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Crystallization and preliminary X-ray crystallographic analysis of a carbonyl reductase from *Candida parapsilosis*

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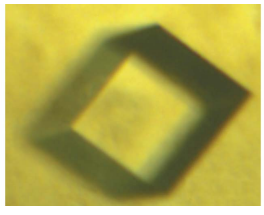


A novel short-chain NADPH-dependent (*S*)-1-phenyl-1,2-ethanediol dehydrogenase (SCR) has been crystallized. Two distinct but related crystal forms of SCR were obtained using the hanging-drop vapour-diffusion method and a reservoir solution consisting of 18%(*w*/*v*) polyethylene glycol 2000 monomethyl ether and 8%(*v*/*v*) 2-propanol as the precipitant. The crystals were rhomboid in shape with average dimensions of 0.3 × 0.3 × 0.4 mm and diffracted to a resolution of 2.7–3.0 Å. The crystal forms both belong to space group $P2_12_12_1$ and have unit-cell parameters a = 104.7, b = 142.8, c = 151.8 Å and a = 101.1, b = 146.0, c = 159.8 Å. The calculated values of V_M, rotation-function and translation-function solutions and consideration of potential crystal packing suggest that there are eight protein subunits per asymmetric unit.

1. Introduction

The NADPH-dependent *S*-specific carbonyl reductase (also known as short-chain carbonyl reductase or SCR) from *Candida parapsilosis* plays an essential role in the reduction of 2-hydroxyacetophenone to produce optically active 1-phenyl-1,2-ethanediol with high productivity and chiral purity (Cao *et al.*, 2006; Fig. 1). The product 1-phenyl-1,2-ethanediol is a versatile chiral building block for the synthesis of numerous pharmaceuticals, agrochemicals, pheromones and liquid-crystal products (Cao *et al.*, 2006; Liese *et al.*, 1996). The protein was identified from the primary microorganism. Its coding gene *scr* has been cloned and overexpressed in *Escherichia coli* and the recombinant enzyme has recently been characterized (Nie *et al.*, 2007).

Sequence analysis suggests that SCR is a member of the short-chain dehydrogenase/reductase (SDR) family, the three-dimensional structures of a number of members of which have been determined, including those of *R*-specific alcohol dehydrogenase from *Lactobacillus brevis* (RADH; Schlieben *et al.*, 2005), mannitol-2-dehydrogenase from *Agaricus bisporus* (MtDH; Horer *et al.*, 2001), *Drosophila lebanonensis* alcohol dehydrogenase (DLADH; Benach *et al.*, 1998) and human liver dehydrogenase (HLADH; Eklund *et al.*, 1990). SCR shares a high degree of similarity (about 35%) to MtDH and RADH. The SDR-family members possess a Ser-Tyr-Lys triad, an N-terminal TG*XXX*G*X*G motif and a characteristic Rossmann-fold structure that is essential for the catalytic mechanism (Oppermann *et al.*, 2003; Persson *et al.*, 2003). The known enzymes from a range of species provided initial indications and subsequent details of the catalytic mechanism. Determining the three-dimensional structure of SCR would greatly improve our understanding of the substrate

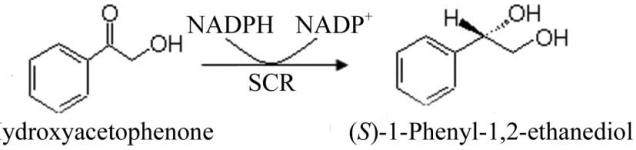


Figure 1
The stereoselective reduction of 2-hydroxyacetophenone to (*S*)-1-phenyl-1,2-ethanediol by SCR.

specificity and catalytic mechanism of the SDR family. Towards this goal, we have now crystallized the recombinant SCR and performed a preliminary X-ray crystallographic study of its structure. In this work, we describe two crystal forms of native SCR.

2. Materials and methods

2.1. Microorganisms and chemicals

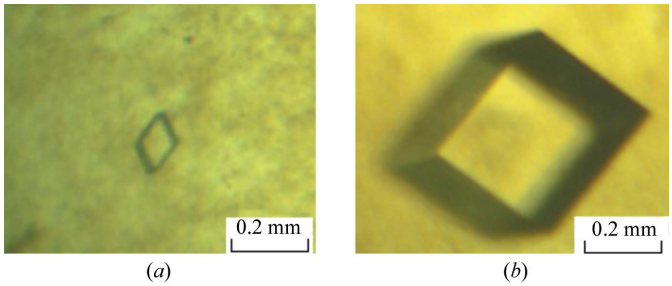
C. parapsilosis strain CCTCC M203011 was obtained from American Type Culture Collection (ATCC, USA). The organism was cultivated as described previously (Nie *et al.*, 2007). The enzymes, vectors, marker DNA, oligonucleotides and other reagents for DNA cloning and amplification were from Takara Bio Co. Ltd, Japan and Novagen Co., USA.

2.2. Expression and purification

The coding region for the SCR cDNA (accession No. DQ675534) was cloned into the expression vector pET21 linearized with *Bam*HI and *Xho*I and expressed as His_6-tagged protein in *E. coli* strain BL21 (DE3) as previously described by Nie *et al.* (2007). The recombinant protein was purified using three procedures. Firstly, it was purified by affinity chromatography on an Ni^{2+} Sepharose column (His-Trap Kit, Pharmacia). The pooled fractions were then loaded onto a Resource Q column (1 × 1 cm) equilibrated with 20 m*M* Tris–HCl pH 8.5 buffer using an ÄKTA Protein Purifier system (Pharmacia, Uppsala, Sweden). This was followed by Superdex 200 (HiLoad 26/60, preparation grade) chromatography in buffer containing 20 m*M* Tris–HCl pH 8.5 and 150 m*M* NaCl. The enzyme was purified to homogeneity as judged by Coomassie Brilliant Blue staining of SDS–PAGE gels.

2.3. Crystallization and data collection

Initial crystallization conditions were screened by the hanging-drop vapour-diffusion method using commercial crystallization screening kits (Crystal Screen 1) from Hampton Research (USA). The crystals of apo-SCR used for diffraction were grown in a buffer containing 18%(*w*/*v*) polyethylene glycol 2000 monomethyl ether (PEG 2K MME) and 8%(*v*/*v*) 2-propanol pH 8.5. A droplet was prepared by mixing equal volumes (1.0 + 1.0 µl) of a protein solution containing 20 mg ml^{-1} SCR in a buffer consisting of 20 m*M* Tris–HCl pH 8.5 and 150 m*M* NaCl and reservoir solution. After 5 d growth, single crystals of approximate dimensions 0.3 × 0.3 × 0.4 mm were soaked in a cryoprotectant solution consisting of 24%(*w*/*v*) PEG 2K MME and 6%(*w*/*v*) sucrose prior to data collection. Diffraction data were collected on beamline BL5A at the Photon Factory synchrotron facility (KEK, Tsukuba, Japan) and were processed with the *HKL*-2000 program package (Otwinowski & Minor, 1997).



(*a*) (*b*)

Figure 2
Crystals of SCR grown using (*a*) crystallization condition No. 41 from Hampton Crystal Screen 1 and (*b*) optimized conditions. The latter was used for diffraction experiments and data collection and had maximum dimensions of 0.3 × 0.3 × 0.4 mm.

Table 1
Crystallographic data-collection statistics.

Values in parentheses are for the highest resolution shell.

	Form 1	Form 2
Wavelength (Å)	1.0	1.0
Temperature (K)	100	100
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 104.7, b = 142.8, c = 151.8	a = 101.1, b = 146.0, c = 159.8
Matthews coefficient (Å^3 Da^{-1})	2.4	2.5
Solvent content (%)	48	50
No. of molecules in ASU	8	8
Resolution (Å)	41.7–2.7 (2.9–2.7)	48.2–3.0 (3.2–3.0)
Observed reflections	817392	3827584
Unique reflections	65214	77121
Redundancy	7.3 (7.4)	6.6 (6.7)
Completeness (%)	99.9 (100.0)	80.3 (100.0)
R_{merge}† (%)	14.2 (31.7)	12. 6 (30.5)
Average $I/\sigma(I)$	11.2 (6.6)	23.7 (3.5)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ are the observed intensity and the mean intensity of related reflections, respectively.

3. Results and discussion

Recombinant SCR protein was concentrated to 10–25 mg ml^{-1} in a buffer containing 20 m*M* Tris–HCl pH 8.0 and 150 m*M* NaCl and centrifuged at 14 000 rev min^{-1} for 15 min prior to crystallization. Crystals were initially obtained by the hanging-drop method using crystallization condition No. 41 from Crystal Screen I (Hampton Research, USA; Fig. 2*a*). An incomplete factorial screen was set up using six pH values (6.5, 7.0, 7.5, 8.0, 8.5 and 9.0), four concentrations of PEG 2K MME [16, 18, 20 and 25%(*w*/*v*)] and three concentrations of 2-propanol [8, 10 and 12%(*v*/*v*)]. Crystals of diffraction quality with typical dimensions of 0.3 × 0.3 × 0.4 nm were harvested after 5 d growth from drops containing 20 mg ml^{-1} protein and reservoir solution (200 µl volume) consisting of 18%(*w*/*v*) PEG 2K MME and 8%(*v*/*v*) 2-propanol pH 8.5 (Fig. 2*b*). Since the crystals showed signs of radiation damage after irradiation for a few hours at room temperature, it was essential to find an effective cryoprotectant solution in order to collect a usable data set from a single crystal. Among other things, we tried to add glycerol to the mother liquor 5%(*v*/*v*) at a time until it reached 25%(*v*/*v*) concentration. However, its effect on diffraction was detrimental. A good cryoprotectant solution was found by adding 6%(*w*/*v*) sucrose to the 24%(*w*/*v*) PEG 2K MME solution. A home-made fine loop of eye-surgery thread was used to fish the crystal out of the cryoprotectant solution. A native data set was collected from a single flash-frozen crystal at 100 K. Diffraction data were collected on beamline BL5A of the Photon Factory synchrotron facility (KEK, Tsukuba, Japan) using a 1 Å wavelength radiation source and were processed using the *HKL*-2000 program package (Otwinowski & Minor, 1997). Two crystal forms were identified. They both belonged to the orthorhombic space group $P2_12_12_1$, with distinct but related unit-cell parameters: a = 104.7, b = 142.8, c = 151.8 Å for the first form and a = 101.1, b = 146.0, c = 159.8 Å for the second form. Statistics of data collection and processing to 2.7 and 3.0 Å resolution, respectively, are summarized in Table 1. The Matthews coefficient V_M (Matthews, 1968) was calculated to be 2.4 and 2.5 Å^3 Da^{-1}, respectively, assuming the possible presence of eight molecules in the asymmetric unit. The solvent contents of the crystals were approximately 48% and 50%,

respectively. The overall merging *R* factor on intensities for the two crystal forms were 14.2 and 12.6%, respectively, with excellent completeness and signal-to-noise ratio. Structure solution by the molecular-replacement (MR) method should be feasible as homologous structures that share more than 30% similarity are available. At this stage, a polyalanine model of SCR based on MtDH (Horer *et al.*, 2001) has been created and used in molecular-replacement attempts. Molecular model building and refinement are in progress. The current *R* factors of the two crystal forms are 31.0 and 40.6% for all reflections in the resolution ranges 41.7–2.7 Å and 48.2–3.0 Å, respectively. Other crystal forms of this enzyme were also obtained and are currently being investigated.

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