Biological Science

5A, 6A, 17A, NW12A/2009G512

Metal mobility in catalytic sites of *Pseudomonas stutzeri* L-rhamnose isomerase

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Introduction

L-Rhamnose isomerase (L-RhI) catalyzes the reversible isomerization of L-rhamnose to L-rhamnulose. *Pseudomonas stutzeri* L-RhI can catalyze not only the isomerization of L-rhamnose, but also that between D-allose and D-psicose. For the aldose–ketose isomerization by L-RhI, a metal-mediated hydride-shift mechanism has been proposed, but the catalytic mechanism is still not entirely understood. To obtain new insights into the overall catalytic reaction mechanism, the X-ray structures of *P. stutzeri* L-RhI in the specific metal-bound form, and inactive mutant forms of *P. stutzeri* L-RhI (S329K and D327N) in a complex with substrate/product, were determined [1]. We report here the X-ray structure of *P. stutzeri* L-RhI in the Mn^{2+}-bound form.

Materials and Methods

A metal-free enzyme was prepared by treating the purified enzyme with 5 mM EDTA. The metal-free enzyme solution was incubated in the presence of 1 mM $MnCl_2$, $CuSO_4$, $CoCl_2$ or $ZnSO_4$. Using the enzyme solution incubated with specific metal ion, each metal-bound crystal was obtained. X-ray diffraction data were collected on the 5A, 6A, 17A and NW-12A beam line in the Photon Factory. The data were processed using the programs HKL2000 and the CCP4 program suite. Each structure was determined by a molecular replacement method with the program MOLREP in the CCP4 program suite, using the structure of *P. stutzeri* L-RhI (PDB code 2HCV) as a start model. Further modeling was performed with the program X-fit in the XtalView program system, and the structure was refined using the program CNS.

Results and Discussion

The overall structures were almost the same as reported previously [2]. The enzyme forms a tetramer (Mol-A, -B, -C, and -D), having four catalytic sites as shown in Figure 1. The monomeric structure contains two metal ions to form the catalytic site. The Cu^{2+}-, Co^{2+}- and Zn^{2+}-bound forms have the same metal-coordinated structure in all four molecules, however, the Mn^{2+}-bound form has two metal-coordinated structures, as shown in Figure 2. In Mol-A and/or Mol-D (AD-form), the structural Mn^{2+} (Mn1) is coordinated by six coordination bonds from Glu219(OE), Asp254(OD), His281(ND), Asp327(OD) and two water molecules (W1 and W2), and the catalytic Mn^{2+} (Mn2) is coordinated by His257(NE), Asp289(OD1) and four water molecules (W2,W3,W4 and W5). This metal-coordinated structure is equivalent to those found in the other metal-bound forms. In Mol-B and/or Mol-C (BC-form), Mn1 is coordinated in the same way as in Mol-A and Mol-D, but Mn2 is coordinated by His257(NE), Asp289(OD1), Asp289(OD2), Asp291(OD) and two water molecules (W6,W7). The distance between Mn1 and Mn2 changes from 5.2 A˚ (BC-form) to 4.2A˚ (AD-form). Temperature factors of Mn2 for four molecules are significantly higher than those of Mn1, supporting the high mobility of Mn2 in the enzyme. It is likely that the positions of the catalytic metal ions of *P. stutzeri* L-RhI vary between the AD- and BC-forms.

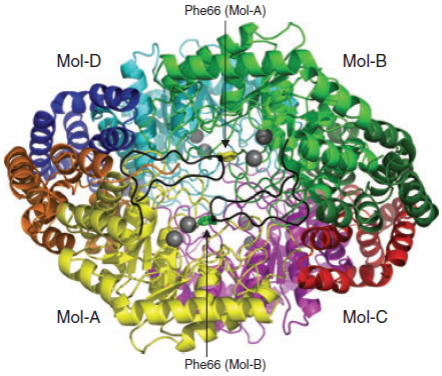

Figure 1 Tetrameric structure of *P. stutzeri* L-RhI

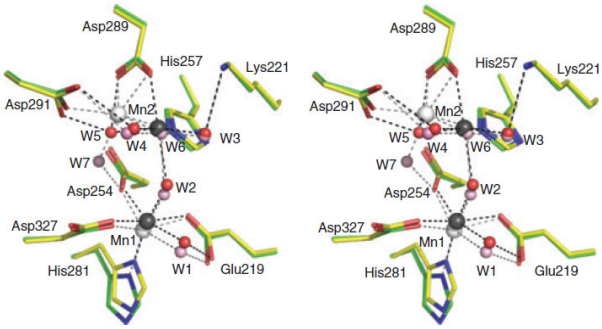

Figure 2. The two forms of Mn^{2+}-bound structure of *P. stutzeri* L-RhI

References

[1] H. Yoshida et al., FEBS J. 277, 1045-1057 (2010).
[2] H. Yoshida et al., J. Mol. Biol. 365, 1505-1516 (2007).

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