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Crystallization and preliminary X-ray analysis of a hydantoinase from *Arthrobacter aurescens* DSM 3745

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Abstract

L-Hydantoinase from *Arthrobacter aurescens* DSM 3745 has been purified to homogeneity and crystallized from polyethylene glycol solutions in a form suitable for X-ray diffraction analysis. The crystals have been grown by the sitting-drop variant of the vapour-diffusion method. X-ray diffraction studies show that the crystals belong to the monoclinic space group $P2_1$ with $a = 111.2$, $b = 74.4$, $c = 146.5$ Å and $\beta = 106.7°$. Its asymmetric unit contains four monomers related by 222 symmetry. The crystals diffract to at least 2.6 Å.

1. Introduction

Hydantoinases are cyclic amidases (E.C. 3.5.2) (Webbs, 1992) which catalyse a reversible ring-opening hydrolysis of 5′-monosubstituted hydantoins. They are used as biocatalysts for the stereospecific production of optically active compounds which are valuable synthons for the synthesis of antibiotics, sweeteners, pesticides, pharmaceuticals and biologically active peptides (Syldatk & Pietzsch, 1995).

According to their stereoselectivity, hydantoinases have been classified as L- (Yamashiro, Kubota & Yokozeki, 1988), non- or D-enantiomer specific (Möller, Syldatk, Schulze & Wagner, 1988; Yokoseki & Kubota, 1987). D-enantiomer specific hydantoinases have been described in animals, plants and bacteria and were considered as dihydropyrimidinases (E.C. 3.5.2.2) which are described to be directly involved in the catabolic metabolism of pyrimidines (Vogels & Van der Drift, 1976). However, a D-hydantoinase from *Agrobacterium* species (Runser & Meyer, 1993) has been purified that does not exhibit dihydropyrimidine amidohydrolase activity. Additionally, natural functions of either L- or non-specific hydantoinases remain unclear.

Biochemical properties and substrate specificities of various hydantoinases are well characterized (for a review see Syldatk, Müller, Siemann, Krohn & Wagner, 1992) but, to the best of our knowlegde, no structural information on atomic resolution has yet been published. In order to obtain information on the tertiary structure of a stereospecific hydantoinase, we crystallized the L-hydantoinase from *Arthrobacter aurescens* DSM 3745. We expect to gain insight into the relationship between the structure of the enzyme, its stereospecificity as well as substrate specificity. Details of its reaction mechanism and other various closely related cyclic amidases may also be obtained and could throw light on their natural functions.

2. Materials and methods

L-Hydantoinase was purified to homogeneity using ammonium sulfate precipitation, hydrophobic interaction and anion-exchange chromatography (Erck, 1993). Homogeneity of the enzyme was checked by standard sodium dodecyl sulfate polyacrylamide gel electrophoresis, isoelectric focusing and MALDI-MS. The purified enzyme was concentrated by ultrafiltration to a concentration of 10 g l^{-1} using an Omega cell membrane (Filtron) with an exclusion limit of 30 kDa. Crystallization was achieved by the sitting-drop variant of the vapour-diffusion method (Ducruix & Giegé, 1992) using Cryschem crystallization plates. A 2 µl droplet containing 10 mg ml^{-1} protein in 0.1 *M* Tris pH 8.0 was mixed with an equal volume of reservoir solution, as described below. Each well contained 1 ml of reservoir solution. Initial conditions were established by the factorial experiment (Jancarik & Kim, 1991) using a Biomek automated laboratory station (Beckman, Mannheim, Germany). X-ray difraction data sets were collected with a Siemens electronic area detector and Cu $K\alpha$ radiation. The X-rays were generated by a Rigaku rotating anode, operating at 50 kV and 100 mA. They were monochromized by a graphite lattice and sharpened by a 0.3 mm collimator. Crystals were mounted in glass capillaries with a drop of mother liquor on a Rigaku three-axis goniometer. The crystal-to-detector distance was 130 mm. The frame width was 10 min arc in with an exposure time of 10 min. All measurements were carried out at 283 K. The crystals were stable in the X-ray beam for more than 5 d. Data were processed using the *XENGEN* 2.0 software (Howard *et al.*, 1987) and the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). For determination of non-crystallographic symmetry, self-rotation functions were calculated using *X-PLOR* 3.1 (Brünger, 1992).

3. Results and discussion

Crystals could be grown from solutions containing polyethylene glycol as precipitant. The optimized conditions were as follows:

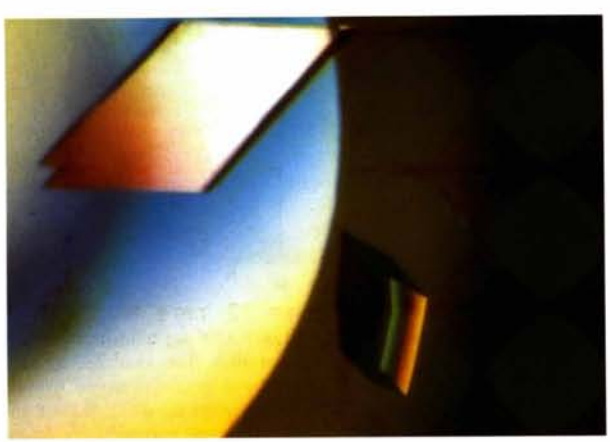

Fig. 1. Typical crystals of L-hydantoinase from *Arthrobacter aurescens* DSM 3745

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Table 1. *Native data set statistics of Arthrobacter aurescens DSM 3745 L-hydantoinase*

D (Å)	$I/\sigma(I)$*	No. of observed reflections	No. of unique reflections	Percentage observed (%)	Multiplicity	R_{sym} (%)
8.06	16.1	5780	2341	94.3	2.5	1.5
5.76	14.2	10811	4229	99.9	2.6	3.0
4.74	13.5	13145	5383	99.8	2.4	4.5
4.10	13.3	14938	6304	99.4	2.4	6.0
3.57	11.1	15888	7096	99.2	2.2	7.4
3.35	8.9	15978	7719	97.9	2.1	8.9
3.10	6.5	15563	8128	95.0	1.9	10.4
2.90	4.5	14471	8335	90.7	1.7	11.9
2.74	3.3	12287	8009	82.2	1.5	13.3
2.60	2.5	5400	4219	41.2	1.3	14.8
Total	8.5	124261	61763	86.6	2.0	9.1

*$I/\sigma(I)$ was calculated with *TRUNCATE* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

the reservoir solution consisted of 16–18%(w/v) polyethylene glycol 8000, 0.25 M Li_2SO_4 and 0.5 mM $MnCl_2$ in 0.1 M 2-(N-morpholino)ethanesulfonic acid/Tris(hydroxymethyl)-aminomethane buffer pH 8.5. The sitting drop, before mixing with reservoir, consists of 10 g l^{-1} L-hydantoinase in 0.1 M Tris buffer pH 8.0. Crystals grew to their full size (0.3 × 0.4 × 0.7 mm) within two weeks at 292 K. The photograph (Fig. 1) shows the typical rhombic prismoidal habit of the crystals. The crystals diffract X-rays to at least 2.6 Å resolution. A data set which was complete up to 3 Å resolution was collected (Table 1) merging diffraction data from two different crystals. These belong to the monoclinic space group $P2_1$ with lattice constants of a = = 111.2, b = 74.38 and c = 146.51 Å and β = 106.69°. The diffraction data could be scaled to a symmetry R value of 9.1% [$R_{sym} = \sum_{hkl}\sum_i I_i(hkl) - \langle I_i(hkl)\rangle / \sum_{hkl}\sum_i I_i(hkl)$] (Table 1).

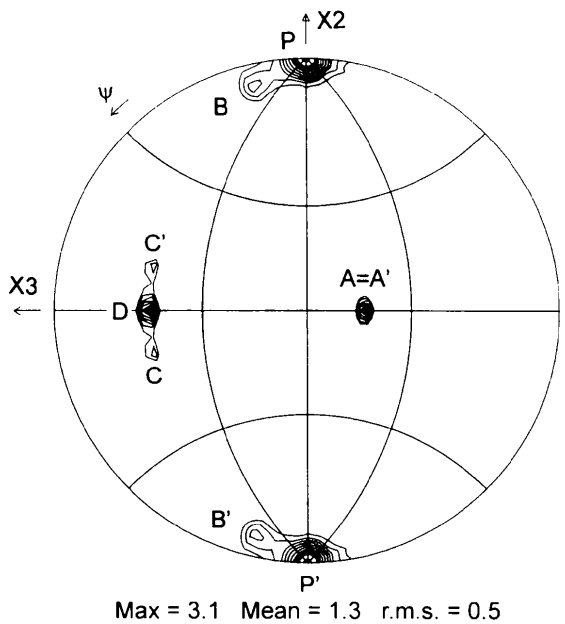

Fig. 2. Self-rotation function at κ section = 180° for reflexions between 15 and 5 Å and a Patterson vector of 40 Å. Two local 222 systems [(A,B,C), (A',B',C')] are connected by a crystallographic twofold screw axis which runs along $X2$ (crystallographic b axis). Peaks A and A' are mapped onto one another as they are perpendicular to the crystallographic twofold screw axis. For the same reason they are accompanied by a Klug peak (D).

Assuming one homotetrameric enzyme of a molecular mass of 200 kDa (according to MALDI-MS) in the asymmetric unit, a crystal packing parameter (Matthews, 1968) of V_m = 2.9 Å^3 Da^{-1} was calculated corresponding to 58% solvent. A plot of a 180° self-rotation function (Fig. 2) showing two 222 systems rotated by crystallographic symmetry is consistent with that assumption.

With the aim of solving the tertiary structure of hydantoinases, molecular replacement calculations are in progress using the alpha-subunit of urease from *Klebsiella aerogenes* (Jabri, Beth, Hausinger & Karplus, 1995) which shows a sequence identity of 22% over 514 overlapping amino acids (May, Siemann, Wiese & Syldatk, 1996).

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