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Crystallization and preliminary X-ray crystallographic analysis of D-phenylglycine aminotransferase from *Pseudomonas stutzeri* ST201

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D-Phenylglycine aminotransferase (D-PhgAT) catalyzes the reversible transamination of D-phenylglycine to L-glutamate with 2-oxoglutarate as the amino-group acceptor. Crystals of substrate-free *Pseudomonas stutzeri* D-PhgAT bound to the cofactor pyridoxal-5′-phosphate (PLP) were obtained by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant. The crystals belong to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 75.155$, $c = 147.554$ Å. The asymmetric unit contains one molecule of D-PhgAT and has a solvent content of 50.0%. A complete native X-ray diffraction data set was collected from a single crystal at 100 K to a resolution of 2.3 Å.

1. Introduction

D-Phenylglycine aminotransferase (D-PhgAT), a vitamin B_6-dependent enzyme, catalyzes the stereospecific amino-group transfer of D-phenylglycine to 2-oxoglutarate to yield L-glutamate by a reversible ping-pong kinetic mechanism (Wiyakrutta & Meevootisom, 1997; Kirsch *et al.*, 1984). The enzyme requires pyridoxal phosphate (PLP) as a coenzyme and has substrate specificity for D-phenylglycine and D-4-hydroxyphenylglycine. However, neither D- nor L-aromatic nor branch-chained amino acids can be utilized as substrates. Using the 'stereo-inverting' transamination property of D-PhgAT, the amino nitrogen can be channelled directly between D-phenylglycine and L-glutamate, a central molecule in cellular nitrogen metabolism, without the need for additional amino-acid racemases for phenylglycine or glutamate. D-PhgAT, found as a homodimer of molecular mass 92 kDa, has been purified from *Pseudomonas stutzeri* ST201 (Wiyakrutta & Meevootisom, 1997). The enzyme is most active at alkaline pH, with maximum activity at pH 9–10, and can be inhibited by typical inhibitors of pyridoxal phosphate-dependent enzymes. The D-PhgAT encoding gene, isolated from the plasmid pBPL-ph, was cloned into the expression vector pET-17b and the expression host BL21(DE3) (Laowanapiban, 2001). From a BLASTp search (Altschul *et al.*, 1997) using the deduced amino-acid sequence, the 453-residue D-PhgAT was found to be homologous to the aminotransferase subgroup II (Mehta *et al.*, 1993) or aminotransferase subclass II (Schneider *et al.*, 2000), including glutamate-1-semialdehyde aminomutase (GSAAT) and ornithine aminotransferase (OAT) with sequence identity of 25–30 and 21%, respectively. Notably, D-PhgAT lacks sequence homology to D-amino-acid aminotransferase (DAAT), having a sequence identity of only 16%.

In general, the amino-group transfer reaction catalyzed by aminotransferases is highly stereo-conserved (Sugio *et al.*, 1995; Peisach *et al.*, 1998) and there is little information regarding transamination reactions between two amino acids which have opposite configuration (van den Tweel *et al.*, 1986, 1988). Therefore, we have initiated a crystallographic investigation of the stereospecific reaction mechanism, as well as of the substrate specificity of the D-PhgAT enzyme.

2. Experimental

2.1. Overexpression and purification

pEPL-transformed *E. coli* BL21(DE3) cells were grown in LB medium with 50 mg l^{-1} ampicillin at 303 K. When the culture reached an absorbance at 600 nm of 2.0, IPTG was added to 0.4 m*M* and the culture was further incubated for 3 h. The cells were harvested by centrifugation, resuspended in 50 m*M* phosphate buffer pH 7.0 with 1 m*M* PLP and 1 m*M* PMSF and subsequently disrupted by a sonicator. The protein was purified by ammonium sulfate precipitation at 25–45% saturation, followed by chromatography on a phenyl-agarose CL-4B column and a DEAE anion-exchange column. The combined active fractions were desalted and concentrated to about 10 mg ml^{-1} using a Centricon Plus-20 ultrafiltration membrane (Amicon, Beverly, MA, USA).

2.2. Crystallization and X-ray data collection

Initial crystallization conditions were obtained using the vapour-diffusion technique

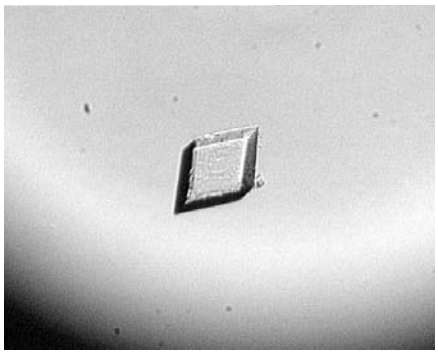

Figure 1
Photograph of a recombinant D-PhgAT trigonal crystal (approximate dimensions 0.2 × 0.2 × 0.2 mm).

in a 24-well tissue-culture plate at 277 and 298 K. In each drop, 2 µl of 10 mg ml^{-1} D-PhgAT solution in 10 m*M* Tris pH 7.8, 100 m*M* NaCl, 1 m*M* EDTA was mixed with an equal volume of reservoir solution. Promising conditions were discovered using $(NH_4)_2SO_4$ and pH-screening grids at 277 K and were further optimized with respect to pH and precipitant concentration. The optimal condition was found to consist of 200 m*M* phosphate buffer pH 6.2 and 28–30% saturated $(NH_4)_2SO_4$. These rhombohedral crystals typically grew to 0.2 × 0.2 × 0.2 mm within a few weeks (Fig. 1).

2.3. Data collection

A 2.3 Å resolution data set from D-PhgAT in complex with PLP was collected at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory on beamline X8C. A single crystal (0.1 × 0.1 × 0.1 mm), cryoprotected by a 60 s soak in a reservoir solution containing 35% $(NH_4)_2SO_4$, 200 m*M* phosphate buffer pH 6.2 and 30% glycerol, was flash-frozen in the cryogenic nitrogen stream using a nylon loop. The crystals of D-PhgAT complexed with PLP possessed trigonal symmetry, with unit-cell parameters $a = b = 75.155$, $c = 149.554$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. The reflection data were collected on an ADSC Quantum-4 CCD detector using X-ray radiation ($\lambda = 0.9795$ Å) with a crystal-to-detector distance of 200 mm and a 0.5° oscillation angle per image, with an exposure time of 60 s for each image and a total of 360 frames (Fig. 2). The diffraction data were integrated, scaled and reduced with *MOSFLM* and *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994) (Table 1). The Laue symmetry and systematic absences showed a clear threefold screw axis, identifying the space group as either $P3_121$ or $P3_221$. The data set was 99.8% complete, with a scaling R_{sym} of 0.065 for 22 083 unique reflections in the resolution range 20.0–2.3 Å (Table 2). Although D-PhgAT exists as a dimeric protein in solution, there is one molecule of D-PhgAT in the asymmetric unit. This revealed that in the crystalline state each monomer of the dimeric enzyme adopts a similar conformation, unlike the asymmetric dimeric crystal structures of glutamine-1-semialdehyde aminomutase (Hennig *et al.*, 1997) and ornithine aminotransferase (Shen *et al.*, 1998). Assuming one monomer of 47.5 kDa D-PhgAT in the asymmetric unit, the calculated volume per unit mass, V_M (Matthews, 1968), is 2.46 Å^3 Da^{-1}, corresponding to a solvent content of 50.0%.

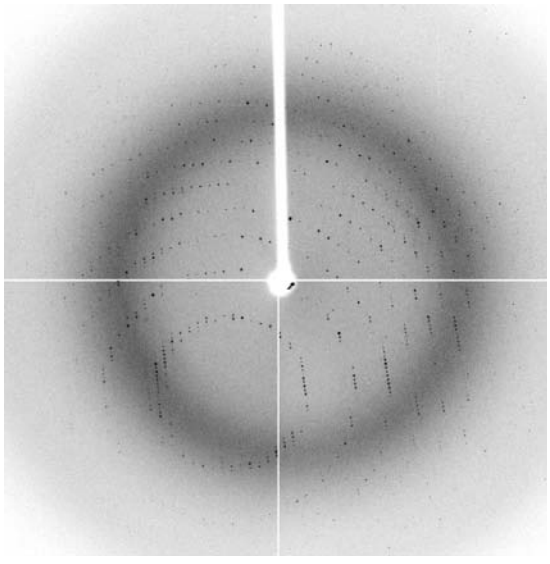

Figure 2
A typical 0.5° oscillation X-ray diffraction pattern of a D-PhgAT crystal.

Preliminary phases for D-PhgAT have been obtained by a molecular-replacement technique using a modified GSA-AT structure. A full description of the procedure for obtaining phase information will be published elsewhere.

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Table 1
Unit-cell parameters and data-collection statistics.

Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å)	$a = b = 75.155$, $c = 147.554$
Resolution (Å)	20–2.3
Temperature	100
Wavelength (Å)	0.9795
Total No. of reflections	487524
No. of unique reflections	22083
Data completeness	99.8
R_{sym}	0.065
Average $I/\sigma(I)$	8.7
Multiplicity	10.7
No. of molecules in asymmetric unit	1
V_M (Å^3 Da^{-1})	2.46
Solvent content (%)	50.0

Table 2
Statistics of data collection from a D-PhgAT crystal by resolution shell.

Resolution (Å)	No. of unique reflections	Completeness (%)	R_{sym}
7.27	753	95.1	0.040
5.14	1346	100	0.041
4.20	1688	100	0.040
3.64	1985	100	0.045
3.25	2218	100	0.056
2.97	2444	100	0.082
2.75	2660	100	0.112
2.57	2826	99.9	0.152
2.42	2960	99.9	0.203
2.30	3203	100	0.270

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