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Crystallization and analysis of the subunit assembly and quaternary structure of imidazoleglycerol phosphate dehydratase from *Saccharomyces cerevisiae*.

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Abstract

Imidazoleglycerol phosphate dehydratase (IGPD) from *Saccharomyces cerevisiae* has been crystallized in the presence of a range of divalent cations using the hanging-drop method of vapour diffusion with ammonium sulfate or polyethylene glycol (PEG) 4000 as the precipitants. X-ray precession photographs have established that the crystals formed with ammonium sulfate (form *A*) belong to the space group *F*432, with cell parameter a = 177.5 Å and a single subunit in the asymmetric unit. A preliminary data set collected to 6 Å resolution on a two-detector San Diego Multiwire area detector has established that the crystals formed with PEG 4000 (form *B*) belong to either of the special pair of space groups *I*23 or $I2_13$, with cell parameter a = 131.0 Å. A self-rotation function has been calculated using these data and indicates that the cell axes show pseudo fourfold symmetry consistent with a dimer in the asymmetric unit in this crystal form. Light-scattering studies indicate that in the presence of Mn^{2+} and a number of other divalent cations IGPD undergoes assembly to a particle of molecular weight approximately 500 kDa. Given the subunit molecular weight of 23 kDa together with the symmetry of the crystals it would indicate that the most likely quaternary structure for this enzyme is based on a 24-mer in 432 symmetry.

Introduction

Imidazole glycerol phosphate dehydratase (IGPD) is a manganese-dependent enzyme which catalyses the dehydration of imidazole glycerol phosphate to imidazole acetol phosphate (Ames, 1957). The sole function of IGPD is in the biosynthesis of histidine where the enzyme immediately precedes the amino transferase step (Ames & Mitchell 1955; Ames, 1957). IGPD was originally thought to be the target of the herbicide Amitrole (3-amino-1,2,4-triazole) (Hilton, Kearney & Ames, 1965) but this now appears unlikely (Burns, Buchanan & Carter, 1971). More recently IGPD has been shown to be the target of the triazole phosphonates, a novel class of experimental herbicides (Hawkes *et al.*, 1993). The enzyme appears to have an unusual catalytic mechanism since there is no imine or carbonyl group α to the departing proton and the involvement of a high-energy diaza-fulvene intermediate has been proposed (Hawkes *et al.*, 1993). The enzyme activity has been identified from a number of sources, including plants, fungi and bacteria. Sequencing studies have indicated that the molecular weight of the IGPD subunit from yeast (Struhl, 1985; Weinstock & Strathern, 1993; Burke & Gould, 1994), *Trichoderma harzianum* (Goldman *et al.*, 1992), wheat (Mano *et al.*, 1993) and *Streptomyces coelicolor* (Limauro, Avitabile, Capellano, Puglia & Bruni, 1990) is generally around 23 kDa. However, in *Escherichia coli* and *Salmonella typhimurium*, the enzyme which carries the IGPD activity is a 40 kDa bifunctional enzyme composed of the dehydratase and the histidinol phosphate phosphatase, steps seven and nine, respectively, in the histidine biosynthetic pathway (Struhl & Davis, 1977; Carlomagno, Chiarotti, Alifano, Nappo & Bruni, 1988). Alignment of the amino-acid sequences show that there is significant homology between the entire polypeptide chain of the *S. cerevisiae* enzyme and the C-terminal portion of the *E. coli* enzyme, with an overall identity of 34.5%. Across the 12 IGPD's for which sequences are available, 35 residues are identical (15.5% identity) and a further 22 positions can be seen where the aligned sequences are strongly conserved.

Cell culture and protein purification

In order to initiate structural studies on IGPD, a semisynthetic yeast IGPD gene was overexpressed in *E. coli* using the strong leftward promoter from bacteriophage λ and an efficient ribosome-binding site. Expression was regulated by a plasmid encoded cI^{857} (temperature sensitive) repressor gene and IGPD expression was induced by shifting the fermentation temperature from 310 to 315 K. The cells were harvested 2 h post-induction and the recombinant enzyme was purified, without significant loss of activity, by fractional precipitation with ammonium sulfate, gradient elution from Mono Q anion-exchange resin and gel filtration down Superdex 200. The enzyme was initially purified as the 'apo' form in buffer containing EDTA. After reaction with excess Mn^{2+}, the enzyme was further purified and exchanged into metal–TME buffer down Superdex 200. The metalloenzyme thus generated contained approximately 1.3 Mn atoms per subunit and had a specific activity of *ca* 70 µmol imidazole acetol phosphate formed min^{-1} mg^{-1} at 303 K (Hawkes *et al.*, 1995).

Crystallization of form *A* crystals

Crystallization of form *A* crystals was carried out using the hanging-drop method of vapour diffusion by mixing 5 µl of the protein solution with 5 µl of the precipitant and equilibrating the drops over the precipitant at 290 K. Trials with ammonium sulfate in the concentration range 30–36% saturated in 0.1 *M* bis-tris-propane buffer, pH 8, containing 0.35 m*M* Mn^{2+}, 0.2 *M* KCl and 10 m*M* β-mercaptoethanol with a protein concentration of 12 mg ml^{-1} resulted in the formation of large pyramidal crystals. These crystals were generally imperfectly formed and often had a combination of both well and poorly defined edges and faces. They had a maximum dimension of 0.7 mm and could be stabilized in the crystallization buffer above with 40% ammonium sulfate. Crystals with a similar morphology could also be produced using enzyme purified in the presence of cobalt or cadmium instead of manganese. X-ray precession photographs of the form *A* crystals showed that

they were poorly ordered, diffracting only to 6 Å resolution and that they belong to the cubic system, point group 432 with *hkl* reflections systematically absent when either $h + k$, $h + l$, or $k + l$ are odd confirming that the space group is $F432$. The cell dimension is a = 177.5 Å and the cell volume is, therefore, 5.59×10^6 Å^3. If the asymmetric unit contains a single subunit (M_r = 23 kDa) then the V_m is 2.5 Å^3 Da^{-1} which is well within the range given by Matthews (1977) whereas values for a dimer or trimer fall outside the range.

Crystallization of form *B* crystals

Crystallization of form *B* crystals was carried out as above using PEG 4000 as the precipitant in the range 14–25% in 0.1 *M* citrate buffer, pH 6, containing 0.35 m*M* $MnCl_2$, 0.1 *M* KCl and 10 m*M* β-mercaptoethanol with a protein concentration of 6 mg ml^{-1}. This resulted in the formation of flat rhombohedral crystals of maximum dimensions 0.2 × 0.5 × 0.5 mm. Test data were collected on the two-detector San Diego Multiwire area detector (Hamlin, 1985) and Rigaku AFC6 goniostat system mounted on a Rigaku RU 200 rotating Cu anode generator running at 50 kV, 100 mA with a 0.3 × 3 mm cathode, graphite monochromator and 0.5 mm collimator. Data were collected to 6 Å using ω-scans of 0.15° per frame, using the 'rachetting' protocol of Xuong, Nielsen, Hamlin & Anderson (1985). A total of 9572 observations were made on one crystal. Data were processed using the software written by Howard, Nielsen & Xuong (1985) to provide a unique set of 748 reflections with a merging R factor of 9.7%. Examination of the diffraction patterns indicate that the form *B* crystals belong to the cubic system point group 23 with cell dimension a = 131.0 Å and a cell volume of 2.25×10^6 Å^3. Examination of these data revealed that reflections with indices $h + k + l$ = odd are systematically absent identifying the space group as one of a special pair either $I23$ or $I2_13$. If the asymmetric unit contains a dimer or a monomer then the V_m is 2.0 or 4.0 Å^3 Da^{-1}, respectively, which are both within the range given by Matthews (1977) whereas the value for a trimer falls outside the range. A self-rotation function calculated with the program *POLARRFN* (W. Kabsch, unpublished work; Collaborative Computational Project, Number 4, 1994) using data from the form *B* crystals, from 10 to 6.5 Å and a 35 Å radius of integration, showed peaks equal to 96% of the origin on the κ = 90° section in the direction of the cell axes (see Fig. 1). This implies that the cell axes show pseudo fourfold symmetry and that the crystals underlying cell symmetry is consistent with a pseudo 432 point group, as observed for the form *A* crystals. This evidence would seem to indicate that the true space group for the form *B* crystals is most probably $I23$ with a dimer in the asymmetric unit.

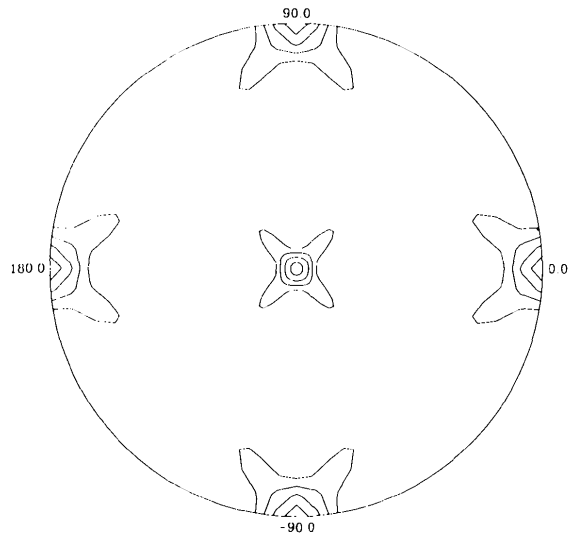

Fig. 1. Section $\kappa = 90°$ of a self-rotation function calculated with *POLARRFN* (W. Kabsch, unpublished work; Collaborative Computational Project, Number 4, 1994) using data from the form *B* crystals, from 10 to 6.5 Å and a 35 Å radius of integration. The peaks shown are the pseudo fourfold axes which are equal to 96% of the origin and occur in the direction of the cell axes.

Light-scattering experiments

The initial purification of IGPD from *S. cerevisae* was carried out in the absence of any metal ions and the molecular weight of this 'apo' form was found to be 70 kDa from gel filtration, implying a trimeric quaternary structure. Whilst the purified enzyme appeared as a single band on sodium dodecyl sulfate gel, dynamic light-scattering experiments using an Oros Instruments 501 detector on solutions of this enzyme showed considerable polydispersity but with a molecular weight comparable with gel-filtration studies. However, light-scattering studies on IGPD solutions in the presence of excess Mn^{2+} showed considerably different behaviour. These studies indicate that the particle has a molecular weight in the region of 500 kDa with a radius of approximately 66 Å. Further studies were carried out with different metal ions and it has been established that the enzyme also assembles to the higher molecular weight form in solutions containing an excess of Co^{2+}, Ni^{2+} or Zn^{2+} (Hawkes *et al.*, to be published). Taken together with the X-ray data these results are consistent with the view that the most likely quaternary structure for this enzyme is based on a 24-mer in 432 symmetry to give a particle of molecular weight of 572 kDa. If we assume that the enzyme is approximately spherical then the cell dimensions of the two crystal forms *A* and *B* indicate a radius of 63 Å (based on the separation between equivalent positions across the face centre) and 57 Å (based on the separation between equivalent positions across the body diagonal), respectively, for the 24-mer. These values are in good agreement with that determined from the light-scattering results.

Unfortunately, neither of the two crystal forms produced to date diffract beyond 6 Å resolution and, therefore, it is not possible at this stage to obtain a high-resolution structure for IGPD, however further crystallization trials are in progress. Attempts to solve the low-resolution structure are currently under way and should provide more details on the subunit organization and assembly.

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