NW12A/2010G698

X-ray crystallographic analysis of Inhibitor-free Structure of L-(2*S*,3*S*)-Butanediol Dehydrogenase

Tomohito Shimegi[1], Takashi Ohtsuki[1], Masami Kusunoki[1], Sadaharu Ui[1,*]
[1]Graduate School of Medicine and Engineering, Univ. of Yamanashi, Takeda, Kofu, Yamanashi 400-8511, Japan

1. Introduction

2,3-Butanediol (BD) is known as a by-product of sugar metabolism in microorganisms. There are three types of stereoisomer of BD (D-, L-, and meso-forms). Since the ratio of BD stereoisomers depends on the organisms and the culture conditions of the fermentation process, various 2,3-butanediol dehydrogenases (BDHs) catalyzing the NAD-dependent redox reaction between acetoin (AC) and BD were identified from many microorganisms. Of these BDHs, meso-BDH from *Klebsiella pneumoniae* IAM 1063, specific for meso-BD and D-AC, and L-BDH from *Brevibacterium saccharolyticum* C-1012, specific for L-BD and L-AC belong to the short-chain dehydrogenase/

reductase (SDR) family, based on their amino acid sequence. These two enzymes share a 50% homology of their amino acid sequence. Therefore, it is very interesting to ascertain exactly how the homologous BDHs distinguish diastereomers.

We previously determined the crystal structures of meso-BDH and L-BDH in complex with NAD^+ and inhibitor mercaptoethanol[1-2]. In order to verify the influence of the inhibitor upon the conformation around the substrate binding pocket, we have tried to analyze crystal structure of L-BDH with NAD^+ only .

2. Results and Discussion

Crystals of L-BDH in complex with NAD^+ were prepared by the sitting drop vapor diffusion method in 50 mM MES buffer (pH 6.0), 30% PEG4000, 20% glycerol at 20°C. X-ray diffraction data collections were performed at Photon Factory BL-NW12A at 95 K. 370,634 measured reflection were reduced to 80,761 unique reflections with an overall R_{merge} of 8.0 %. Crystals belong to the space group $P2_1$ with unit cell dimensions a = 69.8 Å, b = 102.9 Å, c = 119.1 Å, β = 97.4°. This represented 95.5 % completeness and 3.5 redundancy at 2.38 Å. There are two molecules of L-BDH tetramers in the asymmetric unit. The structure of L-BDH in complex with NAD^+ was solved by the molecular replacement method using MOLREP. L-BDH in complex with NAD^+ and mercaptoethanol(PDB code:3A28) was used as a search model. Structure refinement was carried out with the program REFMAC5. Subsequently the molecular model was manually rebuilt using the maps with coefficients of sigma weighted $2F_O$-F_C and F_O-F_C maps with the program Coot of version 0.6.2. The final *R*-value was 18.7 % (R_{free} = 25.2) for the resolution range of 33 – 2.38 Å.

The eight subunits (A-H) in the asymmetric unit can be superimposed with an averaged pairwise RMSD of 0.27 Å for α-carbons. Therefore, the structure of subunit A is taken as the representative of the eight subunits.

The superposition around the substrate binding region of subunit A of the enzyme between with and without the inhibitor is shown in Fig. 1. Except side chains of Trp192 and Phe148 deviating slightly, there is little difference of side chain conformations of the residues around the substrate binding region upon binding of the inhibitor. Based on the determination of the inhibitor-free structure of L-BDH, we will be able to discuss substrate specificity and kinetic of the enzyme using crystal structures of mutated L-BDHs in complex with the inhibitor and free enzymes.

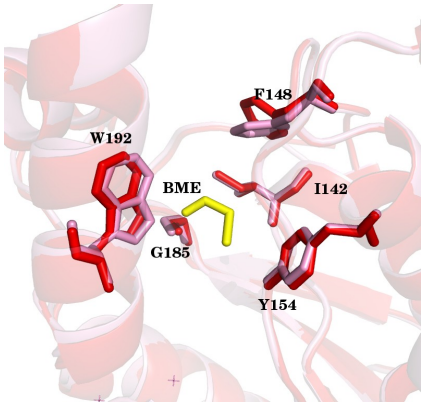

Fig. 1: The superposition around substrate binding region of L-BDH with(red) and without(pink) mercaprtoethanol.

References

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* ui@yamanashi.ac.jp