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Crystallization and preliminary X-ray studies of *meso*-2,3-butanediol dehydrogenase from *Klebsiella pneumoniae* IAM1063

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Meso-2,3-butanediol dehydrogenase (*meso*-BDH) has been crystallized and preliminary X-ray crystallographic characterization of *meso*-BDH crystals has been performed. Single crystals of *meso*-BDH were prepared in two forms by the hanging-drop vapour-diffusion method using polyethylene glycol as a precipitant. Form I crystals belong to space group $C2$, with unit-cell parameters $a = 215.5$, $b = 79.4$, $c = 134.8$ Å, $\beta = 98.22^\circ$, and form II crystals belong to space group $P2_1$, with unit-cell parameters $a = 69.16$, $b = 109.78$, $c = 127.28$ Å, $\beta = 102.29^\circ$. The crystals diffracted to 2.0 and 1.7 Å resolutions, respectively, using synchrotron radiation.

1. Introduction

2,3-Butanediol, which is called an acetoinic compound along with its precursor acetoin, is known to be one of the products of carbohydrate metabolism in microorganisms (Taylor & Juni, 1960; Hohn-Bentz & Radler, 1978). Acetoinic compounds are produced as a major byproduct in the fermentation process of wine, a representative fermented food, and have the possibility of being used to monitor the fermentation progress. Acetoinic compounds have asymmetric C atoms and hence there are two stereoisomers, the D(−) and L(+) forms, for acetoin and three stereoisomers, the D(−), L(+) and *meso* forms, for 2,3-butanediol. However, chemical quantitative analysis of these stereoisomers is not easily applicable to the fermentation industry because of the lack of detection methods. Interestingly, there are three stereospecific dehydrogenases for the specific stereoisomers (Ui *et al.*, 1983, 1984, 1986). They are classified into three types: D(−)-2,3-butanediol dehydrogenase (D-BDH), L(+)-2,3-butanediol dehydrogenase (L-BDH) and *meso*-2,3-butanediol dehydrogenase (*meso*-BDH) (Ui *et al.*, 1984). All three types of 2,3-butanediol dehydrogenases (BDH) are tetrameric enzymes with a molecular weight of approximately 100 kDa (Ui *et al.*, 1997). Of the three BDHs, we have performed cloning and constructed the overexpression systems for the enzyme genes of *meso*-BDH and L-BDH, which exhibit activity with *meso*-2,3-butanediol and L-2,3-butanediol, respectively (Ui *et al.*, 1997, 1998). It was shown that *meso*-BDH and L-BDH have approximately 50% sequence identity (Ui *et al.*, 1998). BDHs belong to the short-chain dehydrogenase/reductase family (Jörnvall *et al.*, 1995) based on their amino-acid sequence analysis. To date, crystal structures of 12 enzymes belonging to this enzyme family have been reported to have similar three-dimensional structures with a Rossmann fold (Andersson *et al.*, 1996; Auerbach *et al.*, 1997; Benach *et al.*, 1998; Breton *et al.*, 1996; Ghosh *et al.*, 1991, 1995; Hülsmeyer *et al.*, 1998; Nakajima *et al.*, 1998; Tanaka, Nonata, Nakanishi *et al.*, 1996; Tanaka, Nonata, Yoshimoto *et al.*, 1996; Varughese *et al.*, 1994). Despite their similar main-chain folds, these enzymes each recognize different substrates, such as steroids, prostaglandins, alcohols and aromatic compounds. The proposed catalytic residues Ser, Tyr and Lys for the dehydrogenation reactions are conserved in this family and similar catalytic mechanisms have been reported (Ghosh *et al.*, 1991; Tanaka, Nonata, Nakanishi *et al.*, 1996). However, there is no comprehensive explanation of the reason why enzymes of very similar protein folds recognize a wide range of substrate structures. It was therefore attempted to determine the crystal structures of *meso*-BDH and L-BDH in order to investigate the recognition mechanism of substrate stereospecificity on a structural basis. This report deals with the preparation of single crystals and preliminary crystallographic studies of *meso*-BDH.

2. Materials and methods

2.1. Protein expression and purification

Meso-BDH from *K. pneumoniae* was produced as a recombinant protein in *Escherichia coli* cells. Cloning and overexpression procedures were performed as described previously (Ui *et al.*, 1997). In order to prepare the enzyme with sufficient quality and in sufficient quantity for crystallization, the purification procedure was slightly modified. Initially, the enzyme was purified by ammonium sulfate fractionation and then by ion-

Table 1
Diffraction data statistics of form I and II crystals.

Values in square brackets and parentheses are for the highest resolution shells (2.07–2.00 and 1.79–1.70 Å for forms I and II, respectively) and standard deviations, respectively.

	Form I	Form II
Space group	*C*2	$P2_1$
Unit-cell parameters		
a (Å)	215.5 (2)	69.16 (3)
b (Å)	79.4 (1)	109.78 (3)
c (Å)	134.8 (2)	127.28 (3)
β (°)	98.22 (4)	102.29 (4)
V_M (Å^3 Da^{-1})	2.68	2.22
No. of subunits per asymmetric unit	8	8
Temperature (K)	100	100
Wavelength (Å)	1.0000	0.9000
Resolution range (Å)	30–2.0	30–1.7
No. of unique reflections	143594	203543
Completeness (%)	92.4 [63.0]	100 [100]
R_{merge}†, R_{meas}‡	0.044 [0.148]	0.054 [0.192]
$I/\sigma(I)$	19.7 [12.9]	7.8 [3.8]

† $R_{merge} = \sum_h \sum_i |I_{h,i} - \hat{I}_h| / \sum_h \hat{I}_h$, where $I_{h,i}$ is the ith measurement of intensity of reflection h and $\hat{I}_h$ is the mean intensity of reflection h. ‡ $R_{meas} = \sum_h [n_h/(n_h - 1)] \times \sum_i |\hat{I}_h - I_{h,i}| / \sum_h \sum_i I_{h,i}$, where n_h, $\hat{I}_h$ and $I_{h,i}$ are the multiplicity, averaged intensity and ith intensity measurement of reflection h, respectively.

exchange chromatography followed by hydrophobic chromatography. The homogeneity of the purified enzyme was checked by SDS–PAGE.

2.2. Crystallization of *meso*-BDH

The purified protein was dissolved in 20 m*M* Tris–HCl buffer pH 8.0 containing 200 m*M* NaCl and 100 m*M* *meso*-2,3-butanediol. The protein solution was concentrated to 10 mg ml^{-1}. A droplet consisting of 5 µl of the protein solution and 5 µl of precipitating solution was equilibrated against 0.25 ml of the same precipitating solution. Initial screening for crystallization conditions by the hanging-drop vapour-diffusion method (McPherson, 1982) was carried out using Crystal Screen I (Hampton Research, USA) as precipitants at both 277 and 293 K. As a result, two forms of *meso*-BDH crystals appeared within two weeks (forms I and II). Tetrahedron-shaped crystals of form I (Fig. 1) were obtained from precipitant solution consisting of 20%(*w*/*v*) PEG 8000, 1% 2-mercaptoethanol, 0.1%(*w*/*v*) β-octylglucoside, 200 m*M* magnesium acetate and 1 mg ml^{-1} NAD^+ in 50 m*M* HEPES buffer pH 7.2 at 293 K. Rod-shaped crystals of form II (Fig. 2) were obtained from precipitant solution consisting of 20%(*w*/*v*) PEG 6000, 1% 2-mercaptoethanol, 200 m*M* magnesium acetate, 20%(*w*/*v*) glucose and 1 mg ml^{-1} NAD^+ in 50 m*M* HEPES buffer pH 7.2 at 293 K.

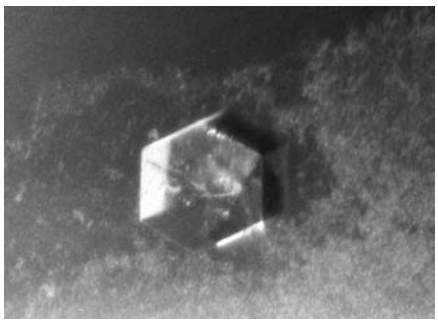

Figure 1
Form I crystal of *meso*-BDH, with dimensions 0.2 × 0.2 × 0.2 mm.

3. X-ray diffraction data collection of *meso*-BDH

X-ray diffraction experiments for form I crystals were performed at 100 K on beamline X25 at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, USA. Diffraction images were recorded on a 2 × 2 CCD detector system. All X-ray data were indexed and integrated using the program *DENZO* and scaled with the program *SCALEPACK* (Otwinowski & Minor, 1997). X-ray data collection for form II crystals was performed on beamline 40B2 at SPring-8, Hyogo, Japan. The crystals were flash-cooled at liquid-nitrogen temperature and were maintained at 100 K. An R-AXIS IV++ detector system (Rigaku) with four imaging plates was used. The X-ray wavelength was adjusted to 0.9000 Å and X-ray diffraction images from a form II crystal were recorded only using one imaging plate in order to avoid systematic errors arising from a sensitivity difference between plates. All diffraction images were recorded as a series of 1° oscillation photographs with 10 s exposure per frame and were processed with the programs *DPS*/*MOSFLM* (Rossmann & van Beek, 1999) and *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

4. Results and discussion

Form I crystals belong to the monoclinic space group *C*2, with unit-cell parameters *a* = 215.5 (2), *b* = 79.4 (1), *c* = 134.8 (2) Å, β = 98.22 (4)° (standard deviations in parentheses were calculated from unit-cell parameters produced by *DENZO*). The allowable V_M value (Matthews, 1968) of 2.68 Å^3 Da^{-1} suggests that eight monomers might be contained in the asymmetric unit (two tetramers). Preliminary crystallographic statistics are shown in Table 1. We calculated self-rotation functions, which gave no significant peaks corresponding to the symmetry of the molecules in the asymmetric unit. Form II crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters *a* = 69.16 (3), *b* = 109.78 (3), *c* = 127.28 (3) Å, β = 102.29 (4)° (standard deviations from *DPS*/*MOSFLM* in parentheses). The merged data set is 100% complete in the resolution range 30–1.7 Å. Assuming eight monomers in the asymmetric unit, the V_M value is 2.22 Å^3 Da^{-1} and the solvent content is 44.6% by volume; these are within the commonly observed range (Matthews, 1968). Preliminary crystallographic statistics are shown in Table 1. The non-crystallographic symmetry of the crystals was explored with self-rotation functions using the program *POLARRFN* (Kabsch, 1976) from the *CCP*4 program suite, which gave significant peaks. The self-rotation revealed three strong twofold axes which were mutually perpendicular, *i.e.* 222 symmetry. The direction of the highest and sharpest peak coincides with that of the crystallographic twofold screw axis. The molecular symmetry of the tetramers is expected to be 222 point symmetry from sequence homology with the other SDRs of known structure. This molecular 222 symmetry is considered to correspond to the 222 symmetry found in the rotation function. The structure analysis of *meso*-BDH is in progress.

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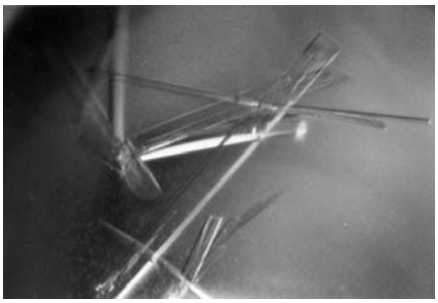

Figure 2
Form II crystals of *meso*-BDH, with typical dimensions 0.1 × 0.1 × 1.0 mm.

and Technology Corporation (ACT-JST) (MK).

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