NE3A/2013G671

X-ray crystallographic analysis of domain-chimeric L-(2S, 3S)-Butanediol Dehydrogenase

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

1 Introduction

2,3-Butanediol (BD) and acetoin (AC, the precursor of BD) are typical metabolic products of oxidative bacteria. 2,3-Butanediol dehydrogenase (BDH) catalyzes an oxidation–reduction reaction between BD and AC in an NAD-dependent manner. There are three types of BD stereoisomers (D-BD, L-BD and *meso*-BD), and there has been considerable interest in BDHs with respect to the isomeric production of BD and AC for possible application of these compounds as chiral moieties in fine chemicals, including drugs and liquid crystals. *meso*-BDH from *Klebsiella pneumoniae* and L-BDH from B*revibacterium saccharolyticum* belong to the short-chain dehydrogenase/reductase (SDR) family[1][2]. Although they share a highly similar structure, their substrate specificities and stabilities differ from each other. Focusing on the structural similarities, a domain-chimeric L-2,3-butanediol dehydrogenase (chimera L-BDH) was obtained by exchanging the domains of the two BDHs in order to potentially combine their beneficial properties[3]. Chimera L-BDH consists of the 'basic' domain from *meso*-BDH, which probably contributes to stability, and the 'leaf-like' domains of L-BDH, which provide the *S*-configuration substrate specificity towards L-BD. As expected, the chimera L-BDH exhibited a higher stability than wild-type L-BDH and had an activity towards L-BD comparable to that of the wild-type enzyme. However, chimera L-BDH also relaxed stereoisomeric recognition and shown significant activity towards other related compounds. These results show not only the potential of the domain-exchange method in enzyme engineering, but also unexpected effects. To understand the reason why chimera L-BDH has this relaxed stereoisomeric recognition, we have tried to analyze crystal structure of chimera L-BDH.

2 Results and Discussion

Crystals of chimera L-BDH in complex with NAD^+ andinhibitor, 2-mercaptoethanolase(ME) were prepared by the hanging drop vapour-diffusion method in 100 mM MES buffer (pH 6.4), 17% PEG6000, 15% glycerol and 1% ME at 20°C. X-ray diffraction data collections were performed at Photon Factory-Advanced Ring NE3A at 95 K. A diffraction data set was collected to 1.58 Å resolution at a wavelength of 1.0 Å, and another data set was collected at a wavelength of 1.8 Å from the same crystal in order to determine the positions of S atoms of ME by the single-wavelength anomalous dispersion (SAD) method. Based on the former data set, the crystal belongs to the space group $C222_1$ with unit cell dimensions a=81.2, b=134.6, c=87.8 Å which is a different space group from tthose of he crystals of *meso*-BDH and L-BDH. The asymmetric unit was expected to contain two monomers of chimera L-BDH, with a Matthews coefficient of 2.25 $Å^3Da^{-1}$, which corresponds to a solvent content of 45.4%. This suggests that a biological tetramer is formed in the crystal lattice by two dimers with crystallographic twofold symmetry, similar to other tetramic SDR-family enzymes. Structure determination via the molecular-replacement method using *meso*-BDH (PDB entry 1geg) or L-BDH (PDB entry 3a28), as search models is currently in progress.

References

[1] M. Otagiri *et al., J. Biochem.* **129**, 205-208 (2001).
[2] M. Otagiri *et al*., *FEBS Lett.* **584**, 219-223 (2010).
[3] T. Shimegi *et al*., *Protein Pept. Lett.* **584**, 219-223 (2011).

* ui@yamanashi.ac.jp