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Crystallization and preliminary X-ray diffraction studies on recombinant diaminopropionate ammonia lyase from *Escherichia coli*

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Diaminopropionate (DAP) ammonia lyase (a PLP-dependent enzyme; EC 4.3.1.15) catalyzes the α,β-elimination reaction of both L- and D-α,β-diaminopropionate to form pyruvate and ammonia. *Escherichia coli* DAP ammonia lyase gene was cloned and over-expressed in *E. coli* and the protein was purified to homogeneity and crystallized using the hanging-drop vapour-diffusion technique. Crystals of two different morphologies were obtained, one of which belonged to the tetragonal space group $P4_12_12$ (or $P4_32_12$), with unit-cell parameters $a = b = 86.01$, $c = 209.56$ Å, and the other to the monoclinic space group $P2_1$, with unit-cell parameters $a = 87.78$, $b = 94.35$, $c = 96.02$ Å, $\beta = 109.73^\circ$. The tetragonal crystals diffracted X-rays to 3.0 Å resolution, while diffraction from the monoclinic form extended to 2.5 Å. Complete X-ray diffraction data sets have been collected for both crystal forms.

1. Introduction

Diaminopropionate ammonia lyase (DAPAL) belongs to the β-family of pyridoxal 5′-phosphate (PLP) dependent enzymes (Mehta & Christen, 2000) and may have structural similarities to other members of the β-family (fold type II) of PLP-dependent enzymes such as D-serine dehydratase (Obmolova *et al.*, 1990) or threonine deaminase (Gallagher, Gilliland *et al.*, 1998). However, earlier studies have revealed that the amino-acid sequence of the pyridoxal phosphate-binding peptide of DAPAL bears no homology to other PLP-dependent enzymes (Nagasawa *et al.*, 1988). The enzyme has been characterized from *Pseudomonas* (Rajagopal Rao *et al.*, 1970; Vijayalakshmi *et al.*, 1975) and *Salmonella typhimurium* (Nagasawa *et al.*, 1988). DAPAL catalyzes the α,β-elimination reaction of both L- and D-α,β-diaminopropionate (DAP) to form pyruvate and ammonia (Fig. 1). The L- and D-isomers of serine are also degraded, although more slowly. DAPAL is unique in that it removes two molecules of ammonia from adjacent C atoms. DAP is the immediate precursor of the neurotoxins 3-oxalyl and 2,3-dioxalyl DAP present in the drought-resistant grain legume *Lathyrus sativus*. The presence of the neurotoxins, which cause neurolathyrism in humans, precludes exploitation of the legume as a major source of food. We have initiated structural studies on *Escherichia coli* DAPAL (ecDAPAL) with a view to gaining insight into the catalytic mechanism and structure–function relationship of the enzyme. A comparison of ecDAPAL with other members of the β-family of PLP-dependent enzymes could provide information on the origin of the reaction and substrate specificities in these enzymes.

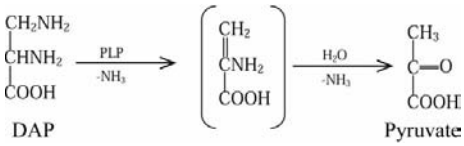

Figure 1
Reaction catalyzed by DAPAL.

2. Cloning, expression and purification

The ecDAPAL gene was cloned and over-expressed according to Khan *et al.* (2003). The expression levels were good and most of the expressed protein was present in the soluble fraction. The soluble fraction was directly loaded onto an anion-exchange DEAE cellulose column previously equilibrated with buffer *A* [20 m*M* phosphate pH 7.0, 1 m*M* 2-mercaptoethanol (βME), 1 m*M* EDTA and 50 μ*M* PLP]. The column was washed extensively with buffer *A* followed by washing with the same buffer containing 0.05 *M* KCl to remove unbound proteins. The enzyme was eluted with buffer *A* containing 0.1 *M* KCl. 5 ml fractions were collected and their absorbance was measured at 280 nm (for the protein) and 414 nm (for PLP). The peak fractions were also examined on SDS–PAGE (Laemmli, 1970). Fractions showing more than 95% purity were pooled. The pooled fractions were dialyzed against an aqueous solution containing 1 m*M* EDTA and 1 m*M* βME for 24 h with three changes. The enzyme was

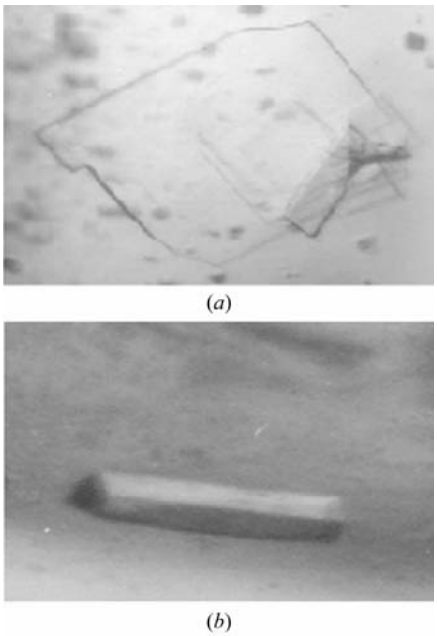

Figure 2
Two different forms of DAP ammonia lyase crystals. (*a*) Tetragonal form, (*b*) monoclinic form.

concentrated by several cycles of low-speed centrifugation using a 10 kDa molecular-weight cutoff Centricon (Amicon) to a final concentration of 20 mg ml^{-1} and was stored in aliquots at 277 K for further use. The purity of the protein was estimated using SDS–PAGE and was found to be nearly homogeneous.

3. Crystallization and preliminary X-ray studies

Crystallization of ecDAPAL was achieved at room temperature using the hanging-drop vapour-diffusion method. Crystallization attempts were carried out with Hampton Research Crystal Screen kits I and II and by using conditions under which PLP-dependent enzymes have been successfully crystallized previously (Markavic Housley *et al.*, 1990; Obmolova *et al.*, 1990; Gallagher, Eisenstein *et al.*, 1998; Janosik *et al.*, 2001). Crystals of dimensions suitable for collecting X-ray diffraction data were obtained by using 20 mg ml^{-1} protein in 40 m*M* Tris–HCl pH 8.3 containing 70 m*M* $MgCl_2$, 50 m*M* Li_2SO_4 and 6 m*M* sodium citrate with 20%(*w*/*v*) PEG 3350 as the precipitant. Crystals (rectangular plates; Fig. 2*a*) appeared in the drop after ∼5 d. The crystals lost some of their order and diffraction quality upon storage for a week or more. However, similar quality crystals were obtained with a protein sample stored at 277 K for several weeks. Another crystal form (thick rods; Fig. 2*b*) was obtained when the concentration of protein was increased from 20 to 35–40 mg ml^{-1}.

Table 1
Data-collection statistics.

Values in parentheses correspond to the last resolution shell.

Space group	$P4_12_12$ or $P4_32_12$	$P2_1$
Resolution range (Å)	20.0–3.0 (3.11–3.0)	20.0–2.5 (2.59–2.50)
No. of reflections	136126	142074
No. of unique reflections	15372	42635
Completeness (%)	93.3 (92.4)	85.4 (85.7)
Multiplicity	8.9	2.7
Mosaicity (°)	0.9	0.9
Average $I/\sigma(I)$	19.0 (6.5)	15.2 (4.4)
$R_{\rm merge}$† (%)	9.8 (34.4)	5.9 (24.0)
$R_{\rm measure}$† (%)	10.5 (36.4)	6.8 (29.0)

† $R_{\rm merge} = 100 \times \sum_h \sum_i^{n_h} |\hat{I}_h - I_{h,i}| / \sum_h \sum_i^{n_h} I_{h,i}$ and $R_{\rm measure} = 100 \times 100 \times \sum_h [n_h/(n_h-1)]^{1/2} \sum_i^{n_h} |\hat{I}_h - I_{h,i}| / \sum_h \sum_i^{n_h} I_{h,i}$ where $\hat{I}_h = (1/n_h) \sum_i^{n_h} I_{h,i}$ and n_h is the multiplicity (Diederichs & Karplus, 1997).

The crystals were mounted in glass capillaries and examined at room temperature for diffraction quality on a Rigaku RU-200 rotating-anode X-ray generator equipped with a MAR Research imaging-plate detector. However, the quality of crystals quickly deteriorated. Useful diffraction data could only be collected at liquid-nitrogen temperature (100 K) following flash-freezing techniques. For this purpose, the crystals were transferred to cryoprotectant solutions containing an increasing concentration of glycerol [5–25%(*v*/*v*)]. Data collection at liquid-nitrogen temperature considerably improved the resolution and prolonged the crystal life in the X-ray beam. Complete data were obtained using single crystals of both forms. The oscillation angle was set to 1°. The exposure time for each frame was 900 s for the plate form and 300 s for the rod form. The resolution of the data from the two crystal forms was 3.0 and 2.5 Å, respectively. The oscillation frames were indexed and the intensities of the Bragg reflections were estimated using the *HKL* program suite (Otwinowski & Minor, 1997). Examination of the systematic absences suggested that the crystals with rectangular plate morphology belong to the tetragonal space group $P4_12_12$ (or $P4_32_12$), with unit-cell parameters $a = b = 86.01$, $c = 209.56$ Å, and those with thick rod morphology belong to the monoclinic space group $P2_1$, with unit-cell parameters $a = 87.78$, $b = 94.35$, $c = 96.02$ Å, $\beta = 109.73°$. The Matthews coefficient (Matthews, 1968) $V_{\rm M}$ is 2.24 and 2.16 Å^3 Da^{-1} (molecular weight 43.3 kDa) for the tetragonal and monoclinic forms, respectively, and indicates that the tetragonal crystal contains two subunits (one dimeric molecule) and the monoclinic crystal contains four subunits (two dimers) in the asymmetric unit. Table 1 lists the relevant X-ray diffraction data statistics.

Amino-acid sequence comparisons suggest that the structure of ecDAPAL might be similar to that of threonine deaminase, another PLP-binding enzyme. However, initial attempts to determine the structure by molecular-replacement by using threonine deaminase (Gallagher, Gilliland *et al.*, 1998) as the starting model did not lead to viable solutions, presumably owing to the low sequence identity (21%). No other protein of known structure shows significant sequence similarity to ecDAPAL. Therefore, attempts to determine the structure by the multiple isomorphous replacement technique are in progress.

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