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Crystallization and preliminary X-ray analysis of an *R*-2-hydroxypropyl-coenzyme M dehydrogenase

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The *R*-2-hydroxypropyl-coenzyme M (2-mercaptoethanesulfonate) dehydrogenase is a key enzyme in the microbial conversion of propylene to the central metabolite acetoacetate. This enzyme is an interesting member of the NAD(P)H-dependent short-chain dehydrogenase/reductase (SDR) family of enzymes, being one of a pair of homologous dehydrogenases that act in concert in a single pathway to convert the *R*- and *S*-enantiomers of hydroxypropyl-coenzyme M to the achiral ketopropyl-coenzyme M product. Crystallization trials have revealed that the highest diffraction quality crystals (better than 2.0 Å resolution) could be achieved when the reaction substrates were added to the enzyme in a stoichiometric excess prior to crystallization.

1. Introduction

The aerobic microorganism *Xanthobacter* strain Py2 is one of several bacteria capable of utilizing short-chain aliphatic alkenes as a sole source of carbon and energy (van Ginkel & de Bont, 1986; Furuhashi, 1986). The pathways for the bacterial metabolism of propylene have been extensively studied in both *Xanthobacter autotrophicus* strain Py2 and actinomycete *Rhodococcus rhodochrous* strain B276 (Ensign *et al.*, 1998; Ensign, 2001; Small *et al.*, 1995; Allen & Ensign, 1998). The conversion of propylene to an enantiomeric excess of *R*-epoxypropane is catalyzed by a di-Fe-containing alkene monoxygenase (Small & Ensign, 1997). The subsequent conversion of epoxypropane to acetoacetate occurs by a three-step pathway consisting of four enzyme components (Allen *et al.*, 1999; Ensign, 2001). The first step results in the conjugation of coenzyme M (CoM) to *R*- or *S*-epoxypropane, forming the corresponding enantiomer of 2-hydroxypropyl-CoM. In the next step, two stereospecific short-chain dehydrogenases catalyze the oxidation of the individual enantiomers of 2-hydroxypropyl-CoM, yielding the common intermediate 2-ketopropyl-CoM (Fig. 1*a*) (Allen & Ensign, 1999). The final step in the pathway is conversion of 2-ketopropyl-CoM to acetoacetate, with concomitant regeneration of free CoM (Clark *et al.*, 2000).

The *R*- and *S*-2-hydroxypropyl-CoM dehydrogenases (*R*- and *S*-HPCDH) are highly specific for the enantiomers of 2-hydroxypropyl-CoM, exhibiting only 0.5–1% activity with the opposing enantiomer (Allen & Ensign, 1999). They exist as dimers with subunit molecular weights of 25 400 and 26 000 Da, respectively. Amino-acid sequence comparisons have revealed that these enzymes belong to the short-chain dehydrogenase/reductase (SDR) family of enzymes (Clark & Ensign, 2002; Ensign, 2001; Allen *et al.*, 1999; Swaving *et al.*, 1995). To date, more than 60 NAD^+- or $NADP^+$-dependent enzymes have been reported. Dehydrogenases in this class are approximately 250 amino acids in length and exist as dimers or tetramers in solution (Persson *et al.*, 1991; Jornvall *et al.*, 1995). The enzymes have been divided into three distinct domains: a conserved N-terminal nucleotide-binding domain, a central domain containing a conserved catalytic triad of Ser, Tyr and Lys and a C-terminal domain that confers substrate specificity. The culmination of structural, kinetic and mechanistic studies on SDR enzymes has resulted in the formulation of a general mechanism of action. Catalysis involves the deprotonation of a Tyr hydroxyl, which then serves as a general base for proton abstraction from the hydroxyl group of the substrate. The deprotonated Tyr general base is then stabilized by the amino group of the Lys side chain. The role of the Ser residue is presumably to increase the acidity of the substrate hydroxyl through hydrogen bonding.

Although the dehydrogenases are in many cases stereospecific, there is only one other case known in which pairs of stereospecific dehydrogenases act in concert in one pathway. Tropinone reductase I and II from *Datura stramonium* (TR-I and TR-II) catalyze the reversible stereospecific reduction of the 3-keto group of tropinone to form the enantiomers tropine (α-hydroxy) and pseudotropine (β-hydroxy), respectively. Structural characterization of TR-I and TR-II has revealed that electrostatic interactions

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between substrates and charged amino acids in the active site are responsible for orienting the substrate into its correct geometry (Nakajima *et al.*, 1993, 1994, 1998). A similar mechanism has been proposed for the binding and oxidation of *R*- and *S*-2-hydroxypropyl-CoM by their respective dehydrogenases based on amino-acid sequence comparisons (Ensign, 2001; Allen *et al.*, 1999). Presumably, differences in the C-terminal domains of the two dehydrogenases are responsible for imparting specificity for the proper substrate and a comparison of the two indicates similar sequence topologies interspersed with a number of notable differences. In three positions, a negatively charged Asp or Glu residue occurs in one of the dehydrogenases at the same position as a positively charged residue Arg or Lys occurs in the other dehydrogenase (Fig. 1*b*). An attraction/repulsion interaction of charged side chains with the negatively charged sulfonate moiety of CoM may provide the means for chiral discrimination. In order to test the hypothesis concerning the mechanism of enantiomeric selectivity in the 2-hydroxypropyl-CoM dehydrogenases, we will determine the structures of the dehydrogenases in the presence of substrates and/or products and in the presence of the inhibitor methylhydroxypropyl CoM (Fig. 1*c*).

2. Crystallization of *R*-2-hydroxypropyl-CoM dehydrogenase

For crystallization, purified recombinant *R*-2-hydroxypropyl-CoM dehydrogenase (*R*-HPCDH) was obtained as described previously (Clark & Ensign, 2002). Using a sparse-matrix crystallization screen (Jancarik & Kim, 1991; Garman & Mitchell, 1996), conditions have been identified that yield diffraction-quality crystals of *R*-HPCDH. Crystals were grown by the hanging-drop vapor-diffusion method at 294 K using 0.1 *M* sodium acetate pH 4.6, 2.0 *M* sodium formate, with equal volumes of protein and precipitating solution. Colorless needle-shaped crystals or very thin plates (0.8–1.2 × <0.1 × <0.1 mm) grew in about one week. Crystals of *R*-HPCDH belong to space group *C*2 (Fig. 2*a*). Although the crystals diffract reasonably well, we have experienced difficulties in identifying the optimal conditions for cryo-cooling for low-temperature data collection such that data to only 3 Å resolution could be collected (Garman & Mitchell, 1996; Petsko, 1975). Crystals of markedly better diffraction quality were obtained using the vapor-diffusion method when the *R*-HPCDH was incubated with NAD^+ and either *R*-2-hydroxypropyl-CoM or *S*-2-hydroxypropyl-CoM (Figs. 2*b* and 2*c*). Substrate binding in the case of these enzymes occurs sequentially, with the binding of NAD^+ required for high-affinity substrate binding. Hanging drops were prepared by mixing the protein solution (*R*-HPCDH with addition of 15 m*M* NAD^+ and 20 m*M* *R*- or *S*-2-hydroxypropyl-CoM) and reservoir solution [0.2 *M* magnesium chloride, 0.1 *M* HEPES pH 7.5, 30%(*w*/*v*) PEG 400 and 0.1 *M* spermine.4HCl] in a 2:1 ratio. Crystals appeared after 3–4 weeks

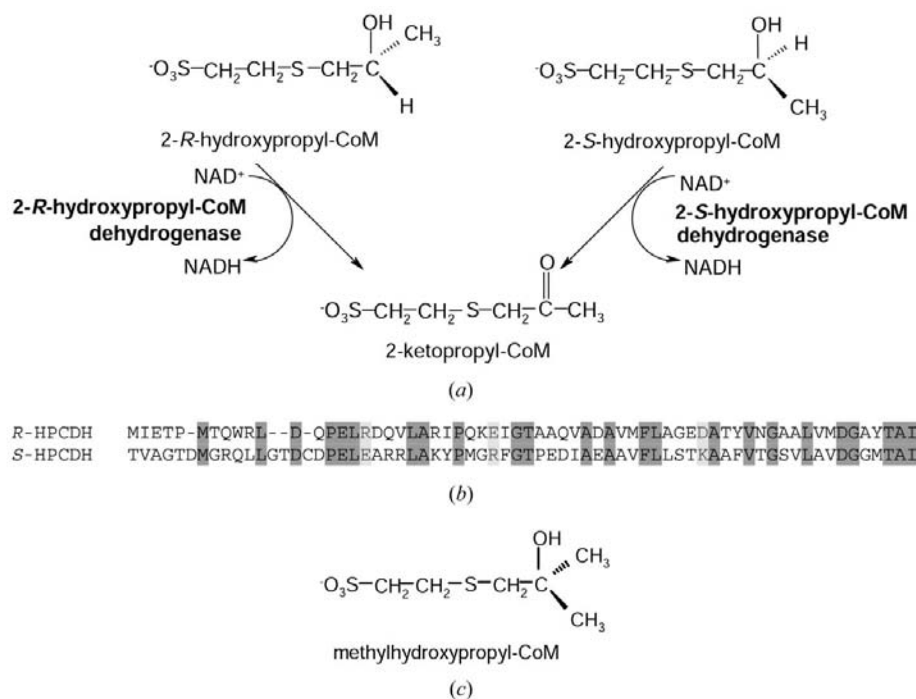
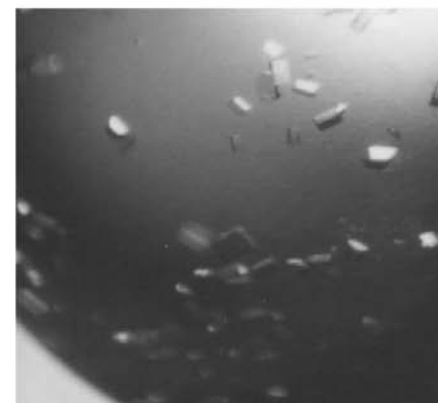


Figure 1
(*a*) The reaction of the *R*- and *S*-HPCDHs in the pathway of epoxide metabolism in the microorganism *Xanthobacter* strain Py2. (*b*) The deduced amino-acid sequence alignment of the C-termini of the *R*- and *S*-HPCDHs, with non-identities differing by charge highlighted in yellow. (*c*) The structure of methylhydroxypropyl-CoM.

(*a*)


(*b*)

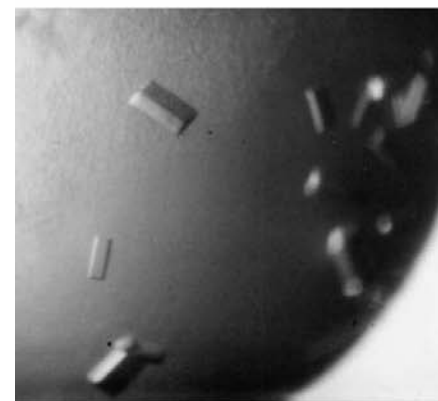

(*c*)

Figure 2
Crystals of *R*-2-hydoxypropyl-CoM dehydrogenase (*a*) in the native form, (*b*) in the presence of NAD^+ and *R*-hydroxypropyl-CoM and (*c*) in the presence of NAD^+ and *S*-hydroxypropyl-CoM.

Table 1
R-HPCDH data statistics.

Values in parentheses indicate values for the highest resolution bin.

Crystals	*R*-HPCDH†‡	*R*-HPCDH† + *R*-HP-CoM§ + NAD^+¶	*R*-HPCDH† + *S*-HP-CoM†† + NAD^+¶
Space group	*C*2	$P2_1$	$P2_1$
Unit-cell parameters			
a (Å)	69.00	64.55	64.44
b (Å)	104.13	108.76	110.28
c (Å)	128.90	68.32	68.98
α (°)	90.00	90.00	90.00
β (°)	92.29	93.75	93.88
γ (°)	90.00	90.00	90.00
Resolution (Å)	20.0–3.0	20.00–1.98	20.00–1.90
Completeness (%)	96.7 (70.2)	94.5 (70.2)	93.9 (95.2)
Observed reflections	45402	133917	149.113
Unique reflections	14825	62112	71201
$I/\sigma(I)$	4.6 (3.7)	9.0 (2.4)	6.5 (2.1)
$R_{\rm merge}$‡‡ (%)	12.2 (18.6)	9.3 (32.7)	8.4 (30.3)

† *R*-HPCDH = *R*-hydroxypropyl-CoM dehydrogenase. § *R*-HP-CoM = *R*-hydroxypropyl-CoM. †† *S*-HP-CoM = *S*-hydroxypropyl-CoM. ‡ Data were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1991). ¶ Data were processed with *MOSFLM* and scaled with *SCALA* (Collaborative Computational Project, Number 4, 1994; Leslie, 1992). ‡‡ $R_{\rm merge} = \sum_{hkl}\sum_i |I_i - \langle I\rangle| / \sum_{hkl}\sum_i |\langle I\rangle|$, where I_i is the intensity for the *i*th measurement of an equivalent reflection with indices *hkl*.

incubation at 294 K. The crystals of *R*-HPCDH with either *R*-2-hydroxypropyl-CoM or *S*-2-hydroxypropyl-CoM are identical in appearance. The crystals grow as colorless rectangular blocks (0.3–0.6 × ~0.2 × ~0.2 mm) and belong to the monoclinic space group $P2_1$.

3. Data collection and analysis

Data to 2.5 Å resolution were collected on our home Cu $K\alpha$ source and data to better than 2 Å resolution were recently collected at the Stanford Synchrotron Radiation Laboratory (Table 1). For data collection, the crystals were flash-frozen on rayon loops in a liquid-nitrogen bath and during data collection the crystals were maintained at 98–108 K. Diffraction data were collected using a Rigaku 3HR (Rigaku, Tokyo, Japan) X-ray generator with a copper-anode source and an R-AXIS IIc imaging-plate area detector (Molecular Structure Corporation, The Woodlands, Texas, USA). Synchrotron data were collected at SSRL beamline 9-1 equipped with a MAR345 imaging-plate detector (marUSA Inc., Illinois, USA). In the case of *R*-2-hydroxypropyl-CoM, the natural substrate for this enzyme, we would anticipate that the enzyme should turnover to form the 2-ketopropyl-CoM product in the presence of NAD^+. Very slow rates of turnover are observed for the *R*-HPCDH-catalyzed dehydrogenation of *S*-2-hydroxypropyl-CoM and it is unclear at this time whether we will observe a substrate-bound or product-bound state with these crystals. We are currently attempting to grow crystals with the product and NAD^+, but we have not yet been able to obtain good diffraction-quality crystals. Since the prospects of obtaining a non-productive complex of substrates and NADH are unlikely owing to the instability of NADH in aqueous solvents, we will utilize the inhibitor methyl-hydroxypropyl CoM (Fig. 1*c*) to approximate the substrate-binding mode. This achiral inhibitor has a methyl group substituted for the hydrogen that undergoes abstraction (Clark & Ensign, 2002). We have been able to obtain crystals that are similar in appearance to the crystals grown in the presence of substrates by substituting the inhibitor for substrate in the crystallization conditions described above. We are currently assessing the diffraction quality of these crystals.

We have initiated the structure determination of *R*-HPCDH. As mentioned previously, there is considerable sequence similarity exhibited between the dehydrogenases and other members of the SDR class. In addition to the tropinone reductases described above, there are numerous examples of the class that have been structurally characterized. The members of the SDR class for which the structures have been determined exhibit a standard nucleotide-binding fold (Rossmann-type fold) and there is significant structural conservation in comparing members of the class (Jornvall *et al.*, 1995; Persson *et al.*, 1991). The overall fold of these enzymes consists largely of an open-twisted β-sheet surrounded by α-helices. We are attempting to exploit the structural conservation of the enzymes in the class to determine the structure of *R*-HPCDH. There are a number of structures available for proteins that exhibit greater than ~30% amino-acid sequence identity with *R*-HPCDH, including the tropinone reductase and alcohol dehydrogenase (Jornvall *et al.*, 1995; Persson *et al.*, 1991). For molecular-replacement searches, the search models will be truncated to eliminate the substrate-binding region, where significant sequence divergence is observed. Calculation of the Matthews coefficient (Matthews, 1968) of the two crystal forms indicates that two dimers of *R*-HPCDH should be present in the asymmetric unit to yield a solvent content that approaches 50%. We will use both crystal forms (*C*2 and $P2_1$) for molecular-replacement searches.

The structure of *R*-HPCDH in the presence of substrates, products and/or inhibitors will provide the basis for rationalizing chiral specificity in *R*-HPCDH. Since *R*-HPCDH and *S*-HPCDH share sequence identity in the C-terminal substrate-binding region, we may be able to generate a homology model for the *S*-HPCDH based on the structure of the *R*-HPCDH. Since we believe the mechanism involves the substitution of a few key charged residues within a region that exhibits significant sequence identity in comparing the two enzymes, this approach is likely to provide a basis for rationalization of the mechanism of chiral discrimination utilized by these dehydrogenases.

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