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Purification, crystallization and preliminary crystallographic analysis of *Arabidopsis thaliana* imidazoleglycerol-phosphate dehydratase

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Imidazoleglycerol-phosphate dehydratase catalyses the sixth step of the histidine-biosynthesis pathway in plants and microorganisms and has been identified as a possible target for the development of novel herbicides. *Arabidopsis thaliana* IGPD has been cloned and overexpressed in *Escherichia coli*, purified and subsequently crystallized in the presence of manganese. Under these conditions, the inactive trimeric form of the metal-free enzyme is assembled into a fully active species consisting of a 24-mer exhibiting 432 symmetry. X-ray diffraction data have been collected to 3.0 Å resolution from a single crystal at 293 K. The crystal belongs to space group *R*3, with approximate unit-cell parameters $a = b = 157.9$, $c = 480.0$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$ and with either 16 or 24 subunits in the asymmetric unit. A full structure determination is under way in order to provide insights into the mode of subunit assembly and to initiate a programme of rational herbicide design.

1. Introduction

Imidazoleglycerol-phosphate dehydratase (IGPD; EC 4.2.1.19) catalyses the sixth step of the histidine-biosynthesis pathway, the dehydration of imidazoleglycerol-phosphate (IGP) to form imidazoleacetol-phosphate (IAP; Fig. 1). This pathway is essential in plants and microorganisms and IGPD has been identified as a possible target for the development of novel herbicides. Herbicides having such a new mode of action would provide useful tools for the management of weeds which have become resistant to currently used classes (Guttieri *et al.*, 1992; Devine, 1997; Preston & Powles, 2002). The mechanism of IGPD catalysis is currently unknown; however, the lack of an adjacent carbonyl or imine group results in a non-acidic leaving hydrogen, which contrasts with most dehydration reactions (Gerlt & Gassman, 1992). The identification of a novel class of triazole phosphonate inhibitors which act against IGPD suggests that the reaction may well occur through a proposed diazafulvene intermediate (Hawkes *et al.*, 1993).

Previous studies on the characterization of IGPD from different species have shown that the apoprotein exists as an inactive trimer but upon addition of manganese (and a limited range of other cations) the trimer assembles further to form a biologically active 24-mer exhibiting 432 symmetry (Hawkes *et al.*, 1995; Tada *et al.*, 1995; Wilkinson *et al.*, 1995). The structure of the trimeric form of the enzyme from the fungus *Filobasidiella neoformans* has recently been determined (Sinha *et al.*, 2004); however, the absence of bound metals in this structure, the presence of disorder in one of the two histidine-rich motifs that have been proposed to be involved in metal binding and catalysis, and the lack of information on the precise mode of subunit assembly prevented a detailed examination of the molecular basis of specificity and catalysis. In this paper, we describe the

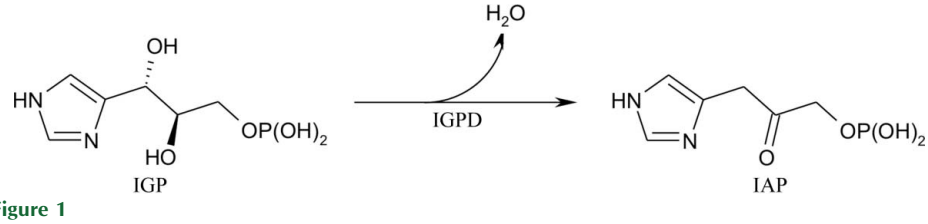

Figure 1
The reaction catalysed by IGPD.

cloning, overexpression, purification, crystallization and preliminary X-ray analysis of the biologically active 24-mer of IGPD from *Arabidopsis thaliana*.

2. Cloning, overexpression and purification of *A. thaliana* IGPD

The *A. thaliana* IGPD gene was PCR amplified and cloned into the pET24 plasmid to form the vector pIGET13, which was subsequently transformed into the overexpression strain *Escherichia coli* BL21 (DE3). A 250 ml flask containing 50 ml LB medium with 50 µg ml^{-1} kanamycin was inoculated with a single colony of the pIGET13-containing strain and grown overnight at 310 K on a shaking tray at 250 rev min^{-1}. 20 ml of this culture was subsequently used to inoculate eight 2 l flasks each holding 500 ml LB medium supplemented with kanamycin and 10 m*M* $MnSO_4$. The cultures were grown at 310 K on shaking trays at 250 rev min^{-1} until the absorbance at 600 nm reached 0.6. Overexpression was induced at this point by adding 0.5 m*M* IPTG and growing at 303 K overnight. To harvest the cells, the cultures were centrifuged at 5000*g* for 20 min at 277 K and the pellets were immediately frozen prior to purification. Analysis of the soluble fraction of the cell paste by SDS–PAGE showed a large band corresponding to the expected molecular weight of the IGPD monomer (22.6 kDa).

Cells containing overexpressed IGPD were thawed, suspended in buffer *A* (40 m*M* Tris–HCl pH 8.0, 2 m*M* EDTA) and disrupted by ultrasonication. Debris was removed by centrifugation at 70 000*g* for 10 min. The supernatant fraction was applied to a column with DEAE-Sepharose Fast Flow (Amersham Biosciences) and proteins were eluted with a linear gradient of NaCl from 0 to 0.5 *M* in buffer *A*. Fractions shown to contain IGPD by SDS–PAGE analysis were combined and the enzyme was precipitated with 1.7 *M* ammonium sulfate. Pellets were collected by centrifugation, dissolved in 1 ml buffer *A* and loaded onto a Hi-Load Superdex 200 column (Amersham Biosciences) equilibrated with 0.1 *M* NaCl in buffer *A*. Proteins were eluted using the same buffer and fractions containing IGPD were combined and concentrated on a VivaSpin concentrator. Under these conditions, the protein eluted in its inactive trimeric state. The enzyme was then loaded onto a Hi-Load Superdex 200 column and equilibrated with buffer *B* (4 m*M* $MnSO_4$, 40 m*M* Tris–HCl pH 8.0, 40 m*M* NaCl, 0.4 m*M* EDTA), under which conditions the protein elutes much earlier, reflecting assembly to the 24-mer. Assembled IGPD was eluted from the column and concentrated on a VivaSpin concentrator to 10–15 mg ml^{-1}. The purity of the final IGPD preparation was estimated to be close to 100% by SDS–PAGE, with a yield of approximately 3 mg enzyme per gram of cell paste.

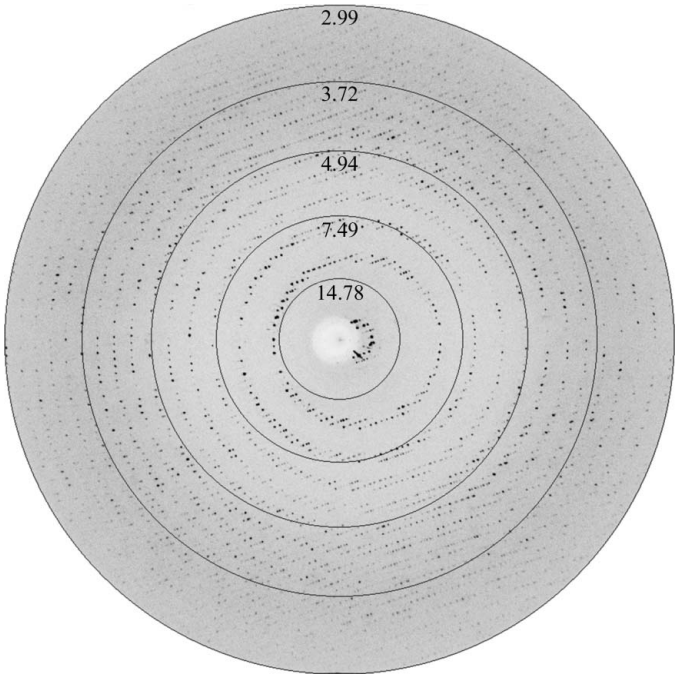

Figure 2
A 1° rotation diffraction image of a crystal of *A. thaliana* IGPD. Diffraction data can be seen to extend to 3.0 Å resolution.

3. Crystallization and preliminary X-ray analysis

During storage of the purified IGPD enzyme in buffer *B* at 277 K for 3 d, pyramidal crystals were observed to have grown with approximate dimensions of 0.3 × 0.15 × 0.15 mm. A single crystal was mounted in a glass capillary tube and data were collected at 293 K to a maximum resolution of 3.0 Å (Fig. 2). Diffraction images were collected at 1° rotations using a Rigaku RU-200 rotating copper-anode source and a MAR Research image-plate detector. Data-collection and processing statistics are given in Table 1.

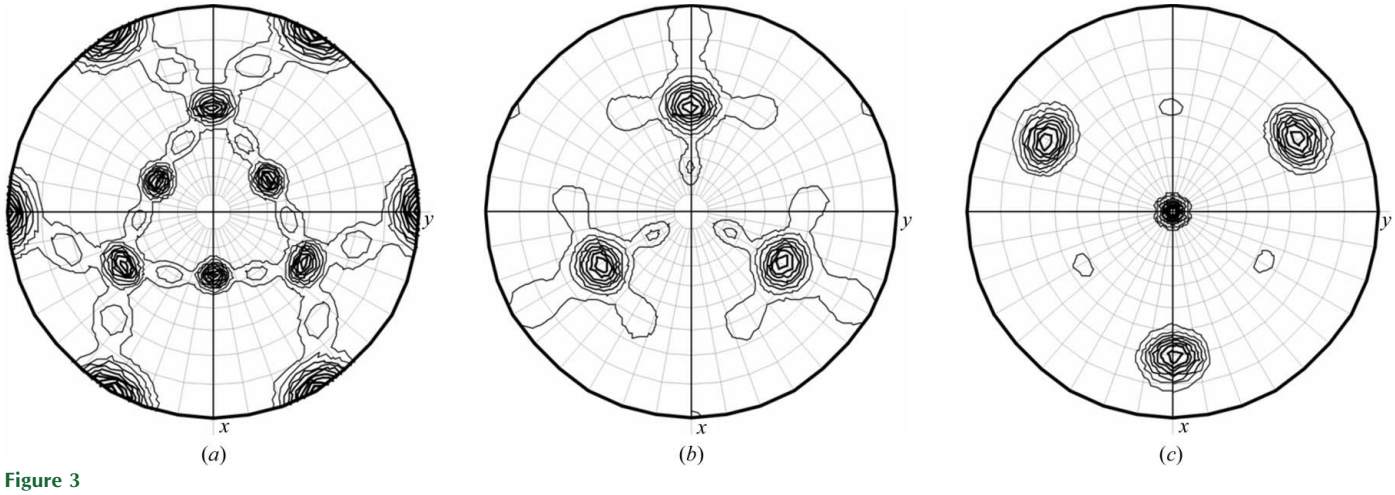

Figure 3
Self-rotation function calculated on data from an *A. thaliana* IGPD crystal. Sections are shown at $\kappa = 180°$ (*a*), $\kappa = 90°$ (*b*) and $\kappa = 120°$ (*c*) to show the presence of non-crystallographic twofold, fourfold and threefold axes, respectively, which appear to be arranged in 432 symmetry.

Table 1
Data-collection and processing statistics.

Values in parentheses refer to the highest resolution shell (3.06–3.00 Å).

Wavelength (Å)	1.542
Space group	*R*3
Resolution (Å)	50.0–3.0
Completeness (%)	98.3 (96.8)
No. of reflections	328806
No. of unique reflections	88754
Redundancy	3.7
$\langle I/\sigma(I)\rangle$	11.7 (3.8)
$I > 3\sigma(I)$ (%)	77.3
$R_{\rm merge}$† (%)	9.7 (22.3)

† $R_{\rm merge} = \sum |I - \langle I\rangle| / \sum I$, where I is the integrated intensity of a given reflection.

Processing of the X-ray diffraction data using *DENZO*/*SCALEPACK* (Otwinowski & Minor, 1997) indicated that the crystal belongs to space group *R*3, with unit-cell parameters in the hexagonal setting of $a = b = 157.9$, $c = 480.0$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. A self-rotation function was calculated in *POLARRFN* (Collaborative Computational Project, Number 4, 1994) using data from 20.0 to 3.0 Å resolution and a radius of integration of 15.0 Å (Fig. 3). Examination of the $\kappa = 90$, 120 and 180° sections reveals the unit cell to contain particles exhibiting the expected 432 symmetry. Given the subunit molecular weight of 22.6 kDa, consideration of the unit-cell volume suggests that the crystal contains either eight, 16 or 24 subunits in the asymmetric unit (AU), corresponding to $V_{\rm M}$ values of 6.4, 3.2 or 2.1 Å^3 Da^{-1}, respectively (Matthews, 1977). Packing considerations suggest that the AU most probably contains either 16 or 24 subunits. A full structure determination of these crystals of IGPD is currently under way in order to provide insight into the role of the manganese ions in subunit assembly and catalysis, and to contribute to the rational development of novel herbicides.

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