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Crystallographic analysis of novel sugar metabolic enzymes from Bifidobacteria

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Introduction

Bifidobacteria are considered to be health-promoting bacteria and have therefore attracted a great deal of attention. 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. 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*-biose I (LNB; Galβ1–3GlcNAc), and *N*-acetyllactosamine (LacNAc; Galβ1–4GlcNAc). Recently, Kitaoka et al. reported the novel metabolic pathway for GNB and LNB in Bifidobacteria [1]. Genes involved in the LNB/GNB metabolism of *B. longum* JCM1217 are homologs of the BL1638–1644 genes of *B. longum* NCC2705. Among these, the product of BL1641 homolog has phosphorolytic activity specific for LNB and GNB, and three downstream genes (BL1642–1644) homologs; lnpB, lnpC, and lnpD) have also been confirmed to encode catalytic enzymes involved in the novel Leloir-like galactose pathway. On the other hand, the three upstream genes (BL1638–1640) are annotated as ATP-binding cassette (ABC)-type sugar transporters, and BL1638 is a solute-binding protein (SBP). Moreover, Bifidobacteria are known to produce various types of extracellular glycosidases [2]. In this study, we focused on structural determination of the novel sugar metabolic enzymes of Bifidobacteria.

Results and Discussion

The crystallization conditions of the gene product of the BL1638 homolog from *B. longum* JCM1217 were searched, and we found that this protein crystallizes only in the presence of LNB or GNB [3]. Therefore, we named the protein galacto-*N*-biose/lacto-*N*-biose I-binding protein (GL-BP). We have determined the crystal structures of GL-BP complexed with LNB, GNB, and lacto-*N*-tetraose (Galβ1–3GlcNAcβ1–3Galβ1–4Glc) were determined [4]. 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.

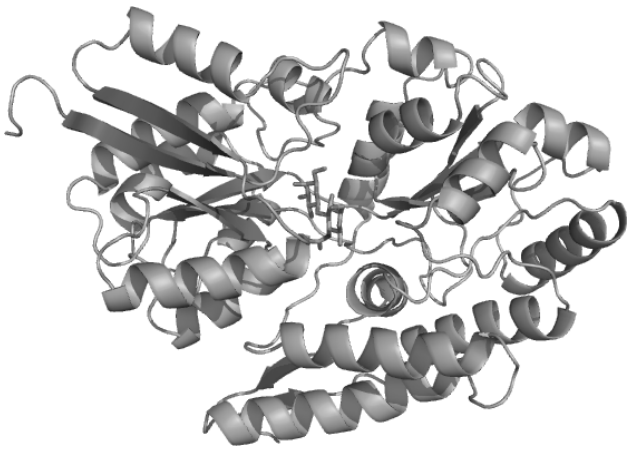

Figure 1 The crystal structure of GL-BP.

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