Bioorganic & Medicinal Chemistry 17 (2009) 4910–4915

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Characterization and synthetic application of a novel β1,3-galactosyltransferase from *Escherichia coli* O55:H7

Xian-wei Liu [a,b], Chengfeng Xia [b,c], Lei Li [a,b], Wan-yi Guan [a,b], Nicholas Pettit [b], Hou-cheng Zhang [a], Min Chen [a,*], Peng George Wang [b]

[a] National Glycoengineering Research Center and The State Key Laboratory of Microbial Technology, Shandong University, LuNeng Keji Dasha B 604, Shanda Nanlu 29-1, Jinan, Shandong 250100, China
[b] Departments of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43202, USA
[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China

ARTICLE INFO

Article history:
Received 15 April 2009
Revised 2 June 2009
Accepted 3 June 2009
Available online 9 June 2009

Keywords:
O-Antigen, Galactosyltransferase
Type 1 chain
Lacto-*N*-tetraose

ABSTRACT

A β1,3-galactosyltransferase (WbgO) was identified in *Escherichia coli* O55:H7. Its function was confirmed by radioactive activity assay and structure analysis of the disaccharide synthesized with the recombinant enzyme. WbgO requires a divalent metal ion, either Mn^{2+} or Mg^{2+}, for its activity and is active between pH 6.0–8.0 with a pH optimum of 7.0. *N*-acetylglucosamine (GlcNAc) and oligosaccharides with GlcNAc at the non-reducing end were shown to be its preferred substrates and it can be used for the synthesis of type 1 glycan chains from these substrates. Together with a recombinant bacterial GlcNAc-transferase, benzyl β-lacto-*N*-tetraoside was synthesized with the purified WbgO to demonstrate the synthetic utility of WbgO.

1. Introduction

Lacto-*N*-biose I disaccharide (2-acetamindo-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranose, Galβ1,3GlcNAc), also known as type 1 chain, is the precursor of a number of important carbohydrate epitopes in human body, such as Lewis A, Lewis B and sialyl Lewis A (SLe^a) antigens.[1] These epitopes are involved in a variety of biological processes including pathogen adhesion,[2] fertilization[3] and tumor metastasis.[4] For example, as a fundamental ligand for E-Selectin, SLe^a mediates the trafficking and recruitment of blood–borne leukocytes during normal and pathological inflammatory responses.[5] SLe^a is also considered as a tumor-associated antigen[6,7] and becomes the most frequently applied serum tumor marker for diagnosis of cancers in the digestive organs.[8]

The Galβ1,3GlcNAc motif is also present in other important oligosaccharides, such as lacto-*N*-tetraose (Galβ1,3GlcNAcβ1,3Galβ1,4Glc, LNT). LNT and their fucosylated and/or sialylated derivatives are among some of the major components in human milk oligosaccharides.[9] Some of them share similar structures with the glycan ligands recognized by human pathogens and are proposed to protect infants against these pathogens via competitive inhibition of the binding between the pathogens and the epithelial ligands.[10,11] Thus, usage of these oligosaccharides as anti-infection therapeutics can be logically suggested.

The glycosyltransferase-catalyzed synthesis of Galβ1,3GlcNAc requires a β1,3-galactosyltransferase (β1,3-GalT). Various genes encoding β1,3-GalT using GlcNAc-R as an acceptor have been identified in both human and other high eukaryotes and the corresponding β1,3-GalT have been characterized.[12,13] A bacterial GlcNAc β1,3-GalT has also been identified in *Streptococcus agalactiae* but has yet to be biochemically characterized.[14] A recombinant human β1,3-GalT has been used to synthesize multi-mg quantities of lacto-*N*-biose I disaccharide and its analogs.[15] But this enzyme was expressed in insect cells which were costly and inefficient. Herein we reported a novel bacterial enzyme expressed in *Escherichia coli* which provides a better source for this type of glycosyltransferase. It is also worthwhile to mention that an alternative enzymatic route has been reported on kilogram-scale production of lacto-*N*-biose I disaccharide with a lacto-*N*-biose phosphorylase,[16] however practical methods for the production of other Galβ1,3GlcNAc containing oligosaccharides such as LNT are still lacking.

E. coli O55:H7 strains are classified as enteropathogenic *E. coli* (EPEC). EPEC is the most common cause of prolonged diarrhea in infants in developing countries.[17,18] In addition, *E. coli* O55:H7 is known to be the closest relative of enterohaemorrhagic *E. coli* (EHEC) O157:H7,[19] which can cause non-bloody diarrhea, bloody diarrhea and hemolytic uraemic syndrome diarrhea and is furthermore one of the dominant intestinal pathogens in developed countries.[20] The O-polysaccharide structure of *E. coli* O55:H7 has been determined,[21] which is a repeating structure of a pentasaccharide containing a Galβ1,3GlcNAc motif (Fig. 1). Among the five different

* Corresponding author. Tel./fax: +86 531 88366078.
E-mail address: chenmin@sdu.edu.cn (M. Chen).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.06.005

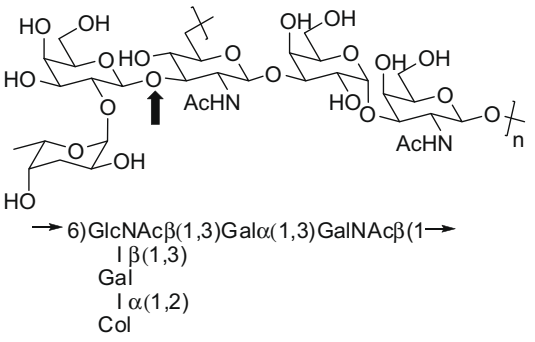


Figure 1. Structure of *E. coli* O55:H7 O-antigen repeating unit. The glycosidic bond catalyzed by β1,3-galactosyltransferase (WbgO) is indicated by an arrow. Col stands for colitose.

glycosidic bonds in the pentasaccharide, four are catalyzed by specific glycosyltransferases during the assembly of the O-antigen repeating units. The last glycosidic bond is formed when the repeating units are polymerized into O-antigen.

The O-antigen biosynthesis gene cluster of *E. coli* O55:H7 has been sequenced and four postulated glycosyltransferases are proposed to be responsible for the assembly of the O-antigen repeating unit, which are WbgO, WbgP, WbgM and WbgN.[22] The WbgN protein belongs to GT family 11,[23] which should be a colitosyltransferase, however the activities of other three glycosyltransferases are unpredictable relying only on bioinformatic methods. In this study, we distinguished the β1,3-GalT among these glycosyltransferases and further characterized this enzyme. Not only does this study enrich the basic knowledge for bacterial GlcNAc β1,3-GalT, but it also provides an alternative enzymatic route for the synthesis of Galβ1,3GlcNAc containing oligosaccharides.

2. Results and discussion

To identify the β1,3-GalT among the three glycosyltransferases from *E. coli* O55:H7, we cloned *wbgO*, *wbgP*, and *wbgM* genes into a glutathione S-transferase (GST) fusing expression vector, pGEX-4T-1. The recombinant enzymes were expressed in *E. coli* and purified by GST affinity chromatography (Fig. 2). Then GalT activity of these enzymes was explored using GlcNAc and *N*-acetylgalactosamine (GalNAc) as acceptors. Among them, only GST-WbgO showed significant GalT activity towards GlcNAc and GalNAc (Table 1). To further determine the function of WbgO, a small scale reaction was performed with *p*-nitrophenyl β-GlcNAc **1** as the sugar acceptor (Scheme 1). The structure of the resultant *p*-nitrophenyl lacto-*N*-bioside (lacto-*N*-biose-β-O-pNP) **2** was analyzed by ESI-MS and NMR spectroscopy. The formation of the β1,3 linkage was confirmed by both 1D and 2D NMR. According to the HMQC and ^{13}C NMR, the δ value of C-3 down shifted to 81.9, which indicated that this hydroxyl was involved in a glycosidic linkage.[24] The assignment of the anomeric carbon of terminus galactose with δ value of 103.6 shows that the anomeric configuration is β, by comparison with the published data.[24] The determination of the configuration was also supported by the ^1H NMR, with the δ value of 4.45 and coupling constant as 7.7 Hz of H-1.[25]

Table 1
The radioactivity reading from galactosyltransferase activity assay for GST-fused WbgO, WbgP and WbgM[a]

Acceptors	Enzymes			
	None	WbgO	WbgP	WbgM
None	57	252	150	161
GlcNAc	—	1053	161	137
GalNAc	—	499	167	158

[a] The activity assays were performed in a total volume of 50 μL with 50 mM Tris–HCl (pH 7.5), 5 mM $MnCl_2$, 0.3 mM UDP-Gal, 0.3 μM UDP-[6-^3H]Gal (10,000 cpm), 2 mM acceptor and 10 μL purified protein (about 5 μg protein) at room temperature overnight. The acceptor and/or enzymes were omitted in the control reaction. The total radioactivity was 9529 cpm.

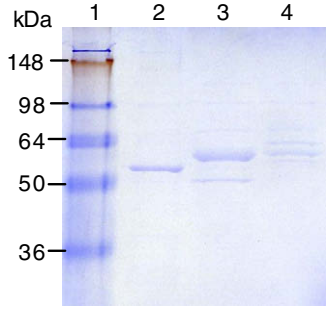
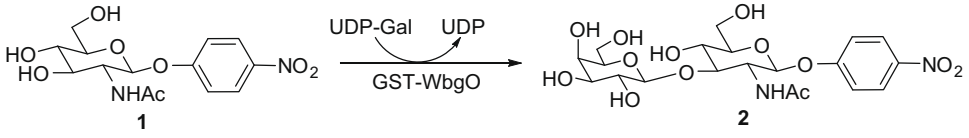


Figure 2. SDS–PAGE analysis of purified GST-fused WbgO, WbgP and WbgM. Lanes: 1, protein standards (the molecular weights are indicated in kDa on the left side); 2, GST-WbgO; 3, GST-WbgP; 4, GST-WbgM.

Expression and purification of WbgO with His6-tag failed using either plasmid pET-15b or pET-22b. On the contrary, expression and purification with pGEX-4T-1 as a GST-fused protein succeeded. The fusion protein GST-WbgO was produced with an N-terminus GST and was purified to homogeneity in one-step GST affinity chromatography (Fig. 2). The purified GST-WbgO showed a molecular weight between 50 and 64 kDa on SDS–PAGE which was consistent with the theoretical value (56.894 kDa). Totally 1.6 mg of GST-WbgO can be purified from 1-L batch fermentation with a specific activity of 67.5 mU mg^{-1} with GlcNAc as the acceptor. One unit of GST-WbgO was defined as the amount of the enzyme that catalyzes the formation of 1 μmol of the sugar product per minute. Cleavage of GST tag from the fusion protein with thrombin was unsuccessful. The cleavage efficiency was low and the resultant WbgO showed undetectable activity. Like many other glycosyltransferases from bacteria,[26,27] even without transmembrane regions, WbgO may be associated with the membrane. It is also possible that WbgO forms protein complex with other enzymes involved in O-antigen biosynthesis. Without those associations in physical environment, the stability and activity may not be able to maintain. Apparently the fusion with GST stabilized WbgO and assisted its activity under artificial conditions. Therefore, GST-WbgO was used for biochemical characterization and enzymatic synthesis in this study.

The effects of pH buffer systems and divalent metal ions on the activity of WbgO were then investigated. As shown in Figure 3A,



Scheme 1. Synthesis of *p*-nitrophenyl lacto-*N*-bioside with recombinant WbgO.

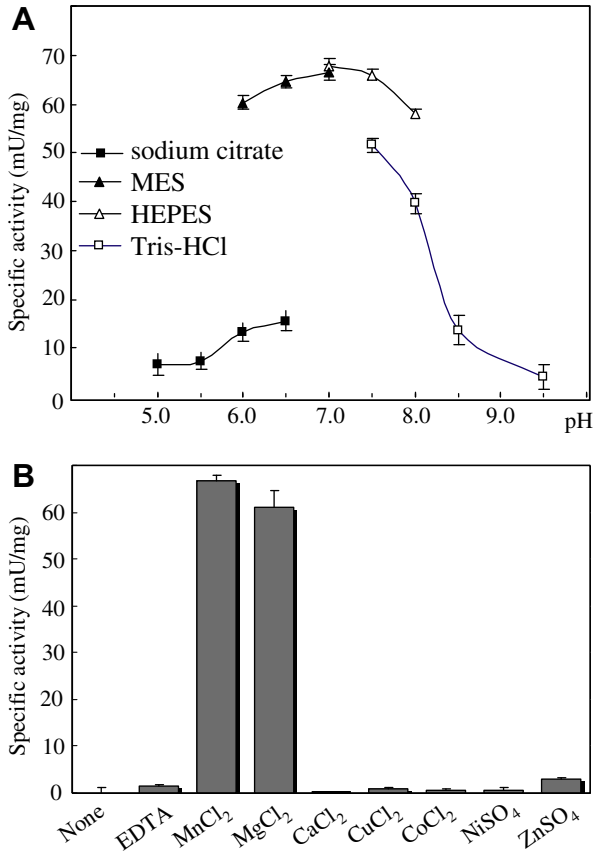


Figure 3. The effects of pH and divalent metal ions on the β1,3-galactosyltransferase activity of GST-WbgO. (A) The pH profile; (B) Effects of divalent metal ions.

the activity assay of GST-WbgO under different pH conditions demonstrates that the enzyme catalyzes the galactosyl-transferring reaction from UDP-galactose (UDP-Gal) to an acceptor under neutral condition (pH 6–8) with optimal activity at pH 7.0. There was sharp decline in enzymatic activity below pH 6.0 or above pH 8.0. In addition, GST-WbgO showed preference for certain buffer systems with the HEPES buffer system being the most efficient. The enzyme displayed comparable activity in MES buffer compared with that in HEPES buffer at the same pH value. In contrast, the enzyme presented lower activity in the Tris–HCl buffer than that in HEPES buffer under the overlapping pH range (pH 7.5 and 8.0). Sodium citrate buffer was found to exert a strong inhibitory effect on the activity of the enzyme as indicated by the distinct lower activity in this buffer at pH 6.5 than that in the MES buffer at the same pH value.

The activity of GST-WbgO was found to be absolutely dependent on specific divalent metal ions (Fig. 3B). Both $MnCl_2$ and $MgCl_2$ significantly supported its activity at the concentration of 5 mM. The activity with $MgCl_2$ was about 90% of that with $MnCl_2$. None of the other divalent metal cations (Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}) were efficient cofactors for this enzyme. In addition, the enzyme showed no activity in the absence of metal ion or in the presence of EDTA. The dependence of a metal ion for its activity indicates that WbgO belongs to the GT-A superfamily.[28] The existence of a DXD motif which is responsible for coordinating the divalent metal ion to bind to the sugar nucleotide is a common structure feature of the GT-A superfamily. Consistent with this feature, a $D^{99}S^{100}D^{101}$ motif was found present in WbgO. This motif is conserved among homologous glycosyltransferases as identified by a BLAST search from the NCBI database (Fig. 4).[29]

A series of acceptors including monosaccharides (GlcNAc, GalNAc, glucose and galactose) and oligosaccharides (lacto-*N*-triose, globotetraose, isoglobotetraose and GlcNAcβ1,3Galβ1,4Glc) were used to explore the acceptor specificity of GST-WbgO. The result (Table 2) suggests that GlcNAc and oligosaccharides with GlcNAc at the non-reducing end (GlcNAc-R) are the predominant acceptors. Furthermore, the trisaccharide lacto-*N*-triose was preferred by the enzyme with a twofold increase of enzymatic activity over GlcNAc. Lacto-*N*-triose is the best among all the acceptors tested in our assay, illuminating the potential of WbgO as a catalyst for the synthesis of biologically important oligosaccharides. GalNAc and oligosaccharides with GalNAc at the non-reducing end are also tolerated by the enzyme displaying relative activities 16–40% that of the monosaccharide GlcNAc. This phenomenon suggests a limited influence of the configuration of the 4-hydroxyl group on acceptor substrate binding. On the other hand, glucose and galactose do not serve as substrates for this enzyme, which indicates the

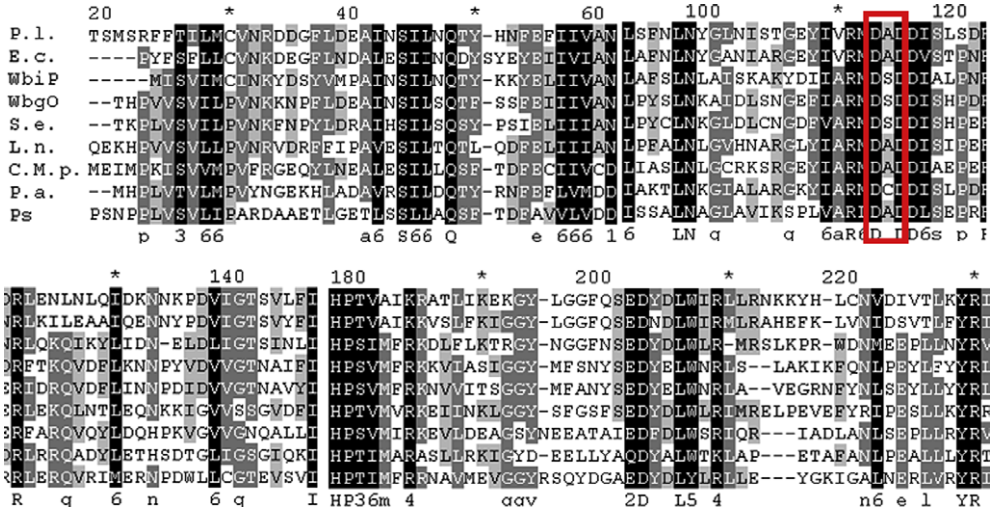


Figure 4. Amino acid sequence alignment of segments from different β1,3-GalTs. The DXD conserved motif was indicated by a box. P.l.: *Photorhabdus luminescens* subsp. laumondii TTO1 (Genbank accession NO.: NP_931977); E.c.: *Escherichia coli* (ABE98422); WbiP: *Escherichia coli* O127:H6 (YP_002329684); WbgO: *Escherichia coli* O55:H7 (AF461121); S.e.: *Salmonella enterica* subsp. salamae serovar Greenside (AAV34525); L.n.: *Lutiella nitroferrum* 2002 (ZP_03696917); C.M.p.: *Candidatus Methanosphaerula palustris* E1-9c (YP_002467214); P.a.: *Prosthecochloris aestuarii* DSM 271 (YP_002015057); Ps: *Pseudovibrio* sp. JE062 (YP_002680025).

Table 2
Acceptor substrate specificity of purified GST-WbgO

Acceptors[a]	Relative activity (%)
GlcNAc	100
GlcNAcβ-O-pNP	73
Lacto-*N*-triose-β-Obn[b]	221
GalNAc	43
Globotetrose-N_3	33
Isoglobotetrose-OMe	34
GalNAcβ1,3Galβ1,4Glc	16
Glucose, galactose	ND[c]

[a] The reactions were performed in a total volume of 50 μL containing 50 mM HEPES buffer (pH 7.0), 5 mM $MnCl_2$, 0.3 mM UDP-Gal, 0.3 μM UDP-[6-^{3}H]Gal (10,000 cpm), 2 mM acceptor and 4 μg (35 pmol) enzymes. The reactions were incubated at 37 °C for 1 h. The acceptor was omitted in the control reaction.

[b] Glycan sequences: lacto-*N*-triose-β-OBn, GlcNAcβ1,3Galβ1,4Glcβ-OBn; globotetrose-N_3, GalNAcβ1,3Galα1,4Galβ1,4Glcβ-N_3; isoglobotetrose-OMe, GalNAcβ1,3Galα1,3Galβ1,4Glcβ-OMe.

[c] ND: not detectable.

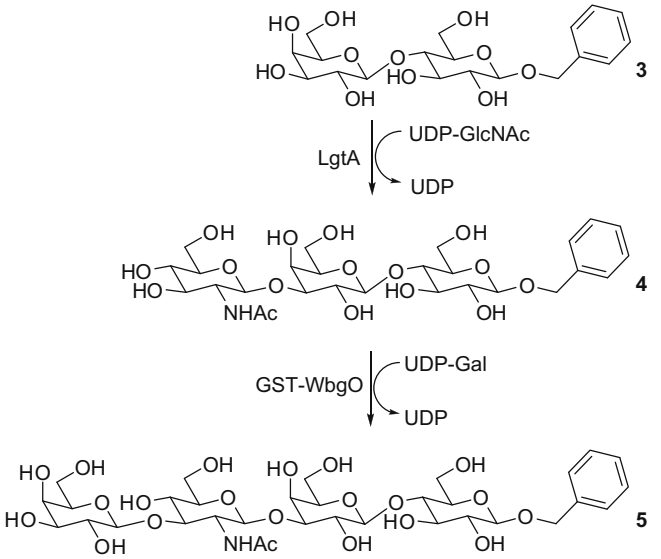
vital role of the 2-acetamido group for the acceptor binding of WbgO.

The kinetic parameters of WbgO were determined for both the donor (UDP-Gal) and some acceptors (GlcNAc, GalNAc and lacto-*N*-triose). The result is found in Table 3. The apparent K_m value for UDP-Gal is 3.4 mM which is comparable with galactosyltransferases from mammalians.[13,30] The difference of kinetic parameters among different acceptors is in line with the result from the acceptor substrate specificity study. The K_m value for lacto-*N*-triose (0.055 mM) is about one-sixth of that for both GlcNAc (0.323 mM) and GalNAc (0.327 mM), and the increased binding affinity for lacto-*N*-triose can explain the relative activities between the three acceptors. The activity difference between GlcNAc and GalNAc appears mainly due to differences in V_{max}.

To demonstrate the synthetical application of WbgO, LNT-β-OBn (compound **5**) was synthesized from lactose-β-OBn (compound **3**) in two steps with a GlcNAc-transferase from *Neisseria meningitidis* (LgtA)[31] and GST-WbgO (Scheme 2). At first, the synthesis of lacto-*N*-triose-β-OBn (Compound **4**) from lactoside **3** with LgtA was carried out with a yield of 86% (19 mg). Then 8 mg resultant trisaccharide **4** was used to synthesize tetrasaccharide **5**. Totally 8.7 mg product was obtained after purification by Bio-Gel P-2 column and lyophilization with a yield of 87%. The product was confirmed by ESI-MS and NMR analysis. It is worth to note that the conversion of **4** to **5** was completed from the TLC detection (Fig. 5). The decrease of yield should be resulted from the loss during purification. The yield can be improved as the synthesis scale is enlarged. Since the Galβ1,3GlcNAcβ-R motif is the basic structure for type I oligosaccharides,[1] WbgO provides an alternative synthetic method for these oligosaccharides. In addition, WbgO also displays remarkable galactosyl-transferring activity towards GalNAc containing oligosaccharides (globotetraose and isoglobotetraose) compared with LgtD, which has been previously used for the synthesis of these oligosaccharides.[32] The remarkable activity toward both oligosaccharides with GlcNAc at the non-reducing end and those with GalNAc at the non-reducing end makes WgbO a more attractive tool for biotechnological applications.

Table 3
Apparent kinetic parameters for the β1,3-galactosyltransferase activity of GST-WbgO

Substrates	K_m (mM)	V_{max} (pmol min^{-1})	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})
UDP-galactose	3.40	490	13.9	4.09
GlcNAc	0.323	179	2.55	7.89
Lacto-*N*-triose	0.055	176	2.50	16.7
GalNAc	0.327	120	1.71	5.23



Scheme 2. Synthesis of benzyl β-lacto-*N*-tetraoside with recombinant LgtA and WbgO.

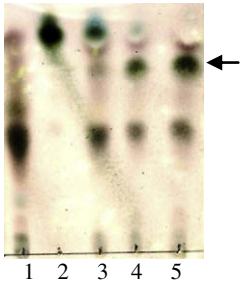


Figure 5. TLC detection of GST-WbgO catalyzed lacto-*N*-tetraoside synthesis reaction. (1) UDP-Gal; (2) lacto-*N*-triose-β-OBn **4**; (3) 4 h reaction; (4) 8 h reaction and (5) 12 h reaction. Arrow indicates the tetrasaccharide product **5**.

3. Conclusion

We identified and characterized a β1,3-galactosyltransferase (WbgO) from *E. coli* O55:H7, which uses GlcNAc and oligosaccharides with GlcNAc at the non-reducing end as its preferred acceptors. This enzyme was overexpressed in *E. coli* BL21 (DE3) with a GST-fused at its N-terminus and purified by one-step affinity chromatography.

WbgO was classified into glycosyltransferase family 2 (GT2), one of the largest GT families. There are many bacterial β1,3-galactosyltransferases identified in this family but few of them use GlcNAc-OR as the predominant acceptor. This is the second GlcNAc β1,3-galactosyltransferase identified in bacteria however it is the first at which has been biochemically characterized. Further more, we demonstrated the potential applications of WbgO in the enzymatic synthesis of important oligosaccharides.

4. Experimental

4.1. Generation of glycosyltransferase expression constructs

The *wbgO*, *wbgP* and *wbgM* genes were amplified by PCR from chromosomal DNA of *E. coli* O55:H7 using primers listed in Table 4. The PCR fragments were digested with *Bam*H I/*Xho* I restriction endonucleases and then ligated into the plasmid pGEX-4T-1 (GE Healthcare Life Sciences, Piscataway, NJ, USA). The confirmed constructs were subsequently transformed into *E. coli* BL21 (DE3) for protein expression.

Table 4
Primers used for PCR cloning of *wbgO*, *wbgP* and *wbgM*[a]

Genes	Primer sequences (5′ to 3′)	
wbgO	1	gcctGGATCCatgataatcgatgaagctg[b]
	2	cggaattCTCGAGtcactttatgtatttacagt
wbgP	1	gcctGGATCCatgattgtgaaaacaataagtg
	2	cggaattCTCGAGttataacttccggataaaaaccac
wbgM	1	gcctGGATCCatggtaaaaattctgcatgtgcacc
	2	cggaattCTCGAGtcaattatttaaatataacgactc

[a] Primer series 1 are forward primers and series 2 are reverse primers.
[b] A *BamH* I restriction site (in capital) is in the forward primers and an *Xho* I site (in capital) is in the reverse primers.

4.2. Expression and purification of glycosyltransferases

E. coli BL21 (DE3) strains harboring the recombinant plasmids were grown in 1 L of LB medium at 37 °C. When OD_{600} reached 0.8, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.2 mM for induction. Protein expression proceeded at 25 °C for 12 h. Cells were harvested by centrifugation at 5000*g* at 4 °C for 15 min. Batch purification of GST-fused proteins with Glutathione Sepharose 4B slurry (GE Healthcare Life Sciences) was performed following the manufacturer's instructions. Protein expression and purification were analyzed by 12% SDS–PAGE. Protein concentration was quantified by the Bradford assay using Bio-Rad Protein Assay reagents (Bio-Rad, Hercules, CA, USA) with standard solutions of bovine serum albumin.

4.3. Activity assays for glycosyltransferases

To determine the GalT activity of recombinant GST-fused WbgO, WbgP and WbgM, the activity assays were performed in a total volume of 50 μL with 50 mM Tris–HCl (pH 7.5), 5 mM $MnCl_2$, 0.3 mM UDP-Gal, 0.3 μM UDP-[6-^{3}H]Gal (10,000 cpm), 2 mM acceptor (GlcNAc or GalNAc) and 10 μL purified protein (about 5 μg protein) at room temperature overnight. For GST-fused WbgO characterization, reactions were performed in a total volume of 50 μL containing 50 mM HEPES buffer (pH 7.0), 5 mM $MnCl_2$, 0.3 mM UDP-Gal, 0.3 μM UDP-[6-^{3}H]Gal (10,000 cpm), 2 mM acceptor and 4 μg (70 pmol) enzymes. The reactions were incubated at 37 °C for 1 h. The acceptor was omitted in the control reaction. The reactions were quenched by adding 150 μL of 10 mM EDTA. Dowex 1X8-400 anion exchange resin (Sigma–Aldrich, St. Louis, MO) was then added as a water suspension (0.8 mL, v/v = 1:1). After centrifugation, the supernatant (0.5 mL) was collected in a 20-mL plastic vial to which 10 mL of Scintiverse BD (Fisher Scientific, Pittsburgh, PA, USA) was added. The incorporated radioactivity was quantified with a Beckmann LS-3801 liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA).

Reactions under different pH conditions were performed in different buffer systems (sodium citrate, pH 5.0, 5.5, 6.0 and 6.5; MES, pH 6.0, 6.5 and 7.0; HEPES, pH 7.0, 7.5 and 8.0; Tris–HCl, pH 7.5, 8.0, 9.0 and 9.5) at the concentration of 200 mM. To determine the dependence of WbgO activity on metal ions, GlcNAc was used as acceptor and the activities were assayed with different metal ion salts ($MnCl_2$, $MgCl_2$, $CaCl_2$, $CuCl_2$, $CoCl_2$, $NiSO_4$ and $ZnSO_4$) at the concentration of 5 mM. The effect of EDTA was tested in the presence of 5 mM $MnCl_2$ and 6 mM EDTA. Other conditions are as same as those described above for GST-fused WbgO characterization.

4.4. Kinetics determination

The apparent kinetics parameters were estimated by activity assays with varied acceptor concentration (0.1, 0.2, 0.5, 1 and 2 mM) along with fixed UDP-Gal concentration (1 mM) or varied UDP-Gal concentration (0.1, 0.2, 0.5, 1 and 2 mM) with 20 mM GlcNAc. The average values from duplicated assays were used to obtain the Lineweaver–Burk plots. The apparent K_m, V_{max} and k_{cat} values were calculated accordingly.

4.5. Synthesis of lacto-series glycoside 2, 4 and 5

The synthesis of lacto-*N*-biose-β-*O*-pNP **2** was performed in a 2.5 mL system containing 17 mg GlcNAcβ-*O*-pNP **1**, 35 mg UDP-Gal, 5 mM $MnCl_2$ and 1.2 mg GST-WbgO in 50 mM HEPES buffer (pH 7.0). The reaction was incubated at room temperature for 48 h.

For lacto-*N*-triose-β-OBn **4** synthesis, 15 mg lactose-β-OBn **3**, 24 mg UDP-GlcNAc and 5 mM $MnCl_2$ were added into 50 mM Tris–HCl buffer (pH 7.5) with 1 U of LgtA, a β1,3-GlcNAc-transferase from *Neisseria meningitidis*.[31] The reaction was allowed to proceed for 12 h at room temperature. LgtA was overexpressed with vector pET15b in *E. coli* BL21 (DE3) and purified as previously described.[31]

WbgO catalyzed LNT-β-OBn **5** synthesis reaction was performed in 50 mM HEPES buffer (pH 7.0) with 8 mg lacto-*N*-triose-β-OBn **4**, 11.5 mg UDP-Gal, 5 mM $MnCl_2$, and 0.8 mg GST-WbgO (total volume: 1.2 mL). The reaction was incubated at 37 °C for 12 h.

All the reactions were eventually heated in boiling water for 5 min. The resultant saccharides were purified by Dowex 1X8-400 anion exchange resin and then Bio-Gel P-2 column (Bio-Rad Laboratories, Hercules, CA, USA). All the reactions were monitored by TLC as previously described.[33]

4.6. Analysis of oligosaccharide products

Products were characterized by 1D (^{1}H and ^{13}C) and 2D (COSY and HMQC) NMR spectra and high resolution mass. The NMR spectra were recorded on a Bruker Avance DRX500 NMR Spectrometer. The ESI-MS was performed on a Bruker MicrOTOF mass spectrometer. The oligosaccharide products were repeatedly dissolved in D_2O and lyophilized before the NMR spectra were recorded at 303 K in a 5 mm tube. All the electronic spectra are available in the Supplementary data.

4.6.1. Lacto-*N*-biose-β-*O*-pNP

^{1}H NMR (500 MHz, D_2O): δ 8.21 (d, J = 9.3 Hz, 2H, $PhNO_2$), 7.16 (d, J = 9.3 Hz, 2H, $PhNO_2$), 5.33 (8.5 Hz, 1H, H-1), 4.45 (d, J = 7.7 Hz, 1H, H-1), 4.15 (dd, J = 10.4, 8.5 Hz, H-2), 3.93 (dd, J = 12.5, 2.1 Hz, 1H, H-6a), 3.92 (dd, J = 10.3, 8.2, 1H, H-3), 3.89 (d, J = 3.4 Hz, 1H, H-4), 3.79 (dd, J = 12.5, 5.2 Hz, 1H, H-5), 3.75–3.68 (m, 4H, H-6b, H-5, H-6), 3.66 (m, 1H, H-4), 3.62 (dd, J = 10.0, 3.3 Hz, 1H, H-3), 3.52 (dd, J = 9.9, 7.7 Hz, 1H, H-2), 1.98 (s, 3H, $NHCOCH_3$); ^{13}C NMR (125 MHz, D_2O): δ 175.0, 161.7, 142.7, 126.1, 116.6, 103.6, 98.4, 81.9, 75.8, 75.4, 72.5, 70.7, 68.6, 68.4, 61.1, 60.5, 54.4, 22.2; HRMS (ESI): $C_{20}H_{28}N_2O_{13}Na$ $[M+Na]^+$, calcd 527.1484, found 527.1476.

4.6.2. Lacto-*N*-triose-β-OBn

^{1}H NMR (500 MHz, D_2O): δ 7.45–7.36 (m, 5H, Ph), 4.90 (d, J = 11.6 Hz, 1H, $PhCH_2$), 4.73 (d, J = 11.6 Hz, 1H, $PhCH_2$), 4.65 (d, J = 8.5 Hz, 1H, H-1), 4.51 (d, J = 8.0 Hz, 1H, H-1), 4.39 (d, J = 7.9 Hz, 1H, H-1), 4.10 (d, J = 2.8 Hz, 1H), 3.94 (dd, J = 12.3, 1.9 Hz, 1H), 3.85 (d, J = 12.6, 1.9 Hz, 1H), 3.78–3.66 (m, 8H), 3.62–3.58 (m, 2H), 3.57–3.51 (m, 2H), 3.43 (t, J = 9.9 Hz, 1H), 3.41 (m, 1H), 3.31 (t, J = 8.3 Hz, 1H), 2.00 (s, 3H); ^{13}C NMR (125 MHz, D_2O): δ 175.0, 136.6, 128.79, 128.76, 128.55, 128.52, 103.0, 102.8, 101.1, 82.0, 78.4, 75.7, 74.9, 74.8, 74.5, 73.6, 72.8, 71.7, 71.6, 70.0, 69.7, 69.6, 68.4, 61.0, 60.5, 60.4, 60.2, 59.6, 55.7, 22.2; HRMS (ESI): $C_{27}H_{41}NO_{16}Na$ $[M+Na]^+$, calcd 658.2318, found 658.2381.

4.6.3. Lacto-*N*-tetraose-β-OBn

^{1}H NMR (500 MHz, D_2O): δ 7.46–7.37 (m, 5H, Ph), 4.91 (d, J = 11.7 Hz, 1H, $PhCH_2$), 4.73 (d, J = 11.7 Hz, 1H, $PhCH_2$), 4.52 (d, J = 8.0 Hz, 1H, H-1), 4.41 (d, J = 7.7 Hz, 1H, H-1), 4.40 (d,

J = 7.9 Hz, 1H, H-1), 4.11 (d, J = 3.1 Hz, 1H), 3.95 (dd, J = 12.3, 1.9 Hz, 1H), 3.89–3.84 (m, 3H), 3.81–3.78 (m, 2H), 3.77–3.70 (m, 5H), 3.69–3.66 (m, 6H), 3.62–3.60 (m, 2H), 3.58–3.54 (m, 2H), 3.51 (m, 1H), 3.45 (m, 1H), 3.32 (t, J = 8.2 Hz, 1H), 2.00 (s, 3H); ^{13}C NMR (125 MHz, D_2O): δ 175.0, 136.36, 128.79, 128.76, 158.55, 128.52, 103.5, 103.0, 102.6, 101.1, 82.1, 82.0, 78.5, 75.3, 75.2, 74.9, 74.8, 74.5, 72.8, 72.5, 71.7, 71.6, 70.7, 70.0, 69.6, 68.6, 68.5, 68.3, 61.1, 61.0, 60.9, 60.5, 60.4, 60.2, 59.8, 54.7, 22.3; HRMS (ESI): $C_{33}H_{51}NO_{21}Na$ $[M+Na]^+$, calcd. 820.2846, found 820.2850.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.005.

References and notes

1. Holgersson, J.; Lofling, J. *Glycobiology* **2006**, *16*, 584.
2. Mitchell, E.; Houles, C.; Sudakevitz, D.; Wimmerova, M.; Gautier, C.; Perez, S.; Wu, A. M.; Gilboa-Garber, N.; Imberty, A. *Nat. Struct. Biol.* **2002**, *9*, 918.
3. Kerr, C. L.; Hanna, W. F.; Shaper, J. H.; Wright, W. W. *Biol. Reprod.* **2004**, *71*, 770.
4. Thurin, M.; Kieber-Emmons, T. *Hybrid. Hybridomics* **2002**, *21*, 111.
5. Lasky, L. A. *Science* **1992**, *258*, 964.
6. Wang, Q. Y. J. *Exp. Clin. Cancer Res.* **2003**, *22*, 431.
7. Hakomori, S. *Adv. Exp. Med. Biol.* **2001**, *491*, 369.
8. Ugorski, M.; Laskowska, A. *Acta Biochim. Pol.* **2002**, *49*, 303.
9. German, J. B.; Freeman, S. L.; Lebrilla, C. B.; Mills, D. A. *Nestle Nutr. Workshop Ser. Pediatr. Program.* **2008**, *62*, 205.
10. Thomas, R.; Brooks, T. *J. Med. Microbiol.* **2004**, *53*, 833.
11. Ramphal, R.; Carnoy, C.; Fievre, S.; Michalski, J. C.; Houdret, N.; Lamblin, G.; Strecker, G.; Roussel, P. *Infect. Immun.* **1991**, *59*, 700.
12. Isshiki, S.; Togayachi, A.; Kudo, T.; Nishihara, S.; Watanabe, M.; Kubota, T.; Kitajima, M.; Shiraishi, N.; Sasaki, K.; Andoh, T.; Narimatsu, H. *J. Biol. Chem.* **1999**, *274*, 12499.
13. Hennet, T.; Dinter, A.; Kuhnert, P.; Mattu, T. S.; Rudd, P. M.; Berger, E. G. *J. Biol. Chem.* **1998**, *273*, 58.
14. Watanabe, M.; Miyake, K.; Yanae, K.; Kataoka, Y.; Koizumi, S.; Endo, T.; Ozaki, A.; Iijima, S. *J. Biochem.* **2002**, *131*, 183.
15. Baisch, G.; Ohrlein, R.; Streiff, M.; Kolbinger, F. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 751.
16. Nishimoto, M.; Kitaoka, M. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 2101.
17. Donnenberg, M. S.; Kaper, J. B.; Finlay, B. B. *Trends Microbiol.* **1997**, *5*, 109.
18. Nataro, J. P.; Kaper, J. B. *Clin. Microbiol. Rev.* **1998**, *11*, 142.
19. Wick, L. M.; Qi, W.; Lacher, D. W.; Whittam, T. S. *J. Bacteriol.* **2005**, *187*, 1783.
20. Yoon, J. W.; Hovde, C. J. *J. Vet. Sci.* **2008**, *9*, 219.
21. Lindberg, B.; Lindh, F.; Lonngren, J.; Lindberg, A. A.; Svenson, S. B. *Carbohydr. Res.* **1981**, *97*, 105.
22. Samuel, G.; Hogbin, J. P.; Wang, L.; Reeves, P. R. *J. Bacteriol.* **2004**, *186*, 6536.
23. Cantarel, B. L.; Coutinho, P. M.; Rancurel, C.; Bernard, T.; Lombard, V.; Henrissat, B. *Nucleic Acids Res.* **2009**, *37*, D233.
24. Vetere, A.; Miletich, M.; Bosco, M.; Paoletti, S. *Eur. J. Biochem.* **2000**, *267*, 942.
25. Fort, S.; Kim, H. S.; Hindsgaul, O. *J. Org. Chem.* **2006**, *71*, 7146.
26. Barreras, M.; Abdian, P. L.; Ielpi, L. *Glycobiology* **2004**, *14*, 233.
27. der Laan, E.; Boots, J. W.; Spelbrink, R. E.; Kool, G. M.; Breukink, E.; Killian, J. A.; de Kruijff, B. *J. Bacteriol.* **2003**, *185*, 3773.
28. Breton, C.; Snajdrova, L.; Jeanneau, C.; Koca, J.; Imberty, A. *Glycobiology* **2006**, *16*, 29R.
29. Altschul, S. F.; Madden, T. L.; Schaffer, A. A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D. J. *Nucleic Acids Res.* **1997**, *25*, 3389.
30. Malissard, M.; Dinter, A.; Berger, E. G.; Hennet, T. *Eur. J. Biochem.* **2002**, *269*, 233.
31. Blixt, O.; van Die, I.; Norberg, T.; van den Eijnden, D. H. *Glycobiology* **1999**, *9*, 1061.
32. Randriantsoa, M.; Drouillard, S.; Breton, C.; Samain, E. *FEBS Lett.* **2007**, *581*, 2652.
33. Li, M.; Liu, X. W.; Shao, J.; Shen, J.; Jia, Q.; Yi, W.; Song, J. K.; Woodward, R.; Chow, C. S.; Wang, P. G. *Biochemistry* **2008**, *47*, 378.