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Crystallization and preliminary X-ray crystallographic analysis of aspartate 1-decarboxylase from *Helicobacter pylori*

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Aspartate 1-decarboxylase (PanD) catalyzes the α-decarboxylation of L-aspartate in the major route of β-alanine production for pantothenate biosynthesis in bacteria. Pantothenate is synthesized in microorganisms, plants and fungi but not in animals and thus the enzymes of its biosynthetic pathway are potential targets for developing agents against these organisms. PanD from the pathogenic bacterium *Helicobacter pylori* has been overexpressed in *Escherichia coli* and crystallized using sodium formate as a precipitant. Crystals diffracted to better than 1.5 Å Bragg spacing upon exposure to synchrotron X-rays. Diffraction data to 1.55 Å have been collected from a crystal grown in the presence of the substrate analogue isoasparagine. The crystal belongs to the tetragonal space group $I422$, with unit-cell parameters $a = b = 81.83$, $c = 93.78$ Å. The asymmetric unit contains one subunit of PanD, with a corresponding crystal volume per protein mass (V_M) of 2.85 Å^3 Da^{-1} and a solvent content of 56.8%.

1. Introduction

Pantothenate (vitamin B_5), the essential precursor of the phosphopantotheine moiety of coenzyme A (CoA) and acyl carrier protein, is synthesized in microorganisms, plants and fungi but not in animals. This makes the enzymes of its biosynthetic pathway potential targets for developing agents against these organisms (von Delft *et al.*, 2001). Pantothenate is produced by the condensation reaction between D-pantoate and β-alanine by the *panC* gene product pantothenate synthase. Pantoate is synthesized from α-ketoisovalerate *via* ketopantoate by sequential reactions catalyzed by ketopantoate hydroxymethyltransferase (PanB) and ketopantoate reductase (PanE). The source of β-alanine depends on the organism. In plants and fungi, it is derived from the degradation of uracil (Brown & Williamson, 1982). However, in bacteria the major route for the formation of β-alanine is the α-decarboxylation of L-aspartate catalyzed by the enzyme aspartate 1-decarboxylase (PanD; Jackowski, 1996).

Aspartate 1-decarboxylase is one of the unusual enzymes that contain a covalently bound pyruvoyl group involved in catalysis (Williamson & Brown, 1979). In the maturation of the pro-protein, *E. coli* PanD is self-cleaved at the Gly24–Ser25 bond (Ramjee *et al.*, 1997) to produce a β-subunit (2.8 kDa) with X—OH at its C-terminus and an α-subunit (11 kDa) with a pyruvoyl group at its N-terminus. The crystal structure of *E. coli* aspartate 1-decarboxylase has been reported (Albert *et al.*, 1998), with three subunits of the tetrameric enzyme cleaved into β-subunits and α-subunits and the fourth subunit containing the uncleaved but rearranged ester intermediate. The proposed Schiff-base intermediate prior to decarboxylation was modelled into the active site.

H. pylori is a Gram-negative bacterium responsible for gastritis, peptic ulcer and gastric cancer (Covacci *et al.*, 1999). Because of its importance as one of the major human pathogens, complete genome sequences of strains 26695 and J99 have been reported (Tomb *et al.*, 1997; Alm *et al.*, 1999). PanD from *H. pylori* is a 117-residue protein which shows 35% sequence identity to *E. coli* PanD. As the first step toward its structure elucidation, we have overexpressed *H. pylori* PanD and crystallized it in the presence of the substrate analogue isoasparagine. The crystallization conditions and preliminary X-ray crystallographic data are reported here.

2. Experimental

2.1. Overexpression and purification

The *panD* gene encoding aspartate 1-decarboxylase from *H. pylori* strain 26695 (HP0034) was amplified by the polymerase chain reaction (PCR) using the chromosomal DNA as template. The forward and reverse oligonucleotide primers were designed using the published genome sequence (Tomb *et al.*,

1997). The PCR product was digested with *Nde*I and *Xho*I and then inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a (Novagen). This construction added a hexahistidine tag to the C-terminus of the recombinant protein. The protein was overexpressed in *E. coli* C41(DE3) cells (Miroux & Walker, 1996). The cells were grown in Luria–Bertani medium to an OD_{600} of 0.6 at 310 K and expression of the recombinant enzyme was induced with 1 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) at 303 K. Cell growth continued at 303 K for 6 h after IPTG induction and calls were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was suspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 0.5 *M* NaCl, 50 m*M* imidazole) and homogenized by sonication. The crude lysate was centrifuged at 70 400*g* (30 000 rev min^{-1}; Beckman 45Ti rotor) for 1 h at 277 K and the cell debris was discarded. The first purification step utilized the C-terminal histidine tag by using an Ni^{2+}-chelated Hi-Trap chelating HP column (Amersham Biosciences). The next step was gel filtration on a HiLoad 16/60 Superdex-200 prep-grade column (Amersham Biosciences) with an elution buffer consisting of 50 m*M* Tris–HCl pH 8.5, 150 m*M* NaCl and 1 m*M* EDTA. Further purification was achieved by ion-exchange chromatography on a Mono Q HR5/5 column (Amersham Biosciences) previously equilibrated with 50 m*M* Tris–HCl pH 8.5 and 1 m*M* EDTA. The protein was eluted with a linear gradient of the same buffer containing 0–1.0 *M* NaCl. The final purification step was achieved by gel filtration on a HiLoad 16/60 Superdex-200 prep-grade column (Pharmacia) previously equilibrated with a buffer solution containing 50 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA and 150 m*M* NaCl. The purified protein was concentrated to 18 mg ml^{-1} using an YM10 membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 5120 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

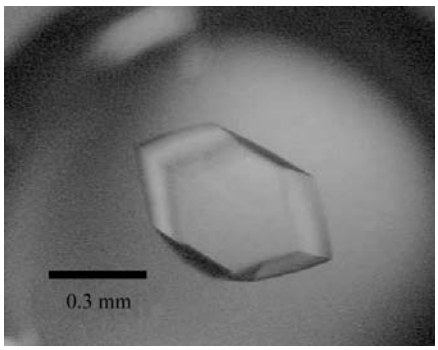

Figure 1
A crystal of *H. pylori* aspartate 1-decarboxylase. Its approximate dimensions are 0.5 × 0.5 × 0.3 mm.

2.2. Crystallization and X-ray data collection

Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well VDX tissue-culture plates (Hampton Research) at 297 K. The substrate analogue isoasparagine was added to the protein solution to a concentration of 20 m*M*. Hanging drops were prepared by mixing 2 µl of protein solution and 2 µl of reservoir solution and were placed over 1.0 ml of reservoir solution. Initial searches for the crystallization conditions were performed using Crystal Screen I (Jancarik & Kim, 1991), Crystal Screen II and MembFac screening solutions (Hampton Research).

For X-ray data collection, a crystal was transferred to a solution consisting of 3.8 *M* sodium formate and 30%(*v*/*v*) glycerol within a minute in two steps before being flash-frozen. X-ray diffraction data were collected at 100 K with a MacScience 2030 image-plate detector at the BL-6B experimental station of Pohang Light Source, South Korea. The synchrotron X-ray wavelength was 0.9795 Å. The crystal was rotated through a total of 70°, with 1.0° oscillation per frame. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

Recombinant *H. pylori* PanD with a C-terminal hexahistidine tag was overexpressed in *E. coli* in a soluble form with a yield of ∼9 mg of homogeneous protein per litre of culture. Crystals were obtained using a reservoir solution consisting of 3.8 *M* sodium formate. They grew to dimensions of 0.5 × 0.5 × 0.3 mm within 3 d (Fig. 1). Crystals diffracted to better than 1.5 Å resolution with synchrotron X-rays and diffraction data were collected to 1.55 Å. A total of 233 538 measured reflections were merged into 22 394 unique reflections with an $R_{\rm merge}$ (on intensity) of 3.2%. The merged data set is 95.6% complete to 1.55 Å. The systematic absences indicate the space group to be *I*422, with unit-cell parameters $a = b = 81.83$ (6), $c = 93.78$ (5) Å, where the estimated standard deviations are given in parentheses. The presence of one subunit of PanD in the asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.85 Å^3 Da^{-1} and a corresponding solvent content of 56.8% (Matthews, 1968). The statistics of data collection are summarized in Table 1. The structure of *H. pylori* PanD has been solved using multi-wavelength anomalous diffraction data and shows that isoasparagine is covalently attached to the pyruvoyl group of Ser25. Structural details will be described in a separate paper.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.61–1.55 Å).

X-ray wavelength (Å)	0.9795 (Pohang Light Source, BL-6B)
Temperature (K)	100
Resolution range (Å)	30.0–1.55
Space group	*I*422
Unit-cell parameters (Å)	$a = b = 81.83$, $c = 93.78$
Total/unique reflections	233538/22394
Completeness (%)	95.6 (90.0)
Mean $I/\sigma(I)$	47.7 (5.7)
$R_{\rm merge}$† (%)	3.2 (15.6)

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h, i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h, i)$ for all i measurements.

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