Structural and Thermodynamic Analyses of Solute-binding Protein from Bifidobacterium longum Specific for Core 1 Disaccharide and Lacto-N-biose I*{5}

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Recently, a gene cluster involving a phosphorylase specific for lacto-N-biose I (LNb; Galβ1–3GlcNAc) and galacto-N-biose (GNb; Galβ1–3GalNAc) has been found in Bifidobacterium longum. We showed that the solute-binding protein of a putative ATP-binding cassette-type transporter encoded in the cluster crystallizes only in the presence of LNB or GNb, and therefore we named it GNB/LNB-binding protein (GL-BP). Isothermal titration calorimetry measurements revealed that GL-BP specifically binds LNB and GNb with K_d values of 0.087 and 0.010 μM, respectively, and the binding process is enthalpy-driven. The crystal structures of GL-BP complexed with LNB, GNb, and lacto-N-tetraose (Galβ1–3GlcNAcβ1–3Galβ1–4Glc) were determined. The interactions between GL-BP and the disaccharide ligands mainly occurred through water-mediated hydrogen bonds. In comparison with the LNB complex, one additional hydrogen bond was found in the GNb complex. These structural characteristics of ligand binding are in agreement with the thermodynamic properties. The overall structure of GL-BP was similar to that of maltose-binding protein; however, the mode of ligand binding and the thermodynamic properties of these proteins were significantly different.

Bifidobacteria are Gram-positive anaerobes naturally present in the dominant colonic microbiota and have been considered to be beneficial for human health. As probiotic agents, Bifidobacteria can prevent or alleviate infectious diarrhea through their effects on the immune system and promote the host resistance to colonization by pathogens (1). Carbon source compounds, including oligofructose, inulin, and raffinose, are used as food additives (prebiotics) to selectively promote the growth of Bifidobacteria in the gut (2, 3). Bifidobacteria predominate the intestinal flora of breastfed infants (4, 5), whereas bottle-fed infants do not show rapid colonization of these organisms (6). It has been widely accepted that oligosaccharides other than lactose in human milk (human milk oligosaccharides, HMOs) play a key role in the growth of Bifidobacteria in the gut (7, 8). However, it remains unknown what structure, in HMOs, constitutes the bifidus factor responsible for increasing the bifidobacterial population. Human milk is reported to contain more than 100 kinds of oligosaccharides, the building blocks of which are the following three basic core disaccharides: lactose (Galβ1–4Glc), lacto-N-tetraose (LNT; Galβ1–3GlcNAcβ1–3Galβ1–4Glc), and β1–3 linkage between LNB and lactose, and fucosylated derivative (Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc) are the major components of HMOs (9–11).

Recently, we found that Bifidobacterium longum IC1217 has a unique metabolic pathway specific for LNB and galacto-N-biose (GNb, Galβ1–3GalNAc), and we presented the hypothesis that the LNB residue in HMOs is the bifidus factor in breastfed infants (12). Because GNb is the common O-glycan core structure in animal glycoproteins, such as mucin in the human intestine, this novel LNB/GNb metabolic pathway is considered to be related to intestinal colonization (12).

The two similar disaccharides, LNB and GNb, are important core structures of oligosaccharides in cell surface glycoconjuga-
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TABLE 1

Data collection and refinement statistics

| Data set            | LNB complex | GNB complex | LNT complex | MAD Peak | MAD Edge |
|---------------------|-------------|-------------|-------------|----------|----------|
| Space group         | C222        | C222        | C222        |          |          |
| Unit cell (Å)       | a = 106.3   | a = 106.4   | a = 106.2   |          |          |
|                     | b = 143.6   | b = 143.4   | b = 143.1   |          |          |
|                     | c = 114.6   | c = 114.5   | c = 113.7   |          |          |
| Beam line           | AR-NW12A    | PF BL-17A   | PF BL-5A    |          |          |
|                     | 1.000       | 1.000       | 1.000       |          |          |
| Wavelength (Å)      |             |             |             | 9.7909   | 0.97964  |
| Resolution (Å)      | 50-1.85 (1.92-1.85) | 50-1.99 (2.06-1.99) | 50-1.65 (1.71-1.65) |          |          |
| Total reflections   | 532,644     | 278,763     | 104,130     |          |          |
| Unique reflections  | 74,846      | 60,673      | 104,130     |          |          |
| Completeness (%)    | 99.0 (99.33) | 99.9 (99.8) | 99.0 (99.7) |          |          |
| Rmerge (%)          | 4.7 (26.4)  | 6.3 (34.5)  | 5.9 (36.9)  |          |          |
| l/σ(l)              | 42.2 (5.2)  | 21.7 (3.5)  | 33.1 (2.6)  |          |          |
| Redundancy (%)      | 7.2 (6.3)   | 4.6 (4.4)   | 4.0 (3.8)   |          |          |
|                    |             |             |             |          |          |

Refinement statistics

| Protein Data Bank code | 2Z8D | 2Z8E | 2Z8F |
|-----------------------|------|------|------|
| Resolution range (Å)  | 29.75-1.85 | 39.13-1.99 | 30.27-1.65 |
| No. of reflections    | 70,377 | 57,449 | 97,852 |
| R-factor/Rmerge (%)   | 18.5/23.8 | 17.9/23.6 | 17.9/21.4 |
| r.m.s.d. from ideal values | 0.017 | 0.017 | 0.011 |
| Bond angles (%)       | 1.516 | 1.548 | 1.340 |
| Average B-factor (Å²) |      |      |      |
| Protein (chain A/B)   | 22.9/25.3 | 24.0/24.4 | 22.1/25.8 |
| Water                 | 34.2 | 32.6 | 36.8 |
| Zinc ion             | 28.0 | 32.0 | 22.3 |
| Sodium ion           | - | - | 14.7 |
| MES                   | 45.0 | 48.5 | 38.4 |
| Ligand (LNB, GNB, or LNT bound to A/B chain) | 16.8/16.7 | 19.3/16.7 | 20.9/27.8 |
| Ramachandran plot (%) |      |      |      |
| Favored (chain A/B)   | 92.9/91.4 | 91.2/92.3 | 91.8/92.9 |
| Allowed (chain A/B)   | 7.9/8.3 | 8.8/7.7 | 8.2/6.8 |
| Disallowed (chain A/B) | 0.0/0.3 | 0.0/0.0 | 0.0/0.0 |

* Values in parentheses are for the highest resolution shell.

gates, and most cancer-associated antigens have structures related to them (13–15). LNB residues are found in the type 1 chains of blood group antigens and in some types of oligosaccharide moieties of glycoproteins and glycolipids. GNB is also called core 1 disaccharide or T-antigen disaccharide. Recently, we showed that the gene product of the BL1638 homolog from B. longum JCM1217 crystallizes only in the presence of LNB or GNB, and we named the protein galacto-N-biose/lacto-N-biose 1-binding protein (GL-BP) (22). In this study, we report that GL-BP specifically binds to GNB and LNB, and we reveal its thermodynamic character and the structural basis for its recognition.

EXPERIMENTAL PROCEDURES

Isothermal Titration Calorimetry Analysis—Isothermal titration calorimetry (ITC) measurements were performed at 25 °C according to standard procedures using a VP-ITC system (MicroCal, Northampton, MA). First, the proteins were dia-lyzed extensively against 20 mM potassium phosphate buffer (pH 7.0), and the ligands were dissolved in the same buffer to minimize the heat of dilution. To obtain the best experimental conditions, several preliminary titration experiments were performed by changing the concentrations of the protein and ligands. During each titration, a protein sample (4.76–50.0 M) was stirred at 300 rpm in a 1.44-ml reaction cell. Then the sample was injected with 7.5–10-μl aliquots of a ligand (0.10–20 mM) 19–24 times at intervals of 360–420 s. The peak values (c = n × protein concentration × Kₐ) were 110, 464, and 4.25 for LNB, GNB, and LNT, respectively. The integrated heat effect was analyzed by means of nonlinear regression using the MicroCal Origin (version 5.0) program. Data fitted by one set of sites yielded the association constant (Kₛ), the enthalpy of binding (ΔH), and the number of binding sites (n). The Gibbs energy
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**TABLE 2**

| Ligand | $K_a$ | $\Delta G^0$ | $\Delta H$ | $-T\Delta S^0$ | $\Delta S^0$ | $n^a$ |
|--------|--------|---------------|------------|----------------|-------------|-------|
| LNB    | $1.15 \pm 0.04^b$ | $-9.65$ | $-32.9 \pm 0.1^b$ | 23.2 | 77.8 | 0.958 ± 0.002$^b$ |
| GNB    | $9.74 \pm 0.73^b$ | $-10.9$ | $-40.6 \pm 0.1^b$ | 29.7 | 99.7 | 1.001 ± 0.002$^b$ |
| LNT    | $0.00910 \pm 0.00065^b$ | $-6.8$ | $-19.2 \pm 0.5^b$ | 12.5 | 41.8 | 0.934 ± 0.018$^b$ |

$^a$ Number of binding sites on the protein.

$^b$ The standard errors of binding parameters were evaluated from a single fit.

*FIGURE 1.* Isothermal titration calorimetry of GL-BP with LNB (left) and LacNAc (right). The thermogram (top panel) and binding isotherm (bottom panel) are shown. Ten microliters (19 injections) of ligand (0.2 mM) were added to the GL-BP solution (10 mM). Data were observed at 25 °C and pH 7.0. The solid line in the bottom panel is a theoretical curve according to the parameters listed in Table 2 for LNB. 1 cal = 4.184 J.

*FIGURE 2.* Ribbon representation of the overall structure of GL-BP complexed with LNB, colored blue at the N terminus and red at the C terminus. Two globular domains, the smaller N-terminal lobe on the left, and the larger C-terminal lobe on the right, are linked by three hinge regions. The ligand LNB molecule is shown as a ball-and-stick model; the three zinc ions are shown as orange spheres.

The enthalpy change, $\Delta H$, determined from the calorimetry experiment, is assumed to be equal to the standard enthalpy change, $\Delta H^0$.

Crystallography—Expression and purification of GL-BP and its co-crystallization with LNB or GNB were performed as reported previously (22). GL-BP in complex with LNT was prepared by co-crystallization using a reservoir solution containing 10 mM LNT. Selenomethionine-substituted protein was expressed in *Escherichia coli* strain B834 (DE3), and the protein was purified and crystallized under conditions similar to those for the native crystals with LNB. The x-ray diffraction data were collected using charge-coupled device cameras on the BL5A and BL17A stations of the Photon Factory, High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The crystals were flash-cooled in a nitrogen stream at 100 K. Diffraction images were indexed, integrated, and scaled using the HKL2000 program suite (23). The structure of GL-BP was determined by a multiwavelength anomalous dispersion method using a selenomethionine-substituted crystal. Twenty four selenium atoms were located, and initial phases were calculated using SOLVE/RESOLVE (24, 25). Initial structural model building was conducted using the program ARP/wARP (26), and the R-factor was improved to 20.4% after 50 cycles of calculation. Manual model rebuilding, introduction of water molecules, and refinement were achieved using Coot (27) and Refmac5 (28). The molecules A and B (residues 37–437 and 36–437, respectively) were numbered based on the full-length gene encoding GL-BP. The clear electron densities corresponding to the ligand molecules (LNB, GNB, or LNT) were found in the $F_{\text{obs}} - F_{\text{calc}}$ maps around the sugar-binding sites of the two GL-BP molecules. In addition to the two ligand molecules, two MES molecules and seven zinc ions were added in the model of the LNB and GNB complex structures. In the LNT complex structures, in addition to two MES molecules, five zinc ions and three sodium ions were added based on the peak height and refined temperature factor. Data collection and refinement statistics are shown in Table 1. Figures were prepared using MolScript (29) and Raster3D (30).

change, $\Delta G^0$, and the entropy change, $\Delta S^0$, of the binding are calculated by $\Delta G^0 = -RT\ln K_a$ and $T\Delta S^0 = \Delta H - \Delta G^0$, respectively, where $R$ is the gas constant, and $T$ is the absolute temperature.
RESULTS AND DISCUSSION

Analysis of Ligand Specificity by ITC—The thermodynamic parameters for binding of GL-BP with oligosaccharides were determined by ITC (Table 2). The isotherms for binding with LNB (Fig. 1, left), GNB, and LNT (data not shown) exhibited normal sigmoidal titration curves, and the binding stoichiometry (n) was ~1 in all cases. In contrast, no apparent heat pulse was observed for LacNAc (Fig. 1, right), lactose, galactose, N-acetylglucosamine, and N-acetylgalactosamine (data not shown). The binding affinity was the highest for GNB and relatively weak for LNB. The affinity for LNT was about 1000-fold weaker than that for GNB. For the three oligosaccharides tested here, the binding process was enthalpy-driven, with unfavorable entropy changes at 25 °C (∆T∆S < 0).

Overall Structure of GL-BP—The structures of complexes of GL-BP with LNB, GNB, and LNT were determined at 1.85, 2.0, and 1.65 Å resolutions, respectively. All crystals contained two molecules (A and B) in the asymmetric unit, and the root mean square deviation (r.m.s.d.) values for C-α atoms between them were less than 0.34 Å in all complex structures. The protein construct used for crystallization consisted of 411 residues (amino acid residues 28–438). Amino acid residues of all structures were determined for the A chain 37–437 and the B chain 36–437; however, residues 409–411 of the B chain were missing, probably because of its flexibility. Hereafter, the descriptions will primarily focus on molecule A.

As generally observed for other SBPs (31), the overall structure of GL-BP consists of two globular domains, N- and C-domains, linked by three hinge regions to form a bilobate structure (Fig. 2). The N-domain consists of six α-helices, and a mixed parallel and antiparallel β-sheet of five strands. The C-domain consists of 10 α-helices, a mixed β-sheet of five strands, and an antiparallel β-sheet of two strands. A homology search of GL-BP using the Dali server (32) revealed that GL-BP is very similar to maltose-binding protein (MPB) and a related protein in the closed form, trehalose/maltose-binding protein from Thermococcus litoralis (Protein Data Bank code 1EU8; Z score = 34.2; r.m.s.d. for 376 C-α atoms = 3.3 Å) (33), and MPB from E. coli complexed with maltotriose (Protein Data Bank code 4MBP; Z score = 33.1; r.m.s.d. for 350 C-α atoms = 3.1 Å) (34). The ABC transporter system of MBP has been classified in the carbohydrate uptake transporter-1 (CUT1) family in the Transport Classification Data Base (35). Because the putative ABC transporter system of GL-BP (BL1638–1640) contains two separate transmembrane components expected to form a heterodimer, it will be also classified in the CUT1 family (36). GL-BP shows modest structural similarity (Dali Z scores > 10) with bacterial SBPs belonging to the SBP_bac_1 family of Pfam (37), such as thiaminase I (38) and binding proteins for iron (39), polyamines (40), sulfate (41), and molybdate (42).

Interactions with GNB and LNB—The crystal structures of the LNB and GNB complexes were almost the same, and the r.m.s.d. values for C-α between these complexes were within 0.24 Å. Co-crystallized sugars were found in the middle of the N- and C-domains and are sequestered from solvent by these
ter (GalNAc) is axial. This causes the difference in conformation of the O-6 hydroxyl group in the same GlcNAc/GalNAc sugar ring. The O-6 hydroxyl group of GlcNAc in LNB is in the gauche-orientation, which prevents steric hindrance with O-4. On the other hand, the O-6 hydroxyl group of GalNAc in GNB is in the trans-orientation and forms a hydrogen bond with two water molecules, one of which also forms an inter-sugar bridging hydrogen bond with the O-6 hydroxyl group of Gal.

The higher affinity of GNB to GL-BP compared with that of LNB can also be explained by comparing the structures of the complexes. In the case of the GNB complex, the axial O-4 hydroxyl group of GalNAc forms a hydrogen bond with a water molecule, which is strongly held by two hydrogen bonds to the main chain O and N-η2 atoms of Arg49. In the case of the LNB complex, the equatorial O-4 hydroxyl group of GlcNAc does not contribute to the protein-sugar interaction. Instead, the O-6 hydroxyl group of GlcNAc forms a hydrogen bond with a water molecule, which is held by a single hydrogen bond to the main chain N atom of Ala84. In total, GL-BP forms an additional hydrogen bond with GNB compared with LNB, and this may result in a larger negative ΔH value, which is advantageous, with a difference of 7.7 kcal mol⁻¹.

Complex Structure with LNT—The Fobs - Fcalc omit map for LNT was clearly observed (Fig. 3C). The overall structure of the LNT complex is very similar to those of the disaccharide (LNB and GNB) complexes, and the r.m.s.d. values for Cα were less than 0.31 Å. The LNB portion of LNT overlaps considerably with the LNB molecule bound to the LNT complex structure (Fig. 4B). No stacking interactions were found in the lactose portion of the LNT; however, there are only two direct hydrogen bonds (supplemental Table). A slight displacement of the loop region containing Lys83–Ser85 residues was observed between the LNT and disaccharide complexes. In the LNT complex, a hydrogen bond between the N-ζ atom of Lys83 and O-γ atom of Ser85 blocks the entrance of the sugar-binding cleft. In contrast, the side chain of Lys83 in the LNT complex is directed to the opposite side, preventing steric hindrance with the lactose portion. As a result, the loop region in the LNT complex is shifted slightly upward, changing the protein to a relatively open state. The O-1 atom of GlcNAc in the LNT complex is also shifted slightly upward, and a hydrogen bond with Glu203 is broken. These results together indicate that specific binding interactions for LNT are not enforced compared with those for LNB, but the lactose portion of LNT might cause...
steric hindrance with the side chain of Lys<sup>83</sup>. Hence, the binding of LNT to GL-BP appears less stable than that to LNB, and this observation may explain the smaller enthalpy change on LNT binding compared with that on LNB binding, the difference being 13.7 kcal mol<sup>-1</sup>. In contrast, the entropic penalty on LNT binding was about 36.0 cal K<sup>-1</sup> mol<sup>-1</sup> smaller than that on LNB binding. The number of water molecules excluded from the cleft on LNT binding should be larger than that on LNB binding, because LNT has a bulky lactose portion. In the LNB complex, seven ordered water molecules were found in the area corresponding to the lactose portion of the LNT complex. Therefore, a larger solvent exclusion effect on LNT binding may reduce the entropic penalty.

Comparison with the Other Solute-binding Proteins—Among the members of SBP_bac_1 family (Pfam), the structural characteristics of MBP from <i>E. coli</i> have been studied extensively (44), and the structures of MBP and related binding proteins (e.g. trehalose/maltose-binding protein and cyclo/maldodextrin-binding proteins) from other sources have also been determined (33, 45–47). MBP from <i>E. coli</i> is known to undergo a large open-close conformational change of the two domains on ligand binding, because the three-dimensional structures of unliganded open (48), liganded close (49), and liganded open (50) states have been reported. In the case of GL-BP, we could not obtain crystals without ligands even after extensive crystallization screening. This result suggests that GL-BP also exhibits a significant structural change on ligand binding.

1-Arabinose-binding protein (ABP) and MBP are prominent examples of sugar-binding proteins with a bilobate structure whose ligand binding process has been extensively studied by both structural and thermodynamic analyses (51–55). ABP of Gram-negative bacteria is found in the periplasm and is an essential component of the osmotic shock-sensitive active transport system for monosaccharides, such as L-arabinose and D-galactose (56). ABP belongs to the Peripla_BP_1 family based on the Pfam classification. ABP and MBP share basically similar structural features as follows: two separate globular domains consisting of a central β-sheet flanked with α-helices, and the ligand binding site, which is located between the two domains (Fig. 5A). However, the folding topology ABP is distinct from that of MBP and GL-BP (49). Interestingly, ABP and GL-BP exhibit similar thermodynamic characteristics on ligand binding, with a large favorable enthalpy and smaller unfavorable entropy (54), although they belong to different Pfam families. The interactions between ABP and ligands are mainly water-mediated or through direct hydrogen bonds, and a small hydrophobic patch is formed by two aromatic residues. Such interactions are commonly found in the binding sites of carbohydrate-binding proteins (57). Thus, the thermodynamic signatures of protein-carbohydrate association are typically enthalpy-driven with unfavorable entropy.

In contrast, the interactions between MBP and malto-oligosaccharide ligands are mainly governed by large hydrophobic patches formed by more than a dozen aromatic residues, which are located near the binding groove (34, 50). Fig. 5 shows a comparison of GL-BP and MBP structures in a liganded closed form complexed with LNT and maltotetraose, respectively. In the MBP-maltotetraose complex structure, the nonreducing
end of the ligand extends to the outside of the cleft, and thus the protein can accommodate a large cyclic ligand (cycloextrin) (50). Although the overall folds of MBP and GL-BP have significant similarity, orientations of the bound sugar chain are completely different (Fig. 5B). These proteins do not share a single subsite for the binding of sugar units. Therefore, GL-BP appears to have been designed for the binding of disaccharides (LNB or GNB), and our structure presents a novel sugar-binding mode of SBP belonging to the CUT1 family. Because the ligand-binding groove of MBP is large and hydrophobic, exclusion of a large number of ordered water molecules on ligand binding is thought to cause a large favorable change in entropy (53). The results of our ITC measurements indicated that the thermodynamic signature of GL-BP on ligand binding is in sharp contrast with that of MBP, although they belong to the same Pfam (SBP_bac_1) and CUT1 families.

Sugar Conformations—Table 3 shows the dihedral angles (φ and ψ) of the glycosidic bonds of oligosaccharides bound to GL-BP or a lectin as well as those of LNT in solution (58). The parameters of LNB and T-antigen (Galβ1–3GalNAcα-O-Ser) bound to the Agaricus bisporus lectin (ABL) (59) are shown in the Table 3. The conformation of LNT bound to GL-BP is similar to that of LNT in solution. Most of the differences in dihedral angles are within 40°. The dipolar map of NMR measurement indicates that the Galβ1–4Glc bond of LNT is stable in a conformation near φ = −60° and ψ = 120° (58). A large number of crystal structures of lactose in complex with lectins, including peanut lectin and human galectin-1 (60, 61), are available, and they adopt similar conformations with φ and ψ values of −50 to −90° and 100° to 130°, respectively. The φ/ψ values of the Galβ1–4Glc bond of LNT bound to GL-BP do not largely differ from these standard values. The conformations of LNB and GNB bound to ABL are also similar to those bound to GL-BP; however, they are more similar to those of the LNB portion of LNT in solution, indicating that the lectin binds the oligosaccharide ligands in a more relaxed conformation.

Comparison with Lectins and Potential Value of GL-BP for Diagnostic Application—To the best of our knowledge, GL-BP is the first example of an SBP having a strong affinity to LNB and GNB; however, a number of lectin structures complexed with these disaccharides have been reported (59, 62–67). A common feature of the binding site of lectins for GNB or T-antigens is a shallow depression on the surface of the protein, and recognition occurs through hydrogen bonds to hydroxyls and stacking interaction to one side of the sugar. This mode of recognition is strikingly different from that of GL-BP, which encloses the nonreducing end of the ligand within two lobes. The binding specificities of these lectins vary. Some lectins recognize exposed T-antigen glycosides only (e.g. peanut agglutinin and ABL) (59, 68); however, some lectins can recognize cryptic T-antigens masked by modifying sugar units such as sialic acids. The binding specificity of GL-BP is higher compared with lectins; it recognizes only the exposed nonreducing GNB or LNB portion without any modifications, and the affinity is stronger compared with lectins. Therefore, GL-BP has a novel sugar-binding characteristic and can be used for diagnostic applications, such as marker proteins for cancer cells (69) or probes for microarrays in glycan profiling (70).

Implications for the Sugar Transport System—The dissociation constant (Kₒ) values of GL-BP to GNB and LNB were 0.010 and 0.087 μM, respectively, about 10–100-fold smaller than the reported Kₒ values of SBPs of transporters for mono- or disaccharides (71). On the other hand, the Kₒ value for LNT was much larger, being 11 μM. Therefore, we conclude that BL1638–1640 is an ABC transporter specific for disaccharides GNB and LNB. Although this operon lacks a nucleotide-binding domain, it is likely that a universal nucleotide-binding domain (BL0673) energizes the sugar-transporting system (21). Colonization of adult human intestine by Bifidobacteria is thought to involve the recognition and metabolism of mucin glycans (72, 73). B. longum JCM1217 has endo-α-N-acetylgalactosaminidase (BL0464 homolog), which specifically cleaves the GNB unit (core 1 sugar) from mucin (74). Moreover, extracellular 1,2-α-L-fucosidase is found in Bifidobacterium bifidum (75). HMO and mucin glycans may be degraded by extracellular glycosidases into disaccharide units, such as LNB, GNB, lactose, and LacNAc, before incorporation by specific transporters. GL-BPs are found in whole cell lysates of several bifidobacterial strains, as revealed by Western blot analysis using anti-GL-BP antibody; however, its homologs are not found in other bacterial genomes in the database except for the

5 J. Wada, T. Katayama, H. Ashida, and K. Yamamoto, unpublished data.

| Glycosidic dihedral angles grouped by linkage type for oligosaccharides bound to proteins or in solution | Chain | Galβ1–3 GalNAc/GalNAc | GalNAcβ1–3Gal | Galβ1–4Glc |
|---|---|---|---|---|
| LNT in solution | A | −60/−119 | −138/94 (−60/−30/110−160) | |
| LNT bound to GL-BP | A | −82/−154 | −76/67 |
| LNB bound to GL-BP | A | −88/−154 |
| GNB bound to GL-BP | A | −90/−146 |
| LNB bound to ABL | A | −78/−136 |
| T-antigen bound to ABL | A | −79/−130 |

a Data were defined according to the IUPAC heavy-atom convention in which φ is defined by Oα−C1−O2−C3−ψ by C2−C3−O2−C3.

b Values were taken from Table V of Ref. 58.

Values were taken from Protein Data Bank code 1Y2W at 1.74 Å resolution (59).

Values were taken from Protein Data Bank code 1Y2U at 1.85 Å resolution (59).

Values were taken from Protein Data Bank code 1Y2W at 1.74 Å resolution (59).
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genome of *Propionibacterium acnes* (50% identity). Thus, our results support the hypothesis that the LNB residues in HMO increase the *Bifidobacteria* population in the intestine of breastfed infants (12). We have already established a technology for producing LNB on a large scale (in kilograms) (76), and the product could be used as a supplement in artificial milk or in glycodevelopmental research.

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