1,2)-Gal-$\beta$1,3-GlcNAc$\beta$. Using the sequence of GTA as a probe to BLAST against the recently completed *H. mustelae* strain ATCC 43772 genome (http://www.sanger.ac.uk/Projects/H_mustelae/), we identified an open reading frame with 40% homology with GTA. We therefore renamed it BgtA (for "bacterial GTA"). The *bgtA* gene was amplified and cloned into the pET28a vector. Expression in *E. coli* BL21 (DE3) and subsequent purification with a Ni-affinity column gave homogeneous target protein. A radioactive-based assay with UDP-GalNAc[6-$^3$H] and the substrate Fuc-α1,2-Gal-$\beta$1,3-GlcNAc$\beta$-$(CH_2)_2N_3$ confirmed that *bgtA* encodes a GalNAc-transferase. A further assay with a panel of acceptors revealed that BgtA has dedicated specificity toward Fuc-α1,2-Gal$\beta$-R sequences (Table S1, Supporting Information). Moreover, BgtA has relaxed specificity (Figure 1B) for the structures appended to the Gal residue, which

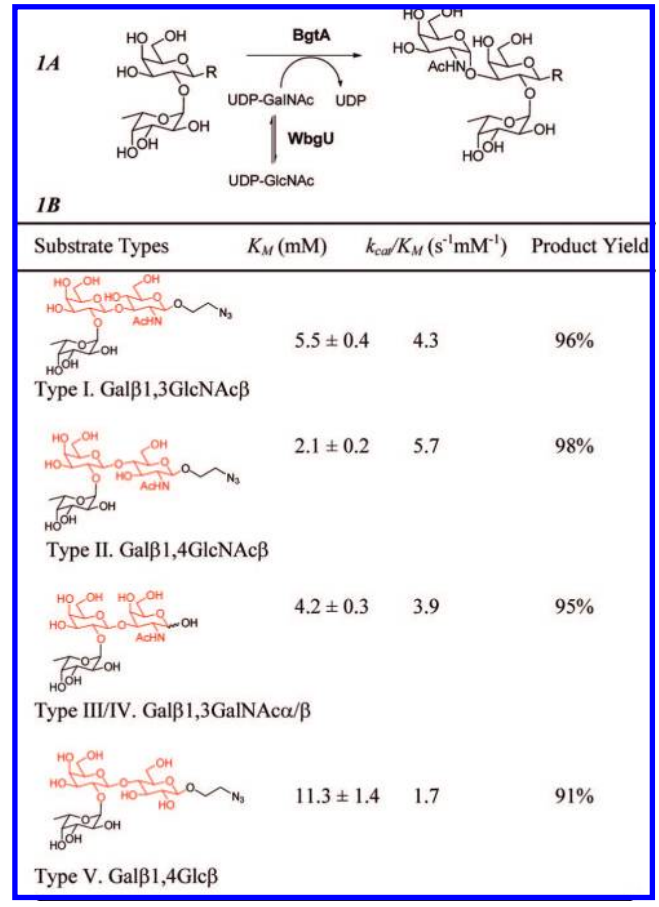


***Figure 1.*** Specificity of BgtA. (A) A one-pot, two-enzyme system for the synthesis of blood group A antigens. (B) Kinetics and yields of BgtA-catalyzed reactions.

collectively make up the five types of naturally existing blood group H core structures.[5] This result underscores the merit of BgtA for the synthesis of different A antigens. The apparent kinetic parameters (Figure 1B) revealed high catalytic efficiency of BgtA. In addition, the flexibility of BgtA was demonstrated in the synthesis of A antigens in vitro using a two-enzyme system (Figure 1A). In this system, previously characterized UDP-GlcNAc/GalNAc C-4 epimerase WbgU[10] was used to generate UDP-GalNAc from the inexpensive UDP-GlcNAc. Yields in excess of 90% were achieved in each reaction. The structures of synthesized A antigens were subsequently analyzed by NMR and MS (Supporting Information).

The properties of BgtA were then exploited to remodel the bacterial cell surface B antigen into the A antigen (Figure 2A). *E. coli* O86 LPS has a type III B antigen structure. Our previous study identified WbnI as a homologue of GTB in the bacterium.[8] Thus,

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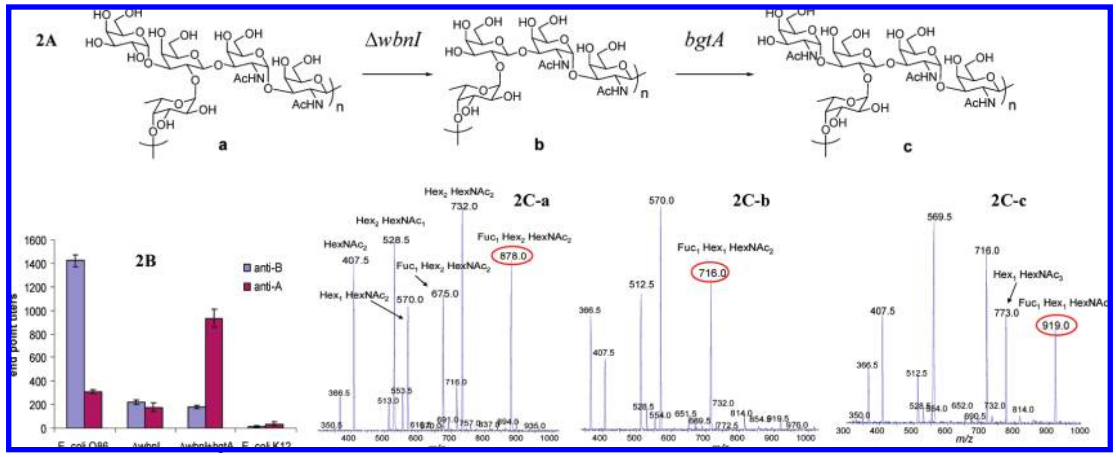


***Figure 2.*** Remodeling *E. coli* cell surface polysaccharides with bgtA. (A) Expected structures of modified polysaccharides (a, *E. coli* O86 wild-type; b, ΔwbnI mutant; c, ΔwbnI/bgtA complement). (B) LPS ELISA with blood group antibodies. (C) MS analysis of polysaccharide structures.

to convert the B structure into the A structure in vivo, we first disrupted the *wbnI* gene from the *E. coli* O86 chromosome.[11] Silver staining revealed the shift of LPS to a lower molecular weight region (Figure S1, Supporting Information). The structure study of polysaccharides from the Δ*wbnI* mutant[11] confirmed an H antigen-like structure. Next, we transformed *bgtA* into Δ*wbnI* mutants for trans-complementation. LPS from the *bgtA*-complemented cells displayed spacing and migration distance similar to those seen in the wild-type LPS; the corresponding bands have shifted to higher molecular weight compared to those from Δ*wbnI* mutants (Figure S1, Supporting Information). However, the amount of polysaccharides was reduced. This observation suggested that BgtA is able to modify the repeating unit; however, the repeating unit polymerase Wzy is less tolerant of the modified structure.[11]

To confirm the structure modification by *bgtA*, we carried out a LPS ELISA assay (Figure 2B). The negative control (*E. coli* K12) displayed minimal basal reactivity toward both anti-A and anti-B antibodies. Wild-type LPS, however, showed more than a 1000-fold increase of anti-B reactivity. It also exhibited a 50-fold increase of anti-A reactivity compared to the background, likely resulting from the cross-reactivity of B and A antigens. The LPS from Δ*wbnI* mutants has reactivity slightly higher than that of the background. The LPS from *bgtA*-complemented cells, however, revealed a strikingly different reactivity. The anti-A activity increased 800-fold compared to the negative control, while the anti-B activity decreased to a level similar to that of Δ*wbnI* mutants. This result demonstrates that *bgtA* can reverse the immuno reactivity of *E. coli* O86, possibly due to the modification of its cell surface polysaccharides.

The polysaccharide structure was further analyzed by CE-MS technique.[12] Full spectra of *O*-deacetylated LPSs from wild-type and mutant cells are shown in the Supporting Information. Information on the repeating unit was obtained by selecting the ion at *m/z* 204.2, which corresponds to the HexNAc oxonium ion, as a precursor to perform precursor ion scan experiments. In the spectrum of *bgtA*-complemented mutant (see Figure 2C-c), the ion at *m/z* 919.0 represents the sugar sequence $Fuc_1Hex_1HexNAc_3$, consistent with the proposed modified repeating unit structure. Similarly, the repeating units of the wild-type and Δ*wbnI* mutant were identified on the basis of *m/z* 878.0 (Figure 2C-a, $Fuc_1Hex_2HexNAc_2$) and 716.0 (Figure 2C-b, $Fuc_1Hex_1HexNAc_2$), respectively. In addition, the mass peaks corresponding to the repeating unit structures were unique in the respective cells, indicating the homogeneous modification.

In conclusion, we report the identification of the first bacterial homologue of GTA. Biochemical characterization of this enzyme (BgtA) demonstrated its high activity and flexible specificity toward the naturally existing blood group core structures, illustrating its synthetic versatility. Moreover, the applicability of BgtA was demonstrated in the engineering of bacterial cell surface polysaccharides. Further detailed mechanistic and structural studies will provide clues to better understand bacterium−host interactions through the adaptation and evolution of cell surface polysaccharide mimicry.

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**Supporting Information Available:** Full experimental details; NMR and MS spectra of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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