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Crystallography of enzymes in the unique sugar metabolism of Bifidobacteria

Masafumi HIDAKA[1], Ryuichiro SUZUKI[1], Takane KATAYAMA[2], Motomitsu KITAOKA[3], Takayoshi WAKAGI[1], Hirofumi SHOUN[1], Hisashi ASHIDA[4], Kenji YAMAMOTO[4], Shinya FUSHINOBU*[1]

[1]Department of Biotechnology, The University of Tokyo, Tokyo 113-8657, Japan
[2]Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Ishikawa 921-8836, Japan
[3]NFRI-NARO, Tsukuba, Ibaraki 305-8642, Japan
[4]Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan

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 endo-α-*N*-acetylgalactosaminidase (EngBF). EngBF catalyses the hydrolysis of *O*-glycosidic bonds in mucin-type *O*-glycan between α-GalNAc and Ser/Thr to release GNB. 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 $Mg(NO_3)_2$, 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 0.9645 Å (remote), 0.9793 Å (peak), and 0.9797 Å (edge). X-ray diffraction data sets were collected using beamlines BL-5A, BL-17A, 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 $MnCl_2$. 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.

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 SO_4) 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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* asfushi@mail.ecc.u-tokyo.ac.jp