5A, 17A, NW12/2008G0149

Structure Determination of Enzymes Involved in the New Carbon Dioxide Fixation System

Akira Nakamura[1], Masahiro Fujihashi[1], Riku Aono[2], Takaaki Sato[2], Yosuke Nishiba[1], Shosuke Yoshida[2], Ayumu Yano[2], Haruyuki Atomi[2], Tadayuki Imanaka[3] and Kunio Miki[1*]

[1] Dept. of Chemsitry, Graduate School of Science, Kyoto University, Sakyo, Kyoto, 606-8502, Japan
[2] Dept. of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[3] College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

1 Introduction

Recently, a novel carbon dioxide fixation pathway is discovered in a thermophilic archaea, *Thermococcus kodakaraensis*. This pathway is composed of three enzymes and involved in AMP metabolism. Ribose-1,5-bisphosphate isomerase (R15P isomerase) is a novel enzyme involved in this pathway and isomerizes ribose-1,5-bisphosphate (R15P) into ribulose-1,5-bisphosphate (RuBP). We here report the crystal structure and molecular mechanism of this enzyme.

2 Experimental

We have determined three structures, unliganded wild-type enzyme (WT (unliganded)), wild-type complexed with its product RuBP (WT-RuBP) and the catalytically inactive mutant C133S in complexed with the substrate R15P (C133S-R15P). The unliganded crystals were obtained using PEG1000 as a precipitant. Crystals of WT-RuBP and C133S-R15P were obtained by co-crystallization method using the same precipitant solutions with WT (unliganded) crystals.

Diffraction datasets were taken at BL5A, BL17A and NW12A beamlines. Phasing was performed with a single anomalous dispersion (SAD) method using a seleno-methionine-labeled unliganded crystals. The WT (unliganded), WT-RuBP and C133S-R15P structures were determined at 2.50 Å, 2.60 Å and 2.85 Å resolution, respectively.

3 Results and Discussion

Six subunits are found in an asymmetric unit of the WT (unliganded) crystal. The oligomerization state is compatible with the gel-filtration analysis, suggesting the determined structure is the physiological state of this enzyme. The monomeric structure consists of two domains, N-terminal α-helical and C-terminal $\alpha\beta\alpha$-sandwich domains. A positively charged cavity is located between the two domains. The three-dimensional structure database search performed by DALI shows that the crystal structure of R15P isomerase resembles to those of PF1008 family proteins as deduced from the primary sequence.

The co-crystallized ligand molecule was bound at the cavity. The positive charge of the cavity is suitable to accommodate the ligand possessing two negatively charged phosphate groups. At the ligand binding-site of WT-RuBP, the conserved residues in PF1008 family, Cys133 and Asp202, are located around the ribulose moiety of the ligand. Mutations of these residues, C133S and D202N, result in non-detectable enzymatic activities. Based on these analyses, we prepared the crystal of C133S-R15P complex and determined the structure.

The Oγ of mutated C133S residue is located 3.3 Å from C2' of the ribose. This oxygen is also interacted with main chain nitrogen The carboxylate of Asp202 interacts with 2'-OH and O4' with 3.1 Å and 2.7 Å, respectively. The carboxylate is surrounded by several hydrophobic residues. These aspects suggest that the thiol of Cys133 and the carboxylate of Asp202 are deprotonated and protonated, respectively, in the enzyme-substrate complex.

The enzymatic reaction is assumed to be proceeded as following. Proton transfer from protonated Asp202 to O4' of the substrate is the first step. Then, proton abstraction from C2' and the cleavage of O4'-C1' bond result in the formation of *cis*-phosphoenolate intermediate. The intermediate is transformed to the product RuBP by the proton transfers from Cys133 to C1' and from O2' to Asp202.

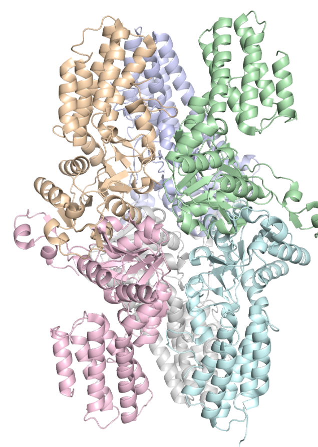

Figure. Overall structure of R15P isomerase hexamer

Reference

[1] A. Nakamura *et al*., J. Biol. Chem., **287,** 20784-20796 (2012).

* miki@kuchem.kyoto-u.ac.jp