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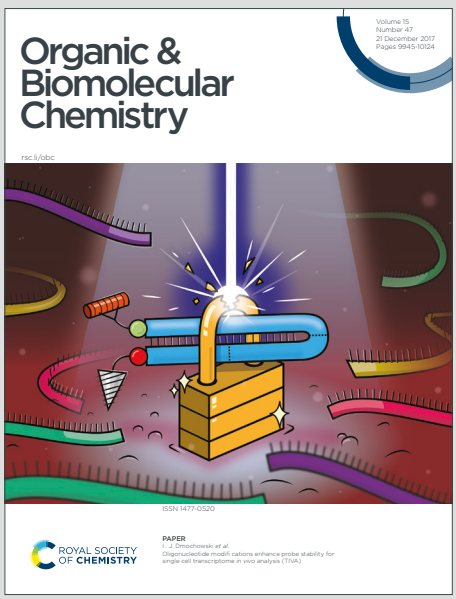

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ARTICLE

Biochemical characterisation of an α1,4 galactosyltransferase from *Neisseria weaveri* for the synthesis of α1,4-linked galactosides

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The human cell surface trisaccharide motifs globotriose and P1 antigen play key roles in infections by pathogenic bacteria, which makes them important synthetic targets as antibacterial agents. Enzymatic strategies to install the terminal α1,4-galactosidic linkage are very attractive but have only been demonstrated for a limited set of analogues. Herein, a new bacterial α1,4 galactosyltransferase from *N. weaveri* was cloned and produced recombinantly in *E. coli* BL21 (DE3) cells, followed by investigation of its substrate specificity. We demonstrate that the enzyme can tolerate galactosamine (GalN) and also 6-deoxygalactose and 6-deoxy-6-fluorogalactose as donors, and lactose and *N*-acetyllactosamine as acceptors, leading directly to analogues of Gb3 and P1 that are valuable chemical probes and showcase how biocatalysis can provide fast access to a number of unnatural carbohydrate analogues.

Introduction

Carbohydrates on animal cell surfaces are often displayed as part of motifs that play a crucial role in biological interactions, such as cell-cell communication, surface recognition and pathogenic infection.[1] Two biologically important trisaccharide motifs are globotriose (i.e., Gb3) and P1 antigens in which galactose is linked by an α1,4-glycosidic bond to lactose and *N*-acetyllactosamine, respectively (Figure 1).[2]

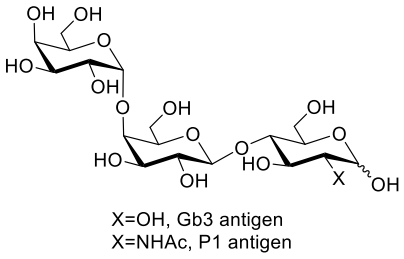

Figure 1. Structure of Gb3 and P1 antigen motifs on cell surface glycans.

The involvement of Gb3 and P1 antigens in a number of biological events has been demonstrated previously, in particular as animal cell surface receptors for toxins and pathogens. Shiga-toxin produced by STEC (shiga-toxin-producing *Escherichia coli*, i.e., *E.coli* O157) was shown to bind to the Gb3 motif of the glycolipid globotriosylceramide, on the intestinal cell surface, and this was recognised as a cause of food poisoning and sometimes even deadly haemolytic uremic syndrome (HUS).[3] Similarly, Shiga-toxin recognises the P1 antigen, which shares the same terminal Gal-α1,4-Gal epitope with globotriose.[4] There is currently only limited therapy available against STEC infection and the interaction between globotriose and shiga-toxin provides an attractive therapeutic target for the development of treatments.[5–9] Furthermore, the invasion of bacterial pathogens including *Neisseria meningitidis* and *N. meningitidis* immunotype L1 into mammalian cells was shown to be mediated by Gb3 in the bacterial lipooligosaccharides. [10] Therefore, Gb3 and P1 antigens, as well as their analogues, have been suggested as competitive inhibitors to prevent infections by pathogenic bacteria.[5,11,12]

Since the isolation of Gb3 and P1 trisaccharides from natural resources is not practical, there is a need for their synthesis and a number of chemical routes have been reported,[13–17] although the requirement for extensive protection/deprotection strategies results in a large number of steps and in low overall yields.

Enzymatic synthetic methods have also been proposed to access both Gb3 and P1 antigens, mainly involving α1,4 galactosyltransferases (α1,4GalTs). Among them, NmLgtC from *N. meningitidis* is the most widely used glycosyltransferase for α1,4-glycosidic bond formation.[18–21] Wang *et al.* studied the acceptor substrate specificity of a truncated enzyme variant

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Electronic Supplementary Information (ESI) available: kinetic studies, characterisation data, copies of the NMR and HRMS spectra. See DOI: 10.1039/x0xx00000x

using UDP-galactose as the donor substrate.[22] In order to decrease the cost of the donor substrate, coupling of the same system with nucleotide sugar regeneration was further investigated by Chen *et al.*, to produce Gb3 antigen derivatives.[23] Furthermore, the gene of α1,4GalT and other required enzymes were cloned into the same plasmid to generate a whole cell biocatalyst for the production of Gb3 and P1 antigens and their derivatives.[24–26] Additionally, α1,4GalTs from *Campylobacter jejuni* [27] and *Neisseria gonorrhoeae* [28] were also cloned and used for α1,4-glycosidic bond formation.

A limitation of enzymatic strategies is frequently a narrow substrate range, which can make access to analogues needed as biochemical probes very limited. Herein, we investigate a new α1,4 galactosyltransferase from *N. weaveri* (NwLgtC) for substrate promiscuity, in particular against deoxy, deoxyfluoro- and deoxyamino- analogues of the donor UDP-Gal. The applicability of this procedure to preparative scale synthesis was demonstrated with the preparation of a new deoxy derivative of Gb3 antigen (Gal6D-α1,4-Lac-*p*NP).

Results and discussion

Amino acid sequence analysis of NwLgtC

Amino acid sequence alignment reveals that NwLgtC displays 46% and 63% identity with NmLgtC from *N. meningitidis* (UniProt: P96945) [22] and NgLgtC from *N. gonorrhoeae* (UniProt: A0A5K1QJC7)[28], respectively (Figure 2). No significant sequence identity was found with CgtD from *C. jejuni* (UniProt: Q8KWQ9).[27]

Gene cloning, protein expression and purification of NwLgtC

The open-reading frame of the LgtC gene from *Neisseria weaveri* was synthesised with codon optimisation for *E. coli* expression and subcloned into pET-28a vector with *N*-terminal His-tag. The recombinant protein was produced in *E. coli* BL21 (DE3) cells using autoinduction TB medium at 25°C for 24 h. Protein purification was performed by Ni-NTA affinity chromatography, pooling all fractions containing the recombinant enzyme. The protein was precipitated by addition of ammonium sulfate (3.2 M final concentration). SDS-PAGE analysis (Figure 3) indicates that the recombinant enzyme possesses an apparent mass of approximately 35 kDa, which is consistent with theoretical molecular weight of 35.6 kDa. The specific activity against UDP-Gal and *p*NP-β-Lac (see assay conditions in the following section) was 66 mU mg^{-1}. This value is significantly lower than that reported for NmLgtC (5 U mg^{-1}) and NgLgtC (13 U mg^{-1}) for the synthesis of unmodified Gb3 antigen using UDP-Gal and lactose as donor and acceptor, respectively (1 U was defined as the amount of enzyme required for the formation of 1 µmol product per minute at 37°C).

In view of a preparative application, the lower activity is certainly an issue, but not extremely critical, since activity and stability can be considerably improved with directed evolution and random mutagenesis approaches, as successfully done for most industrial enzymes. The most likely reasons for the lower activity of the NwLgtC preparation compared to reported homologues lie in the use of a modified substrate (*p*NP-β-Lac instead of Lac) and in the ammonium sulfate precipitation step. Even though the activity might be reduced by such treatment,

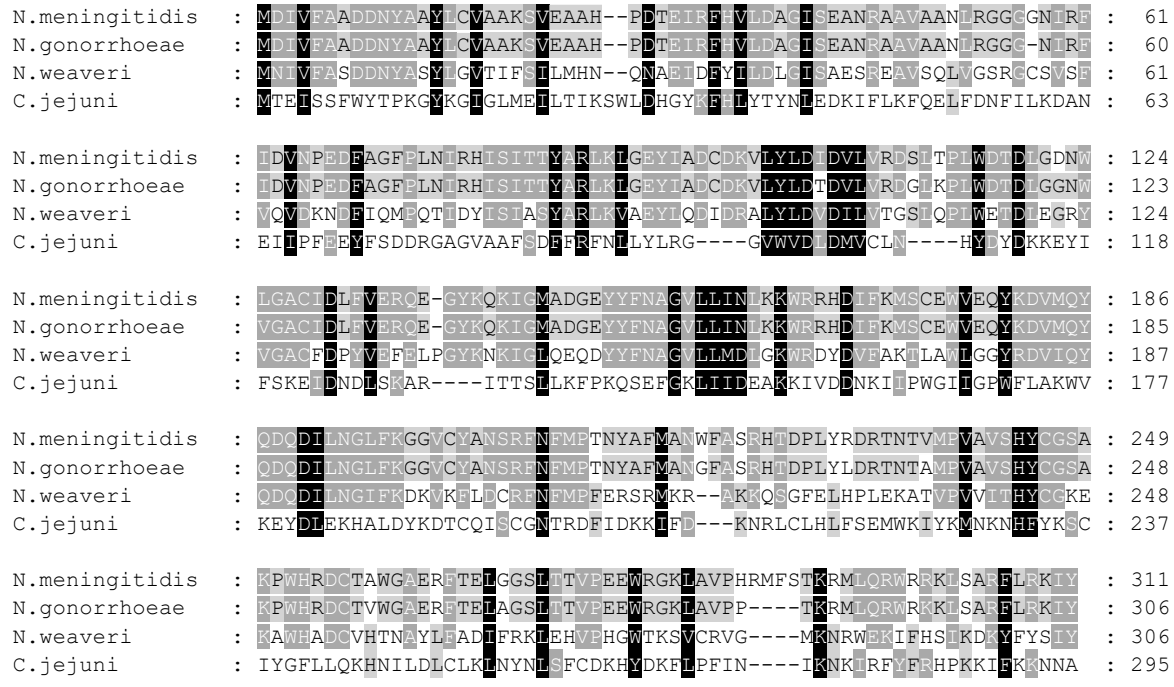

Figure 2. Amino acid sequence alignment of LgtC galactosyltransferases (NmLgtC from *N. meningitidis*, NgLgtC from *N. gonorrhoeae*, NwLgtC from *N. weaveri*, CjCgtD from *C. jejuni*). Highly conserved residues are shown in black.

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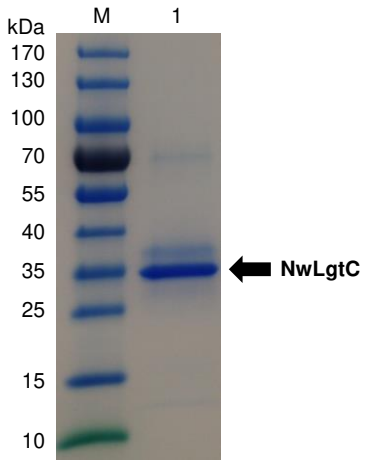

Figure 3. SDS-PAGE analysis of purified NwLgtC. M: protein marker; lane 1: purified NwLgtC.

the loss is more than compensated by the fact that the final preparation can be obtained in large amounts, inexpensively and in a stable form (storage at 4°C, shipment at room temperature, etc.).

Biochemical characterisation of NwLgtC

The influence of pH, temperature and divalent metal ions on the activity of NwLgtC was studied by utilising *p*NP-β-Lac as acceptor. The enzymatic activity increased sharply from pH 4.5 to 5.5, then gradually until 7.0, with a maximum around 7.0-8.0, and decreased for higher values (Figure 4A). This behaviour is comparable with its homologues NmLgtC and NgLgtC. The optimal temperature for the activity of NwLgtC was found to be around 40°C (Figure 4B), which is identical with NgLgtC. The activity declined significantly at temperatures higher than 40°C, with almost no activity left at 70°C. The presence of a divalent cation was confirmed to be essential for the activity of the recombinant enzyme. Mn^{2+} was the best metal ion for such enzyme, followed by Mg^{2+} and Co^{2+} (Figure 4C), which is in agreement with its characterised homologues. NwLgtC was inactive in the presence of Ca^{2+}, Cu^{2+} or Zn^{2+}. Furthermore, the activity could be improved slightly by adding DTT (data not shown), which has also been observed for NmLgtC.[22]

A kinetic study of NwLgtC against UDP-Gal and *p*NP-β-Lac was performed, determining reaction conversions by HPLC. The apparent kinetic parameters were calculated by fitting the data to the Michaelis-Menten model using GraphPad Prism 7 (ESI, Figure S1). The K_m for UDP-Gal and *p*NP-β-Lac were 0.17±0.02 mM and 2.5±0.20 mM, and the corresponding k_{cat} were 0.64 ± 0.02 min^{-1} and 1.11 ± 0.02 min^{-1}. Therefore, the catalytic efficiency values of the enzyme against UDP-Gal and *p*NP-β-Lac were 3.8 mM^{-1} min^{-1} and 0.45 mM^{-1} min^{-1}, respectively. As a comparison, reported literature data for the homologue NwLgtC are: k_{cat} = 14.2 s^{-1}, K_m = 18 μM, k_{cat}/K_m = 0.81 μM^{-1} s^{-1} against UDP-Gal; k_{cat} = 24.0 s^{-1}, K_m = 20 mM, k_{cat}/K_m = 1.20 mM^{-1} s^{-1} against Lac.[19] The increased efficiency reported is likely due to the use of the native acceptor Lac instead of the modified acceptor *p*NP-β-Lac.

Donor substrate specificity study

Initially, a panel of five commercially available nucleotide sugars (UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc and UDP-GlcA) were tested to investigate the donor substrate specificity of NwLgtC, using Lac as the acceptor, under the previously optimised conditions. Only transfer from UDP-Gal was observed by MALDI-ToF analysis (ESI, Figure S12).

In order to study substrate promiscuity in more detail, several nucleotide diphosphate galactose analogues (monodeoxygalactoses, monodeoxy-monofluorogalactoses and galactosamine) were synthesised by a one-pot multienzyme system involving galactokinase (SpGalK), galactose-1-phosphate uridylyltransferase (EcGalPU) and UDP-sugar pyrophosphorylase (BlUSP), in the presence of catalytic amounts of UDP-Glc (Table 1). The formation of UDP-Gal and its analogues was confirmed by HRMS (ESI, Figures S2-S11). To evaluate these as donors for NwLgtC, disaccharides bearing an imidazolium-based tag (i.e., Lac-ITag and LacNAc-ITag) were utilised as acceptor substrates. These were produced by transferring a Gal unit by bacterial β1,4 galactosyltransferase to chemically synthesised Glc-ITag or GlcNAc-ITag, as previously reported.[29] Ionic liquid tagged glycosides are excellent probes for the screening and characterisation of glycoenzymes, since they ionise very readily and produce a dominant mass

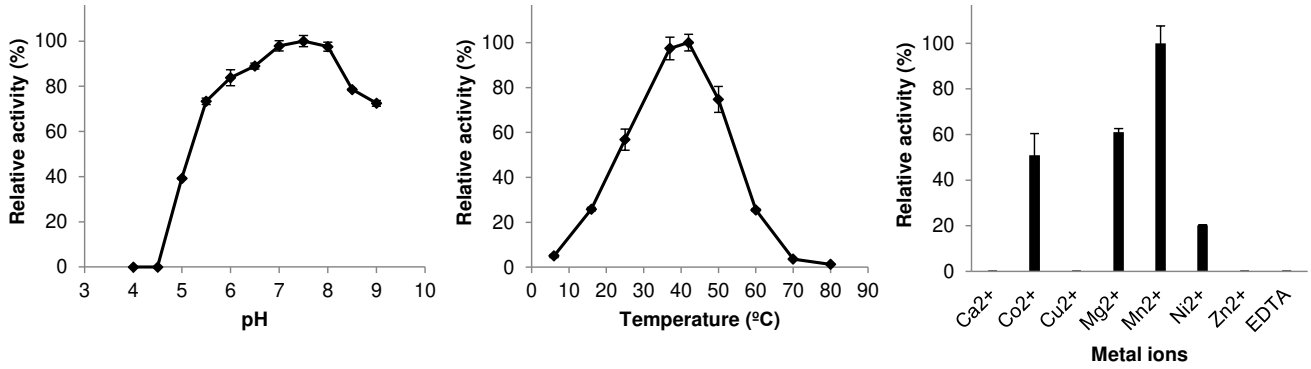

Figure 4. Biochemical properties of NwLgtC. A: effect of pH; B: effect of temperature; C: effect of divalent metal ions.

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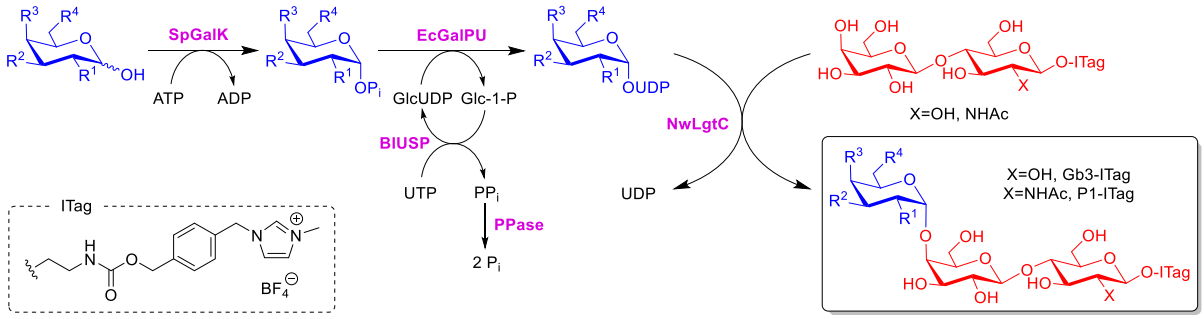
Table 1. Substrate specificity of NwLgtC against a panel of galactose donors and ITag-acceptors.



Substrate	R^1	R^2	R^3	R^4	X = OH Conv. [%][a]	X = NHAc Conv. [%][a]
Gal	OH	OH	OH	OH	76	13
Gal2D	H	OH	OH	OH	n.c.	n.c.
Gal3D	OH	H	OH	OH	n.c.	n.c.
Gal4D	OH	OH	H	OH	n.c.	n.c.
Gal6D	OH	OH	OH	H	65	9
Gal2F	F	OH	OH	OH	n.c.	n.c.
Gal3F	OH	F	OH	OH	n.c.	n.c.
Gal4F	OH	OH	F	OH	n.c.	n.c.
Gal6F	OH	OH	OH	F	41	1
GalN	NH_2	OH	OH	OH	24	1

[a] Determined by MALDI-ToF analysis (n.c. = not converted).

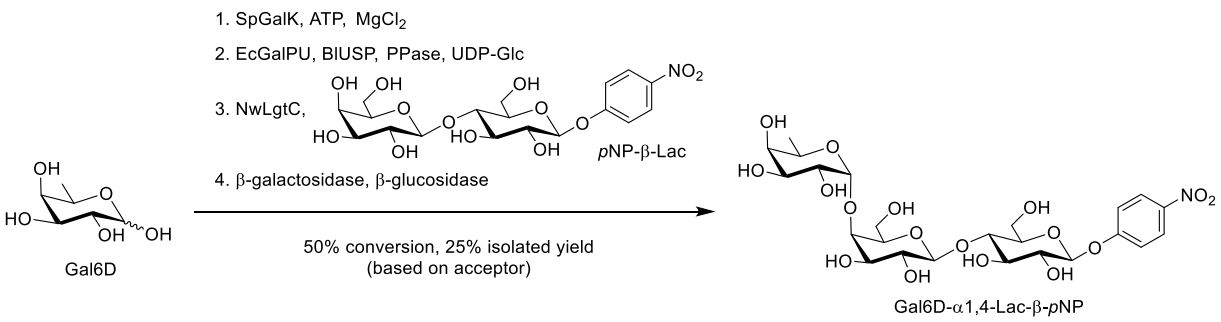
spectrometry signal. This allows for relative quantitative studies by MALDI analysis, by integrating the isotopic envelope peak area of starting substrate and product.[30–33]

According to the results obtained by MALDI-ToF (Table 1), NwLgtC could perform α1,4-galactosylation on both Lac-ITag and LacNAc-ITag using not only galactose, but also 6-deoxygalactose, 6-fluorogalactose and galactosamine (ESI, Figures S13-S20). It is apparent that Lac-ITag acts as a much better acceptor than LacNAc-ITag, giving consistently higher conversion rates (Table 1). Additionally, it was found that Gal2D cannot be transferred by NwLgtC, in contrast with the reported behaviour of NmLgtC.[23] The lower conversions obtained for non-natural donors are very likely due to the decreased efficiency of LgtC in transferring those, rather than the poor conversion of the monosaccharide to the UDP-donor. The efficiency of the one-pot UDP-sugar synthesis was previously established, and also confirmed by HRMS.

The fact that NwLgtC accepts UDP-GalN as a donor is of particular relevance since previous reports demonstrated the inability of other homologues (e.g. NmLgtC) to transfer $GalN_3$, a possible precursor of GalN. Thus, the use of NwLgtC opens up the access to galactosamine-containing Gb3 analogues.

Preparative scale synthesis of Gal6D-α1,4-Lac-β-*p*NP

In order to demonstrate the applicability of the enzyme described herein to preparative synthesis, an extended version of the same three-step multienzyme system discussed above was employed to synthesise a non-natural analogue of Gb3 antigen (Scheme 1). Using Gal6D as precursor of the donor and



Scheme 1. One-pot preparative scale synthesis of Gal6D-α1,4-Lac-β-*p*NP using NwLgtC.

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*p*NP-β-Lac as acceptor, the trisaccharide Gal6D-α1,4-Lac-β-*p*NP could be obtained in 50% conversion (as measured by HPLC), based on the acceptor. After the synthesis, the excess starting material Lac-β-*p*NP was removed by adding β-galactosidase and β-glucosidase. The *p*NP-trisaccharide was then separated from other monosaccharides and *p*-nitrophenol by preparative HPLC. Fractions containing product were collected and dried, affording 1.8 mg product (25% isolated yield, corrected for a small amount of an impurity that coeluted with the product). The structure of the compound was confirmed by NMR and HRMS characterisation (see ESI).

Experimental section

Materials

Galactose, galactosamine, *p*NP-β-lactose, ATP, UTP, UDP-galactose, UDP-glucose, UDP-*N*-acetylgalactosamine, UDP-*N*-acetylglucosamine, UDP-glucuronic acid, 2,4,6-trihydroxyacetophenone (THAP), β-galactosidase and inorganic pyrophosphatase were purchased from Sigma-Aldrich. 2-deoxygalactose, 3-deoxygalactose, 4-deoxygalactose, 6-deoxygalactose, 2-deoxy-2-fluorogalactose, 3-deoxy-3-fluorogalactose, 4-deoxy-4-fluorogalactose, 6-deoxy-6-fluorogalactose were purchased from Carbosynth. β-glucosidase was purchased from Megazyme. Alkaline phosphatase was purchased from Promega. All chemicals and commercially available enzymes were used without any further purification. Galactokinase from *Streptococcus pneumoniae* (SpGalK), galactose-1-phosphate uridylyltransferase from *E. coli* K12 (EcGalPU) and β1,4-galactosyltransferase from *N. meningitidis* (NmLgtB-A) [29] were provided by Prozomix Ltd. The plasmid harbouring UDP-sugar pyrophosphorylase from *Bifidobacterium longum* (BlUSP) was provided by Prof. Josef Voglmeir (Nanjing Agricultural University, China).

Protein expression and purification

The gene of NwLgtC was synthesised by Twist Bioscience (San Francisco, USA) codon-optimised for *E. coli* expression. The synthesised DNA fragment was subcloned into plasmid pET28a, which was subsequently transformed into *E. coli* Top10 competent cells. A few colonies bearing the construct were confirmed by plasmid DNA isolation and gene sequencing. The pET28a-NwLgtC construct was transformed into *E. coli* BL21(DE3) competent cells. Small-scale expression was carried out to determine the production of soluble and active protein. The large-scale expression was performed by inoculating 400 mL autoinduction Terrific Broth medium in a 1 L flask and growing the culture at 25°C for 24 h (final OD_{600} between 10 and 20). The cells were harvested by centrifugation (6000 *g*, 15 min, 4°C), resuspended in HEPES buffer containing NaCl (500 mM) and imidazole (10 mM), and lysed by sonication. The cell debris and intact cells were removed by centrifugation (8000 *g*, 30 min, 4°C). The protein was purified by immobilised metal affinity chromatography (IMAC) on a Ni^{2+}-NTA column (GE Healthcare). The lysate was filtered through a 0.45 µm syringe filter and loaded on the column. Two wash steps were performed using the same buffer containing 10 mM and 50 mM imidazole, respectively. Then, the bound protein was eluted using the same buffer containing 500 mM imidazole. The fractions containing purified protein were combined and concentrated in a centrifugal filter. Protein precipitation was induced by addition of solid ammonium sulfate (to a final concentration of 3.2 M) and the resulting milky white suspension was stored at 4°C until use.

Amino acid sequence of NwLgtC

α1,4 galactosyltransferase cloned from *Neisseria weaveri* LMG 5135 (NwLgtC, UniProt: A0A3S5C3F9)

(MGSSHHHHHHSSGLVPRGSH)MNIVFASDDNYASYLGVTIF
SILMHNQNAEIDFYILDLGISAESREAVSQLVGSRGCSVSFV
QVDKNDFIQMPQTIDYISIASYARLKVAEYLQDIDRALYLDV
DILVTGSLQPLWETDLEGRYVGACFDPYVEFELPGYKNKIGL
QEQDYYFNAGVLLMDLGKWRDYDVFAKTLAWLGGYRDVIQYQ
DQDILNGIFKDKVKFLDCRFNFMPFERSRMKRAKKQSGFELH
PLEKATVPVVITHYCGKEKAWHADCVHTNAYLFADIFRKLEH
VPHGWTKSVCRVGMKNRWEKIFHSIKDKYFYSIY

Biochemical characterisation of NwLgtC

Effect of temperature. A 50 µL reaction mixture containing *p*NP-β-Lac (1 mM), UDP-Gal (2 mM), $MnCl_2$ (10 mM), Tris buffer (50 mM, pH 7.5) and NwLgtC (23 µg) was incubated at varying temperatures (6-80°C) for 1 h. The reaction was quenched by heating at 95°C for 5 min, followed by cooling on ice. The starting material *p*NP-β-Lac was hydrolysed by β-galactosidase and the product was analysed by HPLC. All assays were performed in triplicates.

Effect of pH. A 50 µL reaction mixture containing *p*NP-β-Lac (1 mM), UDP-Gal (2 mM), $MnCl_2$ (10 mM) and NwLgtC (23 µg) in different buffers (50 mM, pH 4.0-9.0) at 37 °C for 1 h. Quenching and analysis were carried out as described above. All assays were performed in triplicates.

Effect of divalent metal ions. A 50 µL reaction mixture containing *p*NP-β-Lac (1 mM), UDP-Gal (2 mM), Tris buffer (50 mM, pH 7.5) and NwLgtC (23 µg) with different divalent metal ions (10 mM, Ca^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}) or EDTA (10 mM) at 37 °C for 1 h. Quenching and analysis were carried out as described above. All assays were performed in triplicates.

Determination of kinetic parameters of NwLgtC

Several 50 µL reaction mixtures in Tris buffer (50mM, pH 7.5) were prepared, containing either different concentrations of the donor substrate UDP-Gal (i.e., 0.1, 0.3, 0.5, 0.7, 1, 2 mM)

with a fixed concentration of *p*NP-β-Lac (2 mM), or different concentrations of *p*NP-β-Lac (i.e., 0.3, 0.5, 1, 2, 4, 6, 8 and 10 mM) with a fixed concentration of UDP-Gal (2 mM). The reactions were started by addition of NwLgtC (23 μg) and incubated at 37°C for 1 h. The reactions were quenched by heating at 95°C for 5 min, followed by cooling on ice. Subsequently, unreacted *p*NP-β-Lac was hydrolysed by incubation with β-glucosidase and β-galactosidase. The kinetic parameters were obtained by fitting the data to the Michaelis-Menten equation using GraphPad Prism 7.03.

Donor substrate specificity study

Commercial nucleotide sugars as donors. A 20 μL reaction mixture containing lactose (1 mM), UDP-sugar (1.5 mM, Gal, Glc, GalNAc, GlcNAc, GlcA), Tris buffer (50 mM, pH 7.5), $MnCl_2$ (10 mM) and NwLgtC (7 μg) was incubated at 37°C overnight. The mixture was analysed by MALDI-ToF (positive mode) using THAP as matrix.

Nucleotide sugars generated enzymatically in situ as donor: A 40 μL reaction mixture containing different monosaccharides (8 mM), ATP (10 mM), Tris buffer (50 mM, pH 8.0), $MgCl_2$ (10 mM), SpGalK (12 μg), UTP (10 mM), BlUSP (3 μg), EcGalPU (1.3 μg) and pyrophosphatase (2 U) was incubated at 37°C overnight to synthesise the UDP-sugars. ITag-Lac and ITag-LacNAc (0.5 mM) were synthesised by using the recombinant bacterial β1,4 galactosyltransferase NmLgtB-A according to published protocol.[29] Subsequently, a 10 μL reaction mixture consisting of ITag-Lac or ITag-LacNAc (0.1 mM), UDP-sugars, $MnCl_2$ (10 mM), Tris buffer (50 mM, pH 7.5) and NwLgtC (30 μg) was incubated at 37°C overnight. The mixture was analysed by MALDI-ToF (positive mode) using THAP as matrix. The conversion rate was calculated by integrating the isotopic envelope peak areas.

Preparative scale synthesis of Gal6D-α1,4-Lac-β-*p*NP

A one-pot three-step telescopic reaction was utilised to prepare Gal6D-α1,4-Lac-β-*p*NP. For the first step (phosphorylation of Gal6D) in a 50 mL Falcon tube a 5 mL reaction mixture was prepared, containing Gal6D (17 mg), ATP (85 mg), $MgCl_2$ (10 mM), $MnCl_2$ (10 mM), Tris buffer (50 mM, pH 8.0) and SpGalK (8 mg). The pH was immediately readjusted to 8.0 by addition of aq. NaOH (5 M), then the solution was incubated at 37°C overnight with gentle shaking. For the second step (UDP-Gal6D formation), 3 mL of a mixture containing UTP (110 mg), Tris buffer (pH 8.0, 50 mM), UDP-Glc (2 mM), BlUSP (3 mg), EcGalPU (1 mg) and pyrophosphatase (30 U) was added to the Falcon tube. The pH was immediately readjusted to 8.0 by addition of aq. NaOH (5 M), then the solution was incubated at 37°C overnight with gentle shaking. For the last step (α1,4 galactosylation with UDP-Gal6D), pNP-β-Lac (5 mg, 10.8 μmol), NwLgtC (22 mg) and alkaline phosphatase (250 U) were added to the Falcon tube, and the solution was incubated at 37°C with gentle shaking. The reaction was monitored by HPLC until no more improvements in conversion could be seen. Then, β-glucosidase and β-galactosidase were added to hydrolyse the excess starting material *p*NP-β-Lac giving Gal, Glc and *p*NP. The product Gal6D-α1,4-Lac-β-pNP was purified by preparative HPLC, and characterised by NMR and HRMS. White solid, 1.8 mg, 27.4% isolated yield (including ~10% of an unidentified glycerol derivative that coeluted with the product), 25% corrected isolated yield (not taking into account the impurity present). ^{1}H NMR (500 MHz, D_2O): δ 8.21-8.17 (m, 2H, ArH), 7.19-7.15 (m, 2H, ArH), 5.22 (d, *J* = 7.8 Hz, 1H, H-a1), 4.78 (d, *J* = 4.1 Hz, 1H, H-c1), 4.46 (d, J = 7.7 Hz, 1H, H-b1), 4.40 (qd, J = 6.7, 0.9 Hz, 1H, H-c5), 3.96-3.92 (m, 1H, H-a6'), 3.90 (d, J = 3.4 Hz, 1H, H-b4), 3.88-3.79 (m, 6H, H-a6'', H-b5, H-b6', H-b6'', H-c2, H-c4), 3.79-3.66 (m, 5H, H-a3, H-a4, H-a5, H-b3, H-c3), 3.63-3.58 (m, 1H, H-a2), dd (dd, *J* = 10.3, 7.9 Hz, H-b2), 1.09 (d, *J* = 6.6 Hz, 3H, H-c6). ^{13}C NMR (125 MHz, D_2O): δ 161.6 (Ar Cquat), 142.6 (Ar Cquat), 126.1 (Ar CH), 116.4 (Ar CH), 103.3 (C-b1), 100.1 (C-c1), 99.2 (C-a1), 78.1 (C-a4), 76.9 (C-b4), 75.5 (C-a5 or C-b5), 75.1 (C-a5 or C-b5), 74.1 (C-a3), 72.5 (C-a2), 72.0 (C-b2 or C-b3), 71.9 (C-c4), 71.8 (C-b2 or C-b3), 69.3 (C-c2), 68.3 (C-c3), 67.1 (C-c5), 60.2 (C-b6), 59.7 (C-a6), 15.2 (C-c6). HRMS (ESI): *m/z* for $[M+Na]^+$ $C_{24}H_{35}NNaO_{17}^+$ calcd. 632.1797, found 632.1802; $[M+K]^+$ $C_{24}H_{35}KNO_{17}^+$ calcd. 648.1537, found 648.1546.

Conclusions

In summary, a novel bacterial α1,4 galactosyltransferase from *N. weaveri* was produced and characterised. The optimal pH and temperature were established (7.5 and 40°C) and the highest enzymatic activity was achieved in the presence of Mn^{2+}. A kinetic study to establish the catalytic efficiency of the enzyme against UDP-Gal and *p*NP-β-Lac was carried out. The donor substrate scope of the enzyme was investigated using commercial and *in situ* synthesised donors, revealing that UDP-Gal, UDP-Gal6D, UDP-Gal6F and UDP-GalN as suitable donor substrates. Among them, to the best of our knowledge, Gal6F and GalN have never been reported as substrates for wild-type α1,4 galactosyltransferases. The transfer of GalN is interesting because the amine group has the potential for orthogonal conjugation and further derivatisation (and because the possible precursor $GalN_3$ was previously shown not to be accepted by LgtC homologues). Furthermore, preparative scale synthesis of Gal6D-α1,4-Lac-β-*p*NP was executed, giving 25% overall yield. Consequently, the current study provides a valuable addition to the array of biocatalysts for glycobiology, in particular for the synthesis of modified α1,4-galactosides to be employed as probes and/or building blocks to study a range of infectious diseases.

Conflicts of interest

There are no conflicts of interest to declare.

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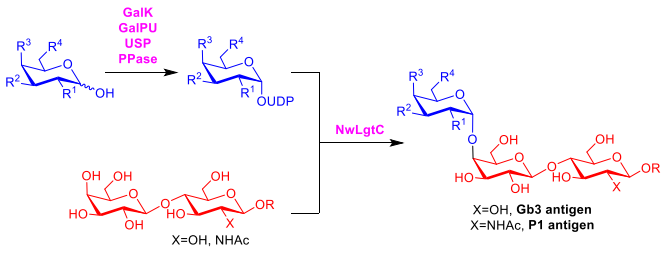
Graphical abstract



Keywords

carbohydrates; globotriose; antigens; galactosyltransferases; biocatalysis