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Analysis of the mechanism for thermostability and catalytic activity of malate dehydrogenase

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Introduction

Malate dehydrogenase (MDH) catalyses reversible conversion between malate and oxoaloacetate using NADH as a coenzyme. In the organisms, MDH catalyses the conversion of malate to oxaloacetate in tricarboxylic acid cycle (TCA cycle). MDH is a homodimer consisted of identical subunit with molecular weight 35 kDa, and is consisted of NAD(H) binding domain (Rossman fold) in its N-terminal region and catalytic domain in its C-terminal region.

Thermus flavus AT-62 strain is a bacteria, which is able to grow over 70°C. Thus, *T. flavus* produces the enzymes with high thermostability. MDH from *T. flavus* (tMDH) is applied to industrial use now. However, the catalytic activity of tMDH at room temperature is low compared with that at high temperature while it is stable at high temperature over 90°C. Therefore, we planned to design MDH, which posseses high thermostability and high catalytic activity simultaneously and analyse the mechanism. We previously determined the crystal structure of malate dehydrogenae from *T. flavus* and showed that high thermostability is derived from the hydrophobic and electrostatic interactions in the intersubunit and interdomain region.

On the other hand, *Deinococcus radiodurans* R-1 strain is a bacteria, which grows in room temperature (optimal growth temperature is 30°C) stands at a near position to *T. flavus* in the phylogenetic tree. MDH from *D. radiodurans* (DrMDH) shows high amino acid identity of 70% with tMDH. Thus, we conducted comparative analysis of the thermal stability and catalytic activity of tMDH and DrMDH by means of kinetic analyses and X-ray crystallography.

Material and Methods

To facilitate purification of DrMDH in a covenient way, we constructed an expression and purification system based on Ni-affinity chromatography. The gene coding for DrMDH with $(His)_6$ tag at its C-terminal end was constructed and introduced into pET (26b(+)) expression system. The crude extract of His-tagged DrMDH were prepared from recombinant *Escherichia coli* and applied to a Ni-NTA resin column pre-equilibrated with buffer B (20 mM Tris-HCl (pH8.0), 500 mM NaCl). The column was washed with buffer B containing 20 mM imidazole. His-tagged DrMDH was then eluted with buffer B containing 500 mM imidazole. The protein fractions were pooled and concentrated to 10 mg/ml. The purity of protein was verified by sodium dodecylsulafate-polyacrylamide gel electrophoresis (15%). Concentration of protein was determined by the methods of Bradford using a protein assay kit.

Crystals of DrMDH were obtained by the vapor diffusion method, mixing 1 µl of the enzyme solution with an equal volume of the reservoir solution, within 1-3 days at 20°C. The reservoir solution contains 2.0 M Ammonium sulfate or 0.1 M Tris-HCl (pH8.0) and 1.6 M Ammonium sulfate.Crystals were grown in 5 days, with a typical dimension of 0.5 mm by 0.5 mm by 0.05 mm.Before data collection, to avoid freezing, the crystals were transferred to a cryoprotectant solution containing 25% glycerol.

Diffraction data were collected with an IP detector on R-axis IV^{++} using 1.54-Å wavelength X-ray from Cu target.

Results and Discussion

Crystal of DrMDH was diffracted to 3-4 Å resolution. Although, the data was somewhat insufficient to determine the high resolution crystal structure. Thus, we are now trying to obtain the crystal with excellent quality of diffraction and searching for the cryoprotection condition to minimize the damage of crystals by solution environmental change.

References

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