Crystallography

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X-ray Structures of *Sporobolomyces salmonicolor* Aldehyde Reductase 2 and Its Complex with NADPH

Shigehiro KAMITORI*[1], Mitsugu YAMADA[1], Hiromi YOSHIDA[1], Keiko KITA[2]
[1]Information Technology Center and Faculty of Medicine, Kagawa University
1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 791-0793, Japan
[2]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University
Uji, Kyoto 611-0011, Japan

Introduction

The red yeast *Sporobolomyces salmonicolor* AKU4429 produces three aldehyde reductases, ARI (323 amino acids, MW= 35,232 Da), ARII (344 amino acids, MW= 37,317 Da) and ARIII (MW= 37,000 Da). ARII can reduce a number of aliphatic and aromatic aldehydes, and ketones, such as camphorquinone and benzaldehydes. The amino acid sequence of ARII is different from that of the other enzymes belonging to the aldo-keto reductase superfamily. To obtain new insights into the classification of ARII and catalytic mechanism underlying the stereoselective reduction of ARII, a three-dimensional structure needs to be elucidated. The crystal structures of unliganded ARII (ARII) and the ARII/NADPH complex (ARII/NADPH).

Materials and Methods

A crystal of ARII was grown by the vapor diffusion method using a protein solution (30 mg/ml ARII in 20 mM Tris-HCl (pH 8.0)) and a reservoir solution (32% (w/v) polyethylene glycol 2000 MME, 100 mM ammonium sulfate and 200 mM sodium acetate (pH 5.0)). A crystal of SeMet ARII was obtained under the same conditions. A crystal of ARII/NADPH was obtained by a co-crystallization method, using the protein solution containing 4 mM of NADPH and the same reservoir solution. X-ray diffraction data were collected using an ADSC/CCD detector system on the 5A, 6A and NW12 beam lines in the Photon Factory. Initial SAD phasing at 2.0 Å of SeMet ARII was performed by locating six selenium sites in the peak dataset using the program SOLVE. After electron density modification, 60% 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 in the XtalView program system, and the structure was refined using the program CNS. Using the structure of SeMet ARII, the structure of ARII was determined by isomorphous replacement and refined at a resolution of 1.8 Å. The structure of ARII/NADPH was determined by molecular replacement using the structure of ARII, and refined at a resolution of 1.6 Å [1].

Results and Discussion

ARII has 12 α-helices with 34% of the amino acid residues and 12 β-strands forming three β-sheet structures with 14% of the amino acids. The structure of ARII can be divided into large domain and small domain, as shown in Figure1. The large domain with the α/β structure is expected to be a NAD(P)H-binding domain and the small domain with five α-helices and two small β-sheets covering the bound NADPH, is expected to be a substrate-binding domain. With the binding of NADPH, structural changes occur in the loop region (Ile91-Tyr101) of the large domain and the loop region (Pro216-Ser222) of the small domain, called as mobile regions, as indicated by red in Figure1. ARII can be classified in the short chain dehydrogenase reductase (SDR) family based on three dimensional structure.

ARII has stereoselectivity in its catalytic reaction, giving rise to excessive production of *(S)*-alcohols from ethyl 4-chloro-3-oxobutanoate. The X-ray structure of the ARII/NADPH complex and preliminary modeling show that the formation of the hydrophobic channel induced by the binding of NADPH is closely related to the stereoselective reduction by ARII [1].

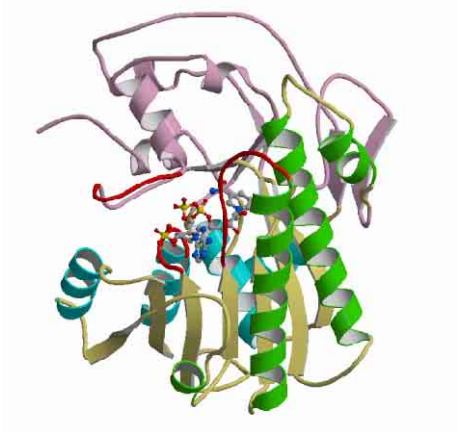

Figure1. The overall structure of ARII/NADPH complex illustrated by the programs MOLSCRIPT and Raster3D. A β-sheet, α-helices on one side of a β-sheet and α-helices on the other side of a β-sheet in the NADPH-binding domain (large domain) are yellow, green and cyan, respectively. The substrate-binding domain (small domain) is shown in light magenta. The bound NADPH is shown by a ball-and-stick model.

References

[1] S. Kamitori et al., J. Mol. Biol. 353, 551 (2005).
*kamitori@med.kagawa-u.ac.jp