Snapshot Structures of Thiamine-Dependent Dehydration Reaction by Phosphoketolase from *Bifidobacterium breve*

Thiamine diphosphate functions as a cofactor in enzymes that catalyze essential reactions in metabolic pathways of all organisms. Phosphoketolase is a thiamine diphosphate-dependent key enzyme in the catabolism of sugar in various microbes. In particular, this enzyme is indispensable for unusually high efficiency of energy production of the glycolytic pathway of beneficial commensal bacteria, bifidobacteria. However, its structure and mechanism have long been enigmatic. We determined the three-dimensional structure of phosphoketolase from *Bifidobacterium breve*. Details of its unique reaction mechanism involving dehydration and nucleophilic attack by phosphate were revealed based on "snapshot" X-ray crystal structures.

Thiamine diphosphate (ThDP) is a biologically active form of thiamine (vitamin B_1). ThDP-dependent enzymes are ubiquitously present in all organisms and catalyze various essential reactions in metabolic pathways. Phosphoketolase is a ThDP-dependent key enzyme in the catabolism of pentose in various microbes including bacteria, filamentous fungi and yeasts. Moreover, it is involved in the central glycolytic pathway of heterofermentative lactic acid bacteria including many beneficial gut microbes. In particular, bifidobacteria, representatives of beneficial commensal bacteria, have an effective glycolytic pathway called "bifid shunt" in which 2.5 mol of ATP are produced per glucose. Therefore, ATP production by the bifid shunt is 1.25-fold more effective than that by the standard glycolytic pathway (Embden-Meyerhof pathway, 2 ATP per glucose). Phosphoketolase is responsible for two catalytic steps in the bifid shunt because of its dual-substrate specificity. Phosphoketolase from bifidobacteria catalyzes the cleavage of xylulose 5-phosphate or fructose 6-phosphate (F6P) utilizing inorganic phosphate (Pi) to produce acetyl phosphate, water, and glyceraldehyde 3-phosphate or erythrose 4-phosphate. Acetyl phosphate is a high-energy metabolite and is subsequently converted by acetate kinase to generate ATP. The first-half reaction of phosphoketolase is similar to that of transketolase. Transketolase is one of the best-studied ThDP enzymes, and its detailed reaction mechanism has been elucidated by a "snapshot" crystallographic study by trapping key intermediates [1]. The reaction of phosphoketolase is distinct from that of transketolase because it has been thought to involve dehydration of an intermediate and subsequent nucleophilic attack of Pi on the dehydrated intermediate. It is noteworthy that the dehydration reaction is not found in other ThDP-dependent enzymes. Although phosphoketolase is an "old" enzyme that was discovered in 1958 [2], its three-dimensional structure was not reported until 2010 in spite of its importance in microbial metabolism.

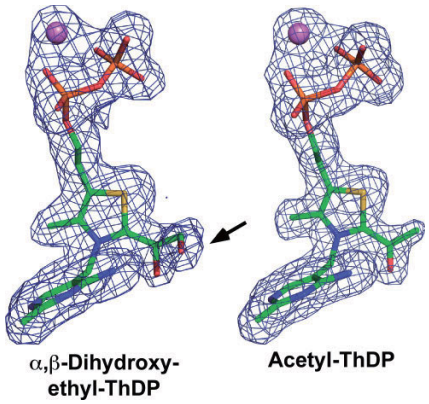

Figure 1
The intermediates before and after the dehydration step of phosphoketolase (α, β-dihydroxyethyl-ThDP and acetyl-ThDP) trapped by X-ray crystallography. The β-hydroxyl group (arrow) disappeared after the in-crystal dehydration reaction.

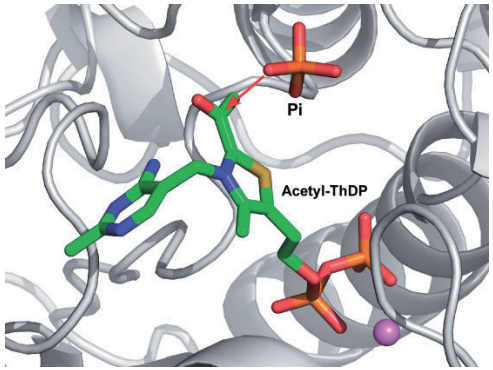

Figure 2
Composite structure of acetyl-ThDP intermediate and inorganic phosphate (Pi) complex. An arrow indicates the nucleophilic attack, which yields the final product, acetyl phosphate.

We determined the crystal structures of phosphoketolase from *Bifidobacterium breve* [3, 4]. The structures of the resting form, the two key intermediates before and after dehydration, the complex with Pi, and four mutant enzymes have been determined by using the BL-5A, 6A, 17A, AR-NE3A, and AR-NW12A. Phosphoketolase is a homodimeric enzyme, and the monomer consists of three α/β-fold domains: N-terminal PP domain, middle Pyr domain, and C-terminal domain. The active site is positioned at the dimer interface between the PP and Pyr domains from different subunits. The intermediates before and after the dehydration (α, β-dihydroxyethyl-ThDP and acetyl-ThDP) were obtained by soaking the crystals in F6P solution for 15 s or 5 min (Fig. 1). The β-hydroxyl group disappeared when the crystal was soaked in F6P solution for >1 min, indicating that the dehydration reaction took place in the crystal. The dehydrated acetyl-ThDP intermediate was stable for at least 1 h in the absence of Pi. The Pi complex structure provided a structural basis of the nucleophilic attack of Pi on the acetyl-ThDP (Fig. 2). Along with kinetic and structural analyses of various mutant enzymes, we succeeded in elucidating almost the entire reaction mechanism of phosphoketolase.

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BEAMLINES

5A, 6A, 17A, AR-NE3A and AR-NW12A

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