Computational and Theoretical Chemistry 1025 (2013) 1–7

Contents lists available at ScienceDirect

Computational and Theoretical Chemistry

journal homepage: www.elsevier.com/locate/comptc

Computational studies on the catalytic mechanism of phosphoketolase

Jing Zhang [a,b], Yongjun Liu [a,c,*]

[a] Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai 810001, China
[b] Key Laboratory of Inorganic Chemistry in Universities of Shandong (Jining University), Qufu, Shandong 273155, China
[c] School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong 250100, China

ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 27 September 2013
Accepted 27 September 2013
Available online 5 October 2013

Keywords:
Phosphoketolase
Density functional theory (DFT) method
Reaction mechanism
THDP-dependent enzyme
Dehydration
Keto–Enol tautomerism

ABSTRACT

Phosphoketolase (PK) is a thiamine diphosphate (THDP) dependent enzyme which plays key roles in the metabolism of heterofermentative bacteria. By using density functional theory (DFT) method, the catalytic mechanism of PK has been studied on simplified models. The calculation results indicate that the formation of 2-α,β-dihydroxyethylidene-THDP (DHETHDP) and erythrose-4-phosphate (E4P) involves one C–C bond formation and one C–C bond cleavage process. Each C–C bond formation or cleavage is always accompanied by a proton transfer in a concerted but asynchronous way. The dehydration process in the reaction of PK is distinct from that of other THDP-dependent enzymes. The Keto–Enol tautomerism process is assisted with a mediator His553. His64, His553 and His97 are found to have the function to stabilize the transition states and intermediates. His64 is a better candidate of B1 catalyst. His553 acts as a proton donor to protonate the carbonyl oxygen, and plays intermediary role in the Keto–Enol tautomerism process. His97 is the probable B2 catalyst in the dehydration process.

1. Introduction

Phosphoketolase (PK) is a prominent enzyme in sugar metabolism, which requires thiamine diphosphate (THDP) and a bivalent cation as cofactors for enzymatic activity [1–4]. PK mainly plays roles in the metabolism of heterofermentative bacteria, and functions as key enzyme in two pathways. One pathway is the phosphoketolase pathway (PK pathway), which is the central pathway in the metabolism of heterofermentative lactic acid bacteria including the genera Lactobacillus and Leuconostoc [5]. The other is the fructose-6-phosphate (F6P) shunt pathway called "bifid shunt" [6]. PK can catalyze the formation of acetyl phosphate (AcP) and erythrose-4-phosphate (E4P) from fructose-6-phosphate (F6P), or the formation of AcP and glyceraldehyde 3-phosphate (G3P) from xylulose-5-phosphate (X5P) utilizing inorganic phosphate (Pi) as acceptor, as shown in the following overall reactions (1) and (2) [7]:

$$\text{fructose-6-phosphate (F6P)} + \text{Pi} \rightarrow \text{erythrose-4-phosphate (E4P)} + \text{acetyl-phosphate (AcP)} \quad (1)$$

$$\text{xylulose-5-phosphate (X5P)} + \text{Pi} \rightarrow \text{glyceraldehyde-3-phosphate (G3P)} + \text{acetyl-phosphate (AcP)} \quad (2)$$

According to substrate preference, PK is divided into two types, namely XPK (EC 4.1.2.9) when specificity is for X5P, and XFPK (EC 4.1.2.22) when both X5P and F6P are accepted [8]. XPK is a key enzyme in the PK pathway of various microbes, but XFPK is only found in bifidobacteria and can catalyze the two steps in the "bifid shunt" pathway owing to its dual-substrate specificity [4]. Another THDP dependent enzyme catalyzing the key step of "bifid shunt" pathway is transketolase (TK), which together with PK belongs to TK family when THDP dependent enzymes are divided into four families based on their primary and tertiary structures [9]. In the past decades, many experimental and theoretical researches have been made for understanding the properties of TK [10–13]. But PK has been little studied since its distribution in the natural world is not very widespread compared with TK [3].

In 2010, the high resolution crystal structures of XFPK from bifidobacterium longum (PDB code: 3AI7) and bifidobacterium breve (PDB code: 3AHC, etc.) were determined [3,4]. These three-dimensional structures provide new insights into the fold of phosphoketolases, binding mode of cofactors, and information of catalytic reaction. PK is a dimeric molecule, which is the functional unit of the enzyme (shown in Fig. 1) [14,15]. Each subunit consists of three domains, including the N-terminal domain (residues 2–378), the middle domain (residues 379–612) and the C-terminal domain (residues 613–814). Residues from both subunits build up two related active sites that are involved in the binding of cofactor THDP. From the active center of Bifidobacterium Longum PK shown in Fig. 2a, we can see that this enzyme shares some similarities with most other THDP enzymes [16–21]. Firstly, THDP adopts the V-like

* Corresponding author at: School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong 250100, China. Tel.: +86 531 883 655 76; fax: +86 531 885 644 64.
E-mail address: yongjunliu_1@sdu.edu.cn (Y. Liu).

2210-271X/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.comptc.2013.09.026

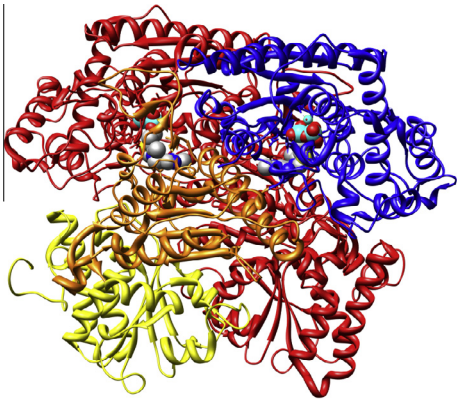

Fig. 1. The overall structure of PK. One subunit is color-coded red. For the other subunit, the three domains are color-coded differently: N-terminal domain is blue, middle domain is orange and C-terminal domain is yellow. Two THDP cofactors are shown as spheres. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

conformation to bring the N4′-amino group of the pyrimidine ring adjacent to the C2 of the thiazolium ring, greatly facilitating the formation of ylide to initiate the catalytic cycle. Secondly, a universally conserved glutamate residue (Glu479) forms a hydrogen bond with the N1′ atom of THDP. It is important to note that PK and TK have similar active site structures in spite that the sequence identity between them is only 17.1%. Several histidine residues in the active site as well as other residues important for catalysis are completely conserved between PK and TK [4].

A possible mechanism of PK has been proposed by Yevenes and Frey [7,22], as shown in Scheme 1. Similar with other THDP-dependent enzymes, THDP must be activated firstly to form THDP ylide to initiate the catalytic cycle. A proton is transferred from the side-chain of carboxyl group of Glu479 to N1′-nitrogen of aminopyridine ring, which aids amino-imino tautomerization at 4′-NH2, and the imino group extracts a proton from the C2-carbon of the thiazolium group (step0). Then, the catalytic cycle starts with the nucleophilic attack of the deprotonated C2-carbon of THDP ylide at the C2″ carbonyl carbon atom of the substrate sugar (F6P or X5P), producing the first covalent intermediate THDP-F6P or THDP-X5P (step1). Next, the scissile C2″–C3″ bond of the sugar moiety of THDP-F6P or THDP-X5P is broke to generate 2-α,β-dihydroxyethylidene-THDP (DHETHDP), and the first product (E4P or G3P) is liberated from the enzyme (step2). A proton acceptor B1 is required to deprotonate the C3″ hydroxyl group of the THDP-F6P or THDP-X5P in this step. The third step is the dehydration process of DHETHDP which requires a proton donor (B2) to protonate the C1″ hydroxyl group (step3). A water molecule is released and enolacetyl-THDP is formed. Enolacetyl-THDP is then converted to 2-acetyl-thiamine pyrophosphate (AcTHDP) by a transformation of enol and ketone (step4). Finally, inorganic phosphate (Pi) attacks the AcTHDP to form acetyl-phosphate (AcP) and the THDP ylide is regenerated (step5). The reaction of PK is distinct from that of other THDP-dependent enzymes at the dehydration process. In the case of TK, the elimination of water from DHETHDP does not occur. Instead, DHETHDP intermediate combines with the incoming aldose to yield a ketose with an extended carbon skeleton.

Some experimental advances have been achieved in understanding the catalytic mechanism of PK. Takahashi et al. [3] superimposed the structures of PK with THDP and TK in complex with THDP-F6P (PDB code: 2R8P) to generate the docking model of PK with THDP-F6P. The docking model suggests that His64 or His320 are possible B1 catalyst that extracts a proton from C3″-hydroxyl group of the sugar moiety of THDP-F6P, while His142 or His553 could be the B2 catalyst that causes the elimination of water from DHETHDP. Shortly afterwards, Suzuki et al. [4] reported the crystal structures of the two intermediates before and after dehydration (DHETHDP and AcTHDP). Observation of the DHETHDP and AcTHDP supports the previous notion that the dehydration occurs in the absence of Pi [7]. Mutagenesis and crystallographic analysis of PK indicate that the most possible candidate of B1 catalyst is His64. But the B2 catalyst remains unclear, and the possible residues are assigned to be His553, the N4′ group of the pyrimidine, or His97. These studies expanded the structural insight into the reaction mechanism of PK, but many questions still remain to be settled besides the assignment of B1 and B2 catalysts. The detailed description of each elementary step, the roles of key pocket residues involved in proton transfer and stabilization of reaction intermediates and transition states, and the energetics of the whole reaction are still not fully understood.

In the present work, we perform a theoretical study on the catalytic mechanism of PK (steps1–4) using hybrid density functional theory (DFT) method on simplified models, which has been successfully employed to study enzymatic systems and gain lots of mechanistic insights [23,24]. Based on the crystal structure of the wild type Bifidobacterium Longum XFPK (PDB code: 3AI7) determined by Takahashi et al. [3] with F6P as substrate, the computational models were constructed, and the residues acting as B1 or B2 catalyst were assigned. In addition, the reaction barriers and energies were calculated.

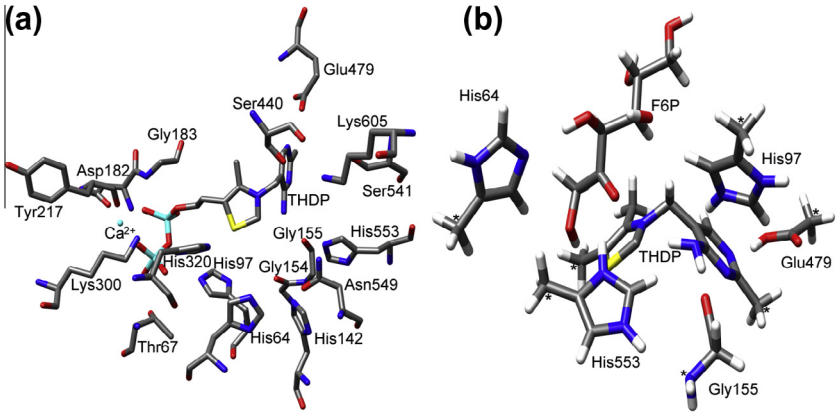

Fig. 2. (a) The active site of PK with the cofactors THDP and Ca^{2+} (PDB code 3AI7). (b) The calculation model employed in the present work. The F6P molecule is separately optimized and then deposited into the active pocket using the Autodock program. The fixed atoms are labeled by asterisks.