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Crystallography of enzymes in the unique sugar metabolism of Bifidobacteria

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Introduction

Bifid shunt is a unique and effective glycolytic pathway of bifidobacteria that produces 2.5 mol of ATP per glucose. Phosphoketolase (PK, EC 4.1.2.22) catalyzes the key steps of the bifid shunt. PK is a thiamine diphosphate (ThDP)-dependent enzyme and catalyzes the cleavage of fructose 6-phosphate (F6P) or xylulose 5-phosphate utilizing inorganic phosphate (Pi) to produce acetyl phosphate (acetyl-P), H_2O and glyceraldehyde 3-phosphate or erythrose 4-phosphate. The PK reaction is different from other ThDP-dependent enzymes because it involves a dehydration step. In order to clarify the reaction mechanism, we have determined the crystal structures of PK from *Bifidobacterium breve* 203 (*Bb*XFPK) [1, 2]. Structures of two key intermediates before and after the dehydration step, α,β-dihydroxyethyl ThDP (DHEThDP) and 2-acetyl-ThDP (AcThDP), and complex with Pi gave an insight into the mechanism of each step of the enzymatic reaction.

Methods

The expression plasmids harboring wild-type and mutant *Bb*XFPK genes were introduced into *Escherichia coli* BL21 CodonPlus (DE3)-RIL. The transformants were cultured and lysed. The crude extracts were purified by 3-step column chromatography.

The purified *Bb*XFPK were crystallized at 20°C using the sitting drop vapor diffusion method by mixing 0.7 µl of a protein solution with 0.7 µl of a reservoir solution composed of 0.1 M Bicine (pH 9.0) and 24% (v/v) polyethylene glycol 6000. Complex structures were obtained by soaking wild-type or mutant *Bb*XFPK crystals in reservoir solution containing 27 mM or 54 mM F6P for various time periods. The crystals were transferred to a reservoir solution containing 20% (v/v) ethylene glycol or 20% (v/v) glycerol and then flash-cooled in a stream of cold nitrogen gas at 100 K. The data set for SeMet-labeled crystal was collected at wavelength of 0.97875 Å (peak). X-ray diffraction data sets were collected using beamlines BL-5A, BL-6A, BL-17A, NE3A, and NW12A.

The data sets were processed and scaled using HKL2000 program suite. SnB and SOLVE/RESOLVE were used for phase determination of Se-SAD. ARP/wARP, Coot and Refmac5 were used for crystallographic refinement. Parameters used in refinement were generated using the PRODRG server.

Results

Four crystal structures of wild-type *Bb*XFPK, ligand-free enzyme, DHEThDP intermediate, AcThDP intermediate, and complex with Pi, were determined. In addition, four mutant *Bb*XFPK structures, H64A, H142A, H320A, and H553A, were determined. The structures provided structural insights into the reaction mechanism including the dehydration and nucleophilic attack by Pi to the AcThDP intermediate. *Bb*XFPK is a homodimeric enzyme and two active sites are positioned at the dimer interface (Figure 1). The overall structure of *Bb*XFPK is basically similar to those of transketolase. On the basis of crystallographic and mutagenesis analyses, we elucidated the critical residues for substrate recognition and indicated the catalytic residues of each step. Our results suggest that dehydration occurs against DHEThDP intermediate to form AcThDP intermediate and then acetyl-P is produced by nucleophilic attack by Pi to AcThDP.

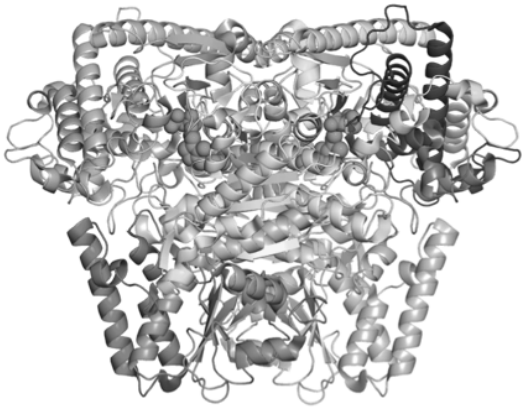

Figure 1 The crystal structure of *Bb*XFPK

References

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