Crystallography of enzymes in the unique sugar metabolism of Bifidobacteria

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Introduction

A unique metabolic pathway specific for galacto-N-biose (Gal-β1,3-GalNAc, GNB) and lacto-N-biose I (Gal-β1,3-Glc-NAc, LNB) has been identified in several strains of bifidobacteria. LNB- and GNB-containing oligosaccharides are abundantly present in human milk and intestinal mucin glycoprotein, respectively. A key intracellular enzyme in the GNB/LNB pathway is GNB/LNB phosphorylase (GLNBP). GLNBP catalyzes the phosphorolysis of GNB and LNB into α-D-galactose 1-phosphate (Gal1P) and corresponding N-acetylhexosamines with anomeric inversion. GLNBP and its homologues are classified into glycoside hydrolase (GH) family 112 in the CAZy Database (http://www.cazy.org/). An important extracellular enzyme linked to the bifidobacterial GNB/LNB pathway is GNB/LNB phosphorylase (GLNBP). GLNBP catalyzes the phosphorolysis of GNB and LNB into α-D-galactose 1-phosphate (Gal1P) and corresponding N-acetylhexosamines with anomeric inversion. GLNBP and its homologues are classified into glycoside hydrolase (GH) family 112 in the CAZy Database (http://www.cazy.org/).

EngBF belongs to the GH101 family. We have determined the crystal structures of GLNBP [1] and EngBF [2].

Methods

GLNBP crystals were obtained at 4°C using the sitting drop vapor diffusion method by mixing 2 μl of a protein solution with 2 μl of a reservoir solution composed of 0.1 M sodium cacodylate (pH 6.5), 0.2 M Mg(NO₃)₂, and 15% (v/v) polyethylene glycol 4000. Complex structures were obtained by co-crystallization. The crystals were transferred to a reservoir solution containing 15% (v/v) glycerol or 15% (v/v) ethylene glycol and then flash-cooled in a stream of cold nitrogen gas at 95 K. The data sets for SeMet-labeled crystals were collected at wavelengths of 0.96416 Å (remote), 0.97923 Å (peak), and 0.97939 Å (edge). X-ray diffraction data sets were collected using beamlines BL-6A and NW12A.

EngBF crystals were obtained at 20°C using the hanging drop vapor diffusion method by mixing 1 μl of a protein solution (25 mg/ml) with 1 μl of a reservoir solution composed of 0.1 M MES-NaOH (pH 6.9), 3% PEG 20000, 25% MPD, 0.2 M NaCl and 0.01 M MnCl₂. The data sets for SeMet-labeled crystals were collected at wavelengths of 0.96416 Å (remote), 0.97923 Å (peak), and 0.97939 Å (edge). X-ray diffraction data sets were collected using beamlines BL-5A and NW12A.

The data sets were processed and scaled using HKL2000. AutoSHARP, SOLVE/RESOLVE were used for phase determination of Se-MAD. ARP/wARP, Coot and Refmac5 were used for crystallographic refinement.

Results

GLNBP structure

The structure of GLNBP was solved as the first three-dimensional structure of GH112 family enzymes. Five crystal structures (ligand-free form, GalNAc complex, GlcNAc complex, quaternary complex with GlcNAc, nitrate, and ethylene glycol, and ternary complex with GlcNAc and SO₄) were determined. The structures provided structural insights into distinct substrate preferences of GLNBP and its homologues from pathogens. The catalytic domain consists of a partially broken TIM barrel fold that is structurally similar to a GH42 β-galactosidase. Anion binding induces a large conformational change by rotating a half-unit of the barrel. This is an unusual example of molecular adaptation of a TIM barrel scaffold to substrates.

EngBF structure

The ligand-free crystal structure of EngBF was determined at 2.0 Å resolution. Automated docking and mutational analyses revealed possible substrate binding mode and important residues for it.

References


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