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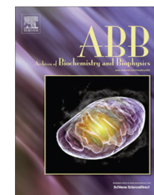

Crystal structures of *S*-HPCDH reveal determinants of stereospecificity for *R*- and *S*-hydroxypropyl-coenzyme M dehydrogenases

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ABSTRACT

(*R*)- and (*S*)-hydroxypropyl-coenzyme M dehydrogenases (*R*- and *S*-HPCDH) are stereospecific enzymes that are central to the metabolism of propylene and epoxide in *Xanthobacter autotrophicus*. The bacterium produces *R*- and *S*-HPCDH simultaneously to facilitate transformation of *R*- and *S*-enantiomers of epoxypropane to a common achiral product 2-ketopropyl-CoM (2-KPC). Both *R*- and *S*-HPCDH are highly specific for their respective substrates as each enzyme displays less than 0.5% activity with the opposite substrate isomer. In order to elucidate the structural basis for stereospecificity displayed by *R*- and *S*-HPCDH we have determined substrate bound crystal structures of *S*-HPCDH to 1.6 Å resolution. Comparisons to the previously reported product-bound structure of *R*-HPCDH reveal that although the placement of catalytic residues within the active site of each enzyme is nearly identical, structural differences in the surrounding area provide each enzyme with a distinct substrate binding pocket. These structures demonstrate how chiral discrimination by *R*- and *S*-HPCDH results from alternative binding of the distal end of substrates within each substrate binding pocket.

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Introduction

Propylene metabolism in the soil bacterium *Xanthobacter autotrophicus* strain Py2 involves the conversion of chiral epoxides to acetoactetate [1–4]. The initial steps in the pathway involve the epoxidation of propylene followed by nucleophilic addition of the small cofactor coenzyme M to the *R*- and *S*-enantiomers of epoxypropane, resulting in a racemic mixture of *R*- and *S*-hydroxypropyl CoM (*R*- and *S*-HPC, respectively). *R*- and *S*-HPC are then converted into a common achiral product 2-ketopropyl-CoM (2-KPC)[1] using two highly stereospecific dehydrogenases, (*R*)-hydroxypropyl-coenzyme M dehydrogenase (*R*-HPCDH) and (*S*)-hydroxypropyl-coenzyme M dehydrogenase (*S*-HPCDH) (Fig. 1). A remarkable feature of *R*- and *S*-HPCDH (40% sequence identity) is the extremely high substrate stereospecificity exhibited by these enzymes (each enzyme displays less than 0.5% activity with the opposite substrate isomer [2]), although each enzyme catalyzes the same chemical reaction.

R- and *S*-HPCDH have been characterized as members of the "classical" short-chain dehydrogenase/reductase (SDR) superfamily of enzymes. SDR enzymes are found throughout eukaryotic and prokaryotic species and constitute one of the largest enzyme families known with over 60,000 members annotated in sequence databases [5]. SDR enzymes are categorized in several enzyme classes including lyases, isomerases, and oxidoreductases, and they act on a broad range of substrates including steroids, sugars, xenobiotics, and aliphatic alcohols [5–7]. Despite the relatively low sequence identity between SDR enzymes (15–30% sequence identity), structural studies have revealed a common scaffold that includes N- and C- terminal domains [8]. The N-terminal domain is a highly conserved Rossmann-fold structure that contains a catalytic triad/tetrad (Tyr–Lys–Ser–Asn in the case of *R*- and *S*-HPCDH) and a GxxxGxG motif involved in NAD(P)(H) binding . The C-terminal domain is more variable, both in terms of sequence and structure, but generally contains a substrate binding loop region that is involved in substrate binding and specificity [8–11]. Although numerous SDR superfamily enzymes exhibit stereospecificity, few stereospecific enzymes have been found to function in concert with a homologous partner-enzyme. *R*- and *S*-HPCDH represent an ideal model system to examine stereospecificity because they perform identical chemistry in the same pathway, yet both enzymes are highly specific for their respective substrates (*R*-HPC or *S*-HPC).

Several biochemical studies, and a product bound structure of *R*-HPCDH has led to a proposed mechanism for *R*- and *S*-HPCDH [12–16]. In this mechanism, the role of the catalytic tetrad residues (Tyr–Lys–Ser–Asn) and the chemistry around the chiral carbon (C2) is analogous to the general mechanism described for other SDR enzymes [6,17]. Specifically, the catalytic tetrad serine assists in positioning the hydroxyl group of the substrate near the

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[1] *Abbreviations used:* 2-KPC, 2-ketopropyl-CoM; *R*-HPCDH, (*R*)-hydroxypropyl-coenzyme M dehydrogenase; *S*-HPCDH, (*S*)-hydroxypropyl-coenzyme M dehydrogenase; SDR, short-chain dehydrogenase/reductase.

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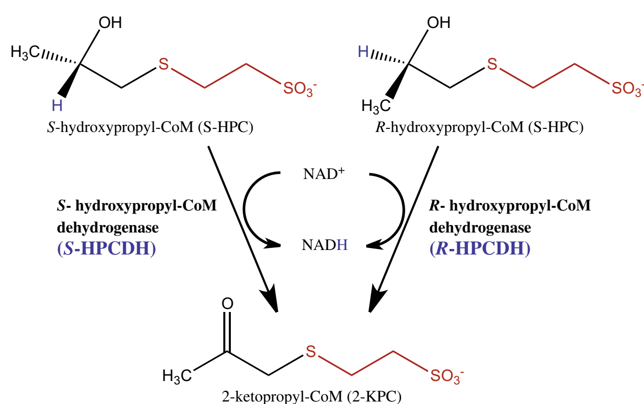

Fig. 1. *R*-HPCDH and *S*-HPCDH convert *R*-and *S*-hydroxypropyl CoM (*R*- and *S*-HPCDH) to a common product 2-ketopropyl-CoM (2-KPC).

catalytic tyrosine. The tyrosine residue in its deprotonated form acts as a general base for proton abstraction from the substrate hydroxyl group, and a hydride is transferred from the C2 carbon of the substrate to NAD^+. Lysine has a dual role in coordinating NAD(H) and in lowering the pK_a of the catalytic tyrosine hydroxyl group through a proton relay involving water molecules and the catalytic tetrad asparagine. The product bound structure of *R*-HPCDH reveals a pair of positively charged amino acids (R152, R196) that coordinate the sulfonate tail of 2-KPC [16]. Kinetic analysis of *S*-HPCDH mutants suggest that positively charged amino acids (R211 and K214) may also contribute to sulfonate binding in the *S*-enzyme [15]. It has been proposed that in each enzyme the sulfonate binding residues are used to properly orient the reactive groups of the substrate at the catalytic site [13,15,16]. Although a crystal structure of *R*-HPCDH is available, there are no structures of *S*-HPCDH. Furthermore, no substrate bound structures of *R*- or *S*-HPCDH are currently available. Here we report high-resolution binary (NAD^+ bound) and ternary (*S*-HPC/NADH bound) complex crystal structures of *S*-HPCDH. These structures provide insight into the structural architecture of the active site and substrate binding path of *S*-HPCDH. In addition, the structures allow for the first structural comparison between *S*-HPCDH and *R*-HPCDH, clarifying structural mechanisms of stereospecificity employed by these enzymes.

Materials and methods

Expression, purification, and crystallization

Three sequence variants of *S*-HPCDH exist on a single megaplasmid in *X. autotrophicus* strain Py2 [18]. The most well behaved *S*-HPCDH variant *in vitro* (previously designated as *S*-HPCDH3) was expressed and purified in *Escherichia coli* as previously described [15]. Protein was concentrated to 13 mg/ml in the presence of 25% glycerol and stored at −80 °C. Protein was thawed on ice and crystallized by sitting drop vapor diffusion at 4 °C at a 1:1 protein:well drop ratio in a well solution consisting of 0.1 M Bis–Tris, pH 6.5, 0.35 M ammonium acetate and 27% polyethylene glycol 3350. Crystals of *S*-HPCDH bound to NAD^+ (binary complex) were grown in the presence of 50 μM NAD^+ and 50 μM *S*-HPC. The crystal was transferred directly into a cryoprotectant composed of the mother liquor, 50 μM *S*-HPC and 10% glycerol, and flash-cooled in liquid nitrogen. *S*-HPC/NADH bound crystals (ternary complex) were grown in the presence of 50 μM NADH and 50 μM *S*-HPC. The crystal was transferred directly into a cryoprotectant composed of the mother liquor without ammonium acetate, 140 μM *S*-HPC and 10% glycerol, and flash-cooled in liquid nitrogen.

Data collection

Diffraction data were generated and collected using a home-source X-ray generator (Rigaku RU-200 and MicroMax-007HF) and detector (Rigaku R-AXIS IV++). Data were processed using the HKL2000 program suite [19]. The *S*-HPCDH crystals belong to space group $P2_12_12$ with unit cell dimensions of a = 116 Å, b = 128 Å, and c = 58 Å. Four monomers (a single tetramer) are in the asymmetric unit. Data were collected to 1.6 Å for each structure. Data statistics are summarized in Table 1.

Structure determination, model building and refinement

The binary complex *S*-HPCDH structure was solved by molecular replacement using the *R*-HPCDH structure ([16], PDB code, 2cfc) as a molecular replacement search model. The ternary complex structure was solved by molecular replacement using the refined binary complex as a search model. Molecular replacement was performed using Phaser [20] from the Phenix program suite [21].

Manual building of the *S*-HPCDH structures was done using the program COOT [22]. Electron density maps were high in quality, permitting unambiguous identification and positioning of the majority of amino acids in each monomer of asymmetric unit. Discontinuous electron density prohibited modeling of the first two N-terminal amino acids in each monomer, and a loop region beginning at position 200 and extending to positions 204–207, depending on the monomer. The one exception is one monomer in the ternary complex structure, in which the entire loop region is

Table 1
Data collection and refinement statistics for *S*-HPCDH structures. Values in parentheses correspond to those in the outer resolution shell.

S-HPCDH structure	Binary complex	Ternary complex
Data collection		
Wavelength (Å)	1.5418	1.5418
Resolution range (Å)	35.0–1.60	35.0–1.60
Outer shell (Å)	1.66–1.60	1.66–1.60
No. of reflections		
Unique	113,574	108,385
Total	708,549	1,179,096
Average redundancy	6.2 (2.8)	10.9 (7.9)
Mean $I/\sigma(I)$	25.8 (1.9)	37.0 (3.3)
Completeness (%)	98.3 (86.0)	93.7 (63.9)
R_{sym} (%)[a]	6.3 (38.9)	5.7 (37.4)
Space group	$P2_12_12$	$P2_12_12$
# of protein molecules/asym. unit	4	4
Unit cell dimensions		
a, b, c (Å)	116.1, 127.9, 58.5	116.0, 128.4, 58.4
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Refinement		
R_{work}/R_{free} (%)[b]	17.1/19.9	16.4/19.5
Atoms in the structure	8,114	8,098
Protein	6,895	6,977
Waters	1027	931
Ligands	192	220
Average B factor (Å^2)	13.8	14.3
Protein	12.5	13.2
Water	22.5	23.4
NAD^+/NADH	11.0	11.0
S-HPC	–	22.4
rmsd bond (Å)/angle (°)	0.008/1.104	0.007/1.114
Protein geometry[c]		
Ramachandran outliers (%)	0	0
Ramachandran favored (%)	98.9	98.1
Rotamer outliers (%)	0	0.9
PDB ID	4GH5	4ITU

[a] $R_{sym} = (\Sigma|(I-\langle I\rangle)|)/(\Sigma I)$, where $\langle I\rangle$ is the average intensity of multiple measurements.
[b] $R_{work} = (\Sigma|F_{obs}-F_{calc}|)/(\Sigma|F_{obs}|)$ and is calculated using all data; R_{free} is the R-factor based on 5% of the data excluded from refinement.
[c] Ramachandran statistics were calculated using the MolProbity server [24,25].

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