5A, 17A, NW12A/2006-G164

Investigation of the Reaction Mechanism of Orotidine-5'-Monophosphate Decarboxylase

Masahiro Fujihashi[1], Shingo Kuroda[1], Lakshmi P. Kotra[2], Emil F. Pai[3] and Kunio Miki[1]
[1]Graduate School of Science, Kyoto University, Sakyo-ku, 606-8502, Japan
[2]Center for Molecular Design and Preformulations and Division of Cell & Molecular Biology, Toronto General Research Institute/University Health Network, Toronto, ON, Canada M5G 1L7
[3]Departments of Biochemistry, Medical Biophysics, and Molecular Genetics, University of Toronto, Toronto, ON, Canada M5G 1L7

1 Introduction

Orotidine-5'-monophosphate decarboxylase (ODCase) converts orotidine-5'-monophosphate (OMP) into uridine-5'-monophosphate (UMP), the final step of *de novo* pyrimidine biosynthesis. This enzyme is known as one of the most proficient enzymes known. The $t_{1/2}$ of the decarboxylation in water is estimated at about 78 million years. On the contrary, the enzyme completes the reaction within milliseconds. The reaction acceleration ratio is 17 orders of magnitude.

Despite the intensive structural, enzymological, computational and chemical investigations, there is still no general agreement regarding the overall mechanism of this enzyme. More than hundred crystal structures from various organisms have been determined so far. They all represent a dimer of TIM-barrel fold subunits. The active site is deeply buried and located at the dimer interface. A characteristic Lys-Asp-Lys-Asp network is found in all determined structures and the motif is considered to play a dominant role in decarboxylation.

We have long been investigated crystallographic and enzymological analyses of this enzyme in order to elucidate the molecular mechanism of this enzyme. Here, we report the importance of the substrate distortion in catalysis.

2 Experiment

Our investigations were performed using ODCase from *Methanothermobacter thermoautotrophicus (Mt*ODcase). Purified *Mt*ODCase (10 mg/ml) was mixed with 5-10 mM of ligands and the mixture was crystallized using the crystallization solution composed of 1.1-1.36 M sodium citrate and 5% (v/v) dioxane at pH 6-8.5. Crystals were dipped in a cryo-protectant buffer consisting of 1.2 M sodium citrate, 15% glycerol and 0.1 M MES-Na at pH 6.5 and flash-frozen in a nitrogen stream at 95 - 100 K. The data collections were performed using beamlines 5A, 17A and NW12A. The crystals diffracted X-rays around 1.5 Å resolution and belonged to the space group $C222_1$ with the cell parameters of a = 53 Å, b = 103 Å and c = 74 Å. Phasing was performed by the fourier synthesis using a previously determined structure of *Mt*ODCase.

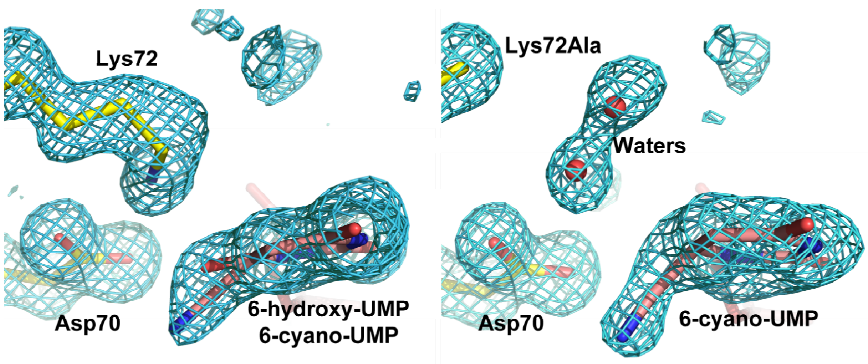
3 Results and Discussion

The left panel of the figure shows the omit electron density map superposed on the atomic model of wild-type *Mt*ODCase with 6-cyano-UMP. The cyano substituent on the C6 position of the pyrimidine ring is apparently distorted from the pyrimidine plane. The distortion is also found in 6-cyano-UMP bound to the mutant of the active site lysine (Lys72Ala mutant, right panel). Similar distortions are found in various structures. These structural features suggest that the active site aspartate (Asp70) located close to the C6 substituent of the pyrimidine ring play a dominant role for the distortion and the catalysis

References

M. Fujihashi *et al*., J. Mol. Biol **387** (2009) 1199.

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



Left: 6-cyano-UMP bound to wild-type *Mt*ODCase. Since ODCase slowly converts 6-cyano-UMP into 6-hydroxy-UMP, both cyano and hydroxy substituents at C6 of pyrimidine ring can be seen in the omit electron density map. The cyano group is apparently distorted from the pyrimidine plane.

Right: 6-cyano-UMP bound to K72A mutant *Mt*ODCase. The cyano group is also distorted in this complex.