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Preparation and preliminary X-ray diffraction studies of a new crystal form of L-asparaginase from *Escherichia coli*

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L-Asparaginase is an enzyme which hydrolyzes asparagine to produce aspartic acid and ammonia. It is an effective chemotherapeutic drug, especially in the treatment of acute lymphoblastic leukaemia in children. The enzyme from *Escherichia coli* was crystallized in a new crystal form with space group $C2$, unit-cell parameters a = 76.3 (0), b = 134.6 (2), c = 64.8 (7) Å, β = 110.5 (1)° and a dimer in the asymmetric unit. Synchrotron-radiation diffraction data have been collected to 1.95 Å resolution.

1. Introduction

L-Asparaginase, the enzyme which hydrolyzes asparagine to produce aspartic acid and ammonia, is isolated from various bacterial sources and is an effective and widely used chemotherapeutic drug, especially in the treatment of acute lymphoblastic leukaemia in children (Chakrabarti & Schuster, 1997; Hill *et al.*, 1967). The enzyme works by depleting the tumour of its source of asparagine from the circulation, as tumours, especially of lymphoid origin, are incapable of intracellular asparagine synthesis and are L-asparaginase dependent. Despite its effectiveness, however, the use of L-asparaginase as a chemotherapeutic agent is somewhat limited because of its toxicity, side effects and the spontaneous acquisition of resistance to this enzyme by tumour cells (Alberts *et al.*, 1999; Ettinger *et al.*, 1997; Sadoff *et al.*, 1997; Tozuka *et al.*, 1997).

Rational modifications of L-asparaginase as a drug, directed toward improvements in stability and lowering of side effects, depend to a large extent on the availability of a well refined three-dimensional structure of the enzyme and a deeper understanding of its conformational flexibility. Crystallographic structures of bacterial L-asparaginases from *Erwinia chrysanthemi* (Miller *et al.*, 1993), *Pseudomonas* 7a (Lubkowski *et al.*, 1994; Jakob *et al.*, 1997), *Wolinella succinogenes* (Lubkowski *et al.*, 1996) and *Escherichia coli* (Swain *et al.*, 1993; Palm *et al.*, 1996) have been described.

The active *E. coli* L-asparaginase is a homotetramer of 142 kDa, with 326 amino-acid residues per monomer. The native *E. coli* enzyme has previously been crystallized in the monoclinic space group $P2_1$ and the structure has been refined to 2.3 Å resolution (Swain *et al.*, 1993), whereas the T98V mutant of the enzyme produced crystals in the orthorhombic space group $P2_12_12_1$ and diffracted to 2.2 Å resolution (Palm *et al.*, 1996). Both crystal forms contain one tetramer of L-asparaginase per asymmetric unit cell.

Here, we report a new crystal form of the native *E. coli* enzyme in the monoclinic space group $C2$ with one dimer per asymmetric unit; the crystals diffract to 1.95 Å resolution at the synchrotron source.

2. Methods, results and discussion

L-asparaginase was purchased in a lyophilized form from Merck, Sharp and Dohme and was used in crystallization trials without further purification. Preliminary screening of the crystallization conditions was performed using a sparse-matrix screen at 291 K (Crystal Screens I and II, Hampton Research Corp.) and refinement of the crystallization conditions was then carried out. The crystals were grown at room temperature using the hanging-drop vapour-diffusion technique by mixing equal volumes (2 µl + 2 µl) of a protein solution concentrated to 20 mg ml^{-1} and a reservoir solution containing 30% 2-methyl-2,4-pentanediol, 4% PEG 3350, 0.1 *M* 2-(*N*-morpholino)ethanesulfonic acid buffer pH 6. Crystals of dimensions 0.5 × 0.3 × 0.2 mm appeared after one week and were brought to the dedicated protein crystallography beamline (Polikarpov, Oliva *et al.*, 1997; Polikarpov, Perles *et al.*, 1997) at the Laboratório Nacional de Luz Síncrotron (Campinas, SP, Brazil) for data collection.

X-ray diffraction data were collected at room temperature using a MAR345 image plate. The synchrotron-radiation wavelength was set to 1.38 Å to optimize the X-ray flux and minimize absorption errors. The first image was subjected to the autoindexing routine of *DENZO* (Otwinowski, 1993), from which the best refined solution was a *C*-centred orthorhombic cell. Following an optimum strategy of

Table 1
Data-collection statistics.

Values for the highest resolution shell (2.0–1.95 Å) are shown in parentheses.

Resolution (Å)	13.0–1.95
Number of observations	63394
Number of unique reflections	40783
$\langle I/\sigma(I)\rangle$	12.7 (2.4)
Multiplicity	1.58 (1.51)
Completeness (%)	91.8 (93.2)
$R_{\rm merge}$ (%)	4.7 (32.5)

data collection suggested by the program *MARHKL*, a total of 70° of data were collected in steps of 1°. The collected images were processed and scaled with *DENZO* and *SCALEPACK* (Otwinowski, 1993). The crystals belong to the space group *C*2 with unit-cell parameters a = 76.3 (0), b = 134.6 (2), c = 64.8 (7) Å, β = 110.5 (1)°. Data-collection statistics shown in Table 1.

The Matthews value (Matthews, 1968) suggested the presence of one dimer per asymmetric unit (V_m = 2.19 Å^3 Da^{-1}). The structure was solved by the molecular-replacement method with *AMoRe* (Navaza, 1994), using a dimer of L-asparaginase refined in space group $P2_1$ (PDB code 3eca) as the search model. The rotation function was calculated using diffraction data in the resolution range 10.0–4.0 Å using a Patterson radius of 36 Å. The rotation function gave a clear solution with a correlation coefficient (CC) of 0.318, with the next highest peak having a CC of 0.16. The translation search was performed in the same resolution range using the Crowther & Blow (1967) translation function. The search gave a strong solution with a CC of 0.799 and an R factor of 27.4%, which was then subjected to 20 cycles of rigid-body refinement against all data between 10.0 and 3.3 Å resolution using the fitting function of *AMoRe*. The resulting R factor and CC were 26.4% and 0.822, respectively. Further refinement of the structure is in progress.

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