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Crystal structure of D-serine dehydratase from *Escherichia coli*

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ABSTRACT

D-Serine dehydratase from *Escherichia coli* is a member of the β-family (fold-type II) of the pyridoxal 5′-phosphate-dependent enzymes, catalyzing the conversion of D-serine to pyruvate and ammonia. The crystal structure of monomeric D-serine dehydratase has been solved to 1.97 Å-resolution for an orthorhombic data set by molecular replacement. In addition, the structure was refined in a monoclinic data set to 1.55 Å resolution. The structure of DSD reveals a larger pyridoxal 5′-phosphate-binding domain and a smaller domain. The active site of DSD is very similar to those of the other members of the β-family. Lys118 forms the Schiff base to PLP, the cofactor phosphate group is liganded to a tetraglycine cluster Gly279–Gly283, and the 3-hydroxyl group of PLP is liganded to Asn170 and N1 to Thr424, respectively. In the closed conformation the movement of the small domain blocks the entrance to active site of DSD. The domain movement plays an important role in the formation of the substrate recognition site and the catalysis of the enzyme. Modeling of D-serine into the active site of DSD suggests that the hydroxyl group of D-serine is coordinated to the carboxyl group of Asp238. The carboxyl oxygen of D-serine is coordinated to the hydroxyl group of Ser167 and the amide group of Leu171 (O1), whereas the O2 of the carboxyl group of D-serine is hydrogen-bonded to the hydroxyl group of Ser167 and the amide group of Thr168. A catalytic mechanism very similar to that proposed for L-serine dehydratase is discussed.

1. Introduction

D-Serine dehydratase (EC 4.2.1.18) (DSD) from *Escherichia coli* strain K12 catalyzes the degradation of D-serine to pyruvate and ammonia (Scheme 1) [1]. D-Serine is a competitive antagonist of β-alanine in the biosynthetic pathway of pantothenate and coenzyme A [2]. Therefore, DSD appears to serve as a detoxifying enzyme in most *E. coli* strains.

To our knowledge, DSD is the only PLP-dependent enzyme with a monomeric quaternary structure. D-Serine dehydratase consists of a single polypeptide chain (M_r 47,901) and has one catalytically essential pyridoxal 5′-phosphate (PLP) per protein molecule ($K_d = 0.03$ μM) [1]. A second low affinity-binding site for the cofactor has also been reported ($K_d \sim 1$ mM) [3]. D-Serine dehydratase is a member of the β-family of the PLP-dependent enzymes and belongs to the fold-type II family [4].

Even though DSD crystallizes readily, the microcrystals initially obtained were not suitable for structure determination due to unfavorable zwilling formation. Single crystals of DSD in complex with 3-amino-2-hydroxyproprionate were obtained to determine the space group [5].

Here, we report two three-dimensional structures of the holoform of DSD as determined by X-ray crystallography to a resolution of 1.97 Å and 1.55 Å, respectively. It is the first crystallographic structure of the holo form of the fold-type II DSD. The crystal structure of D-serine dehydratase from chicken kidney was reported by Tanaka and coworkers [6] instead presents a zinc-dependent enzyme similar

Abbreviations: DSD, D-serine dehydratase; PLP, pyridoxal 5′-phosphate; LSD, rat liver L-serine dehydratase; OASS, O-acetylserine sulfhydrylase; TDH, L-threonine dehydratase; SR, serine racemase; TPS, tryptophan synthase; CBS, cysteine β-synthase; NMDA, N-methyl-D-aspartate; DTE, dithioerythritol; TAPS, N-Tris(hydroxymethyl) methyl-3-propane sulfonic acid.

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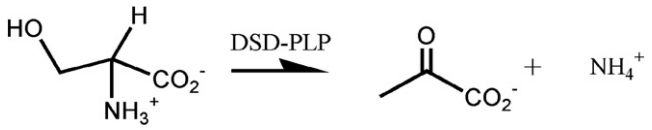

Scheme 1.

to DSD from *Saccharomyces cerevisiae* [7]. The DSD from chicken kidney furthermore belongs to the fold-type III of PLP-dependent enzymes, thus its structure is very different from DSD from *E. coli*, described in this paper. At the same time, Bharath and coworkers [8] reported the crystal structure of D-serine deaminase from *Salmonella typhimurium*. The latter enzyme was crystallized in two structures, an open and closed conformation. However, for both structures no traceable electron density could be observed for the cofactor PLP, indicating that the enzyme likely has a low affinity for the cofactor under the crystallization conditions used. In the deaminase from *S. typhimurium*, modeling of the active site and substrate suggests that Thr 166 may be involved in the abstraction of a proton from the C_α of the substrate. However, the accuracy of the fitted substrate in the deaminase protein seems unclear considering that no unambiguous electron density is observed for PLP in the PLP-D-serine complex [8]. In contrast, our high-resolution structure data provides a highly defined electron density of the cofactor in DSD and is fully developed and all protein partners are established enabling us to propose a putative reaction mechanism for DSD.

2. Materials and methods

2.1. Enzyme

D-Serine dehydratase from *E. coli* was purified from a wild-type DSD expression plasmid [9] using the method described by Schiltz and Schnackerz [10]. 125 g *E. coli* cell paste was suspended in 280 ml potassium phosphate, pH 7.8, containing 1 mM DTE and 1 mM EDTA. The cell suspension was forced three-times through a French press at a pressure of 8000–9000 lb/in^2. Cell debris removed by centrifugation at 27,500×*g* for 30 min. The supernatant crude extract was adjusted to pH 7.3 by slow addition of cold 2.5 M KOH. To remove nucleic acids and nucleoproteins 10% aqueous Polymin P solution was slowly added to bring the protein solution to a final concentration of 1% in Polymin P. After 15 min stirring the suspension was centrifuged for 20 min at 27,500×*g*. The next step was an ammonium sulfate precipitation at 35–55% saturation followed by centrifugation. The pellet was dissolved in a minimum amount of 20 mM potassium phosphate, 0.1 mM EDTA, 0.1 mM pyridoxal phosphate, 1 mM DTE, pH 7.8. Residual ammonium sulfate was removed by dialyzing the protein solution against three-1.5 liter changes of the same buffer. Insoluble material was removed by centrifugation and the clear solution applied to a DEAE-Cellulose column (3.6×30 cm) equilibrated with the dialysis buffer at 4 °C. The enzyme was eluted with a 500 ml gradient of 0–140 mM KCl dissolved in phosphate buffer. Fractions with specific activities greater than 30 units/ml were pooled and concentrated by precipitation with ammonium sulfate (80% saturation). The precipitated protein was dissolved in 70 mM potassium phosphate, 1 mM DTE, 5 μM PLP, 0.1 mM EDTA, pH 7.8. A molecular sieve chromatography on Biogel P-100 (1.6×85 cm) with a flow rate of 2 ml/h followed. Active fractions are pooled and concentrated using an Amicon ultrafiltration system. Further purification was accomplished by crystallization of the enzyme with saturated ammonium sulfate. Specific activities between 120 and 140 units/mg protein were obtained.

2.2. Enzyme assay

The enzymatic activity of DSD was determined at 25 °C as described by Dowhan and Snell [1]. The assay mixture contains 10 mM D-serine, 100 mM potassium phosphate, pH 7.8, 0.15 mM NADH, lactate dehydrogenase (0.1 mg/ml) and DSD. The decrease of NADH absorbance was measured at 334 nm. One unit is defined as the amount of enzyme required to form 1 μmol of pyruvate in 1 min at 25 °C under the above assay conditions.

Table 1
Crystal growth conditions.

Orthorhombic $P2_12_12_1$	Monoclinic $P2_1$
Drop: DSD (10 mg/mL), 1 M Sodium formate in imidazole/maleate buffer, pH 7.0 Well: 2.0 M formate in 0.1 M imidazole/maleate buffer, pH 7.0	Drop: DSD (10 mg/mL), 0.35 M Sodium citrate in 0.1 M imidazole/maleate buffer, pH 7.0 Well: 0.7 M sodium citrate in 0.1 M imidazole/maleate buffer, pH 7.0

2.3. Crystallization and data collection

Different crystal forms of DSD could be obtained. The crystal growth conditions are described in Table 1. Crystals were picked up by a fiber cryo-loop and frozen in a stream of liquid nitrogen for X-ray data collection. Diffraction data from crystals of orthorhombic and monclinic crystal forms were collected at the beamlines EMBL/DESY X31, MPG/DESY BW6 and SRS Daresbury beamline 10.1 using MAR 345 imaging plate, MAR CCD 165 mm, and MAR CCD 225 mm mosaic detectors, respectively. The data collection statistics of the two crystal forms of DSD are summarized in Table 2.

2.4. Structure determination and refinement

The DSD structure was solved by molecular replacement in the orthorhombic space group using the program MOLREP [11]. A solution was found for a model comprising residues 29–317 of the catalytic subunit of allosteric threonine deaminase from *E. coli* (pdb code 1TDJ) [12] with 19% sequence identity to DSD.

The rotation function was calculated at 40–3 Å resolution using full atomic model with default MOLREP 8.2 options. The translation function was found for polyalanine model at 40–5 Å resolution. The solution had correlation of 38.8% and R-factor 59.0% while the wrong translation peaks for correct orientation had correlation not higher than 35.0% and R-factor not lower than 60.3%. For wrong orientations translation peaks had correlation not higher than 33.0% and R-factor not lower than 60.6%.

Next, the solution was subjected to 200 cycles of positional and B-factor refinement at 40–2.0 Å in REFMAC [11,13]. Although Free R

Table 2
Summary of crystallographic data collection statistics.

Crystal form	Orthorhombic	Monoclinic
X-ray source	BW6, MPG/DESY	10.1, SRS
Wavelenght (A)	1.05	0.979
Space group	$P2_12_12$	$P2_1$
a, b, c (A)	a = 143.16, b = 47.74, c = 72.30	a = 73.77, b = 47.80, c = 75.19
Resolution (A)[a]	40–1.97 (2.0–1.97)	50.0–1.55 (1.61–1.55)
Rsym[b] (%)	9.7 (51.5)	85.7 (41.7)
Number of unique reflections	35756	73947
Completeness (%)	99.6 (99.4)	95.7 (80.3)
Redundancy	6.2 (2.9)	4.7 (2.5)
$<I/\sigma(I)>$	19.8 (1.8)	15.9 (2.25)

$I/\sigma(I)$ is the ratio of the mean intensity to the mean standard deviation of intensity.
[a] The data for the highest resolution shell are shown in parentheses.
[b] Rsym = S I − <I>, where I is intensity of reflection and <I> is the intensity averaged from multiple observations of symmetry-related reflections.