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X-ray structure of L-ribose isomerase from *Acinetobacter* sp.

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1 Introduction

Acinetobacter sp. L-ribose isomerase (L-RI) catalyzes a reversible isomerization between L-ribose and L-ribulose. It has been reported as a new enzyme by Shimonishi and Izumori [1], since there was no sequence similarity to known amino acid sequences of available data bases. In previous study, we have succeeded in overexpression, purification and crystallization of recombinant his-tagged L-RI [2]. Here we determined the crystal structure of his-tagged L-RI by using selenomethionine-substituted L-RI.

2 Experiment

The expression and purification of *Acinetobacter* sp. his-tagged L-RI has already been reported [2]. The selenomethionine-substituted his-tagged L-RI (SeMet L-RI) was also prepared in the same manner as his-tagged L-RI but using *E. coli* B834 cells grown in LeMaster broth. By the hanging-drop vapor-diffusion method, crystals of SeMet L-RI were grown in a droplet mixing 2 µl of protein solution (11.5 mg ml^{-1} in 5 mM Tris-HCl, pH 7.5) and 2 µl of reservoir solution (0.2 M NaCl, 20 % (v/v) PEG 400 and 0.1 M HEPES pH 7.5) against 450 µl of the reservoir solution at 293 K. For his-tagged L-RI, the reservoir solution containing additional 10 mM hexaamine cobalt chloride was used. A crystal of SeMet L-RI mounted in a cryoloop was soaked in crystallization solution containing 30 % (v/v) glycerol and flash-cooled in liquid nitrogen at 100 K. His-tagged L-RI crystal was directly flash-cooled without cryoprotectant. X-ray diffraction data were collected on the PF-AR NW12A beam line in the KEK and on the BL26B1 in SPring-8. Diffraction data were processed using the programs HKL2000 and the CCP4 program suite.

After an initial phase of SeMet L-RI determination by MAD method using the program SOLVE, an initial model was built using the program RESOLVE. Further model building was performed with the programs Coot in CCP4 program suite, and X-fit in the XtalView program system, and the structure was refined using the programs Refmac5 and CNS.

3 Results and Discussion

The structure of L-RI is shown in Fig. 1 (a). L-RI has six α-helices and ten β-strands. The metal ion (Mn^{2+}) bound in the active site is located at a cupin-type beta-barrel, coordinated by Glu124 in β5, His117 and His119 in a loop between β4 and β5, His199 in a loop between β8 and β9. The structure of L-RI is similar in structure to that of L-lyxose isomerase from the pathogenic *E. coli* O157:H7 (3MPB). The sequence identity, the r.m.s. deviation for Cα atoms and Z-score of a DALI search between them were 18 %, 2.8 Å and 15.9, respectively.

There are two and one molecules in an asymmetric unit in SeMet L-RI and his-tagged L-RI structures, respectively. Each of them forms tetramer by symmetry operation and that was also suggested by PISA (Fig. 1 (b)), as was reported that L-RI is tetrameric enzyme [1].

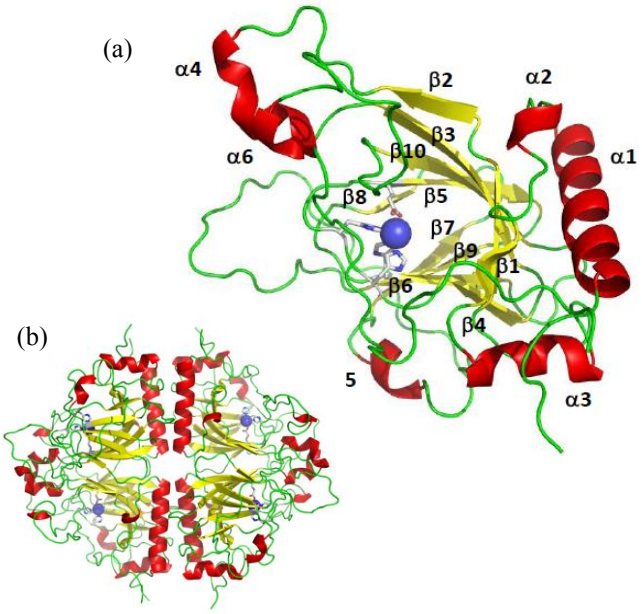

Fig. 1: (a) Monomeric structure and (b) predicted tetrameric structure of his-tagged L-RI.

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References

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