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X-ray Structures of L-Rhamnose Isomerase from *Pseudomonas stutzeri* in Complexes with L-Rhamnose and D-Allose

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Introduction

L-rhamnose isomerise catalyzes the reversible isomerization of L-rhamnose to L-rhamnulose. L-rhamnose isomerase from *Pseudomonas stutzeri* (*P. stutzeri* L-RhI) shows a broader substrate specificity than L-RhI from *Escherichia coli* (*E. coli* L-RhI), catalyzing the isomerization between various aldoses and ketoses, such as between L-rhamnose and L-rhamnulose, L-mannose and L-fructose, L-lyxose and L-xylulose, D-ribose and D-ribulose, and D-allose and D-psicose. Since some of them are so called "rare sugars" which exist in small amounts in nature, *P. stutzeri* L-RhI is exploited for industrial applications in rare sugar production. To elucidate the substrate-recognition mechanism responsible for the broad substrate-specificity of *P. stutzeri* L-RhI, understanding the three-dimensional structure of *P. stutzeri* L-RhI in complexes with its substrates is important. We have reported the crystal structure of *P. stutzeri* L-RhI alone and in complexes with L-rhamnose and D-allose.[1,2]

Materials and Methods

X-ray diffraction data were collected using an ADSC Quantum detector system on the BL-6A beam line in the Photon Factory. Diffraction data were processed using the programs HKL2000 and the CCP4 program suite. Initial SAD phasing at 2.1 Å of *P. stutzeri* SeMet L-RhI was performed by locating 24 selenium sites in the peak dataset using the program SOLVE. After electron density modification, 90 % of amino acid residues could be located in the resultant electron density map, using the program RESOLVE. Further model building was performed with the program X-fit, and the structure was refined using the program CNS. Using the structure of *P. stutzeri* SeMet L-RhI, the structure of *P. stutzeri* L-RhI and those of *P. stutzeri* L-RhI/substrates were determined by molecular replacement method and refined at a resolution of 2.0 Å for *P. stutzeri* L-RhI, 1.97 Å for *P. stutzeri* L-RhI/L-rhamnose and 1.97 Å for *P. stutzeri* L-RhI/D-allose, using the program CNS.

Results and Discussion

The overall subunit structure of *P. stutzeri* L-RhI is shown in Figure 1. *P. stutzeri* L-RhI has fifteen α-helices with 54 % of the amino acid residues and eight β-strands with 8 % of the amino acid residues. The structure can be divided into a large domain (Phe50-Val356) and an additional small domain (N-terminus - Lys49, and Asp357 - C-terminus). The large domain forms the core of the enzyme with the $(\beta/\alpha)_8$ barrel fold. The bound metal ions are located at the centre of the barrel, and several His and Asp residues in β-strands direct their side-chain groups toward the inside of the barrel to coordinate with metal ions. The additional small domain is composed of two α-helices (α0 and α0') in the N-terminal region and five α-helices (α9 - α13) in the C-terminal region.

The complex structures of *P. stutzeri* L-RhI with L-rhamnose and D-allose show that both substrates are nicely fitted to the substrate-binding site. The part of the substrate-binding site interacting with the substrate at the 1-, 2-, and 3-positions is equivalent to *E. coli* L-RhI, but the other part interacting with the 4-, 5-, and 6-positions is different. In *E. coli* L-RhI, the β1-α1 loop creates an unique hydrophobic pocket at the the 4-, 5-, and 6-positions, leading to the strictly recognition of L-rhamnose as the most suitable substrate, while in *P. stutzeri* L-RhI, there is no corresponding hydrophobic pocket where Phe66 from a neighbouring molecule merely forms hydrophobic interactions with the substrate, leading to the loose substrate-recognition at the 4-, 5-, and 6-positions.

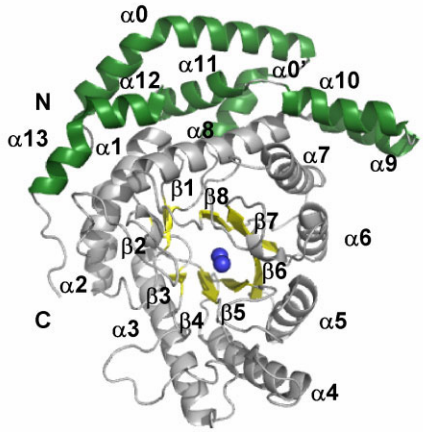

Figure 1. Overall subunit structure of *P. stutzeri* L-RhI illustrated by the program PyMol.

References

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