Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

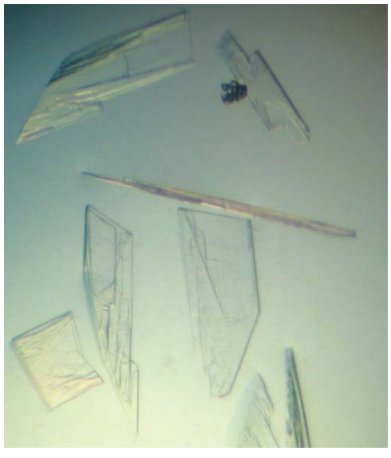
Crystallization and preliminary crystallographic analysis of L-asparaginase from *Erwinia carotovora*

Linnea E. K. Wikman,[a] Julya Krasotkina,[b] Anastasia Kuchumova,[b] Nikolay N. Sokolov[b] and Anastassios C. Papageorgiou[a]*

[a]Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku 20521, Finland, and [b]Institute for Biomedical Chemistry, Russian Academy of Medical Sciences, 559-B, 10 Pogodinskay St, Moscow 119121, Russia

Correspondence e-mail: tassos.papageorgiou@btk.fi

Received 9 February 2005
Accepted 15 March 2005
Online 24 March 2005



Bacterial L-asparaginases have been used as therapeutic agents in the treatment of acute childhood lymphoblastic leukaemia for over 30 y. However, their use is limited owing to the glutaminase activity of the administered enzymes, which results in serious side effects. In contrast, L-asparaginase from *Erwinia carotovora* exhibits low glutaminase activity at physiological concentrations of L-asparagine and L-glutamine in the blood. Recombinant *Er. carotovora* L-asparaginase was crystallized in the presence of L-glutamate by the hanging-drop vapour-diffusion method using 10 mg ml^{-1} purified enzyme, 16–18%(*w*/*v*) PEG 3350 and 0.2 *M* NaF. X-ray diffraction data were collected to 2.6 Å at 293 K using an in-house rotating-anode generator. The crystals belong to the monoclinic $P2_1$ space group, with unit-cell parameters a = 78.0, b = 112.3, c = 78.7 Å, β = 101.9° and a homotetramer in the crystallographic asymmetric unit. A molecular-replacement solution has been found and refinement is currently in progress. The crystal structure may provide leads towards protein-engineering efforts aimed at safer asparaginase administration in leukaemia treatment.

1. Introduction

L-Asparaginase (L-asparagine amidohydrolase; EC 3.5.1.1) is an enzyme that catalyzes the conversion of L-asparagine to L-aspartate and ammonia and to a lesser extent the formation of L-glutamate from L-glutamine. Similar to asparaginases, enzymes known as glutaminase-asparaginases that are able to catalyze the hydrolysis of both L-asparagine and L-glutamine with comparable efficiency have been identified. Crystallographic structures of bacterial L-asparaginases from *Escherichia coli* and *Erwinia chrysanthemi* (Swain *et al.*, 1993; Aghaiypour *et al.*, 2001; Sanches *et al.*, 2003; Lubkowski *et al.*, 2003; Kozak *et al.*, 2002) and L-glutaminase-asparaginases from *Pseudomonas* 7a and *Wolinella succinogenes* (Lubkowski *et al.*, 1994, 1996) have provided insights into the active site of these enzymes. Moreover, plant-type asparaginases have been identified and the sequence similarities of one of them, namely *Lupinus luteus* L1A, to the *E. coli* periplasmic asparaginase EcAIII have been reported (Borek *et al.*, 2004).

Administration of L-asparaginase has been found to reduce L-asparagine levels in the blood and to selectively inhibit malignant growth (Duval *et al.*, 2002). This is explained by the fact that certain tumours, especially acute lymphoblastic leukaemia (ALL) tumour cells, have a decreased or completely absent activity for asparagine synthase and hence are nutritionally dependent on an external supply of L-asparagine for growth. Accordingly, L-asparaginases from *E. coli* and *Er. chrysanthemi* have been used as therapeutic agents since the early 1970s. However, asparaginase-based therapy often produces side effects, including hypersensitivity reactions, leukopenia, neurological seizures, pancreatitis and hyperglycaemia. The toxicity of the administered asparaginases is partially attributed to an intrinsic glutaminase activity of the enzymes, which results in L-glutamine depletion in the blood. Although L-asparaginases from *Er. chrysanthemi* and *E. coli* show the lowest toxicity of the asparaginases with known anti-tumour activities, the need for new asparaginases, especially from *Erwinia* strains, is of great interest for biomedicine and biotechnology. Elimination of the glutaminase activity would

result in better and safer asparaginases for clinical use. L-Asparaginase from *Er. carotovora* (75 and 45% sequence identity to *Er. chrysanthemi* and *E. coli* L-asparaginases, respectively) has been found to possess a relatively low glutaminase activity (2.6% of the asparaginase activity compared with 10% in *Er. chrysanthemi* L-asparaginase), thus providing a promising starting point for protein-engineering studies of the active site of the enzyme (Krasotkina *et al.*, 2004). We have initiated structural studies on *Er. carotovora* asparaginase and report here the crystallization of the enzyme in the presence of glutamate and the preliminary characterization of the crystals.

2. Experimental methods

The protein was expressed and purified as described previously (Krasotkina *et al.*, 2004). The pure protein (MW 34 kDa in SDS–PAGE gel) was concentrated to 10 mg ml^{-1} in 10 m*M* phosphate buffer pH 7.0. Initial crystallization trials were carried out using the hanging-drop vapour-diffusion method at 289 K and Hampton Crystal Screen I. The drops contained 2 µl protein solution and an equal volume of precipitant solution. Inspection of the drops after 1 d revealed the presence of needle-like crystals in several conditions in which PEG was used as the main precipitant. The PEG/Ion screen from Hampton Research was subsequently used to identify the optimum salt condition for the improvement of the crystals. Rod-like crystals and needles appeared under various conditions. In addition, thin plate-like crystals were found to grow in the presence of 0.2 *M* NaF, KF or NH_4F (conditions 1, 2 and 3 of the PEG/Ion screen, respectively). For an initial evaluation of their quality, crystals were placed in quartz capillaries and exposed to X-rays produced by a Rigaku RU-200 generator operated at 50 kV and 100 mA and equipped with Osmic confocal mirrors and a copper target (Cu $K\alpha$; λ = 1.5418 Å). Diffraction data were recorded on a MAR345 imaging-plate detector. The rod-like crystals were found to diffract poorly (∼10 Å). A plate-like crystal was found to diffract to approximately 4 Å resolution and the growth conditions of this crystal were chosen for further optimization. Prior to crystallization, the protein was mixed with sodium L-glutamate solution pH 8.5 to a final concentration of 10 m*M*. Crystals suitable for crystallographic analysis and structure determination were subsequently obtained in the presence of 16–18%(*w*/*v*) PEG 3350, 0.2 *M* NaF (Fig. 1). Different pH values had no effect on the appearance of the crystals. Data to 2.6 Å resolution were collected at room temperature (exposure time 5 min, crystal-to-detector distance 225 mm) from a single crystal. The crystal was translated in the beam twice during data collection and a total of 86 images (1.5° rotation for each image) were recorded. Raw intensities were integrated, scaled and merged using the *XDS* program suite (Kabsch, 1993). Data statistics are given in Table 1.

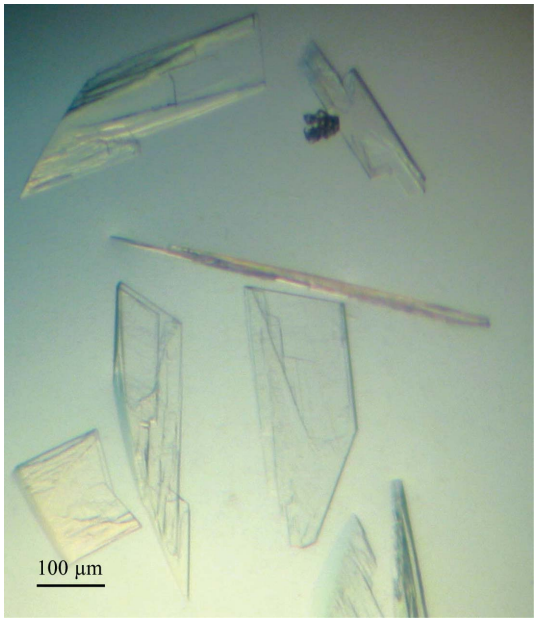


Figure 1
Typical crystals of *Er. carotovora* L-asparaginase grown in the presence of 10 m*M* sodium L-glutamate.

Table 1
Data-collection statistics.

Values in parentheses are for data in the highest resolution shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 78.0, b = 112.3, c = 78.7, β = 101.9
Resolution range (Å)	20–2.6 (2.8–2.6)
Wavelength (Å)	1.5418
Temperature (K)	293
Total reflections	100826
Unique reflections	38608
Completeness (%)	94.5 (91.7)
$R_{\rm merge}$ (%)	12.9 (38.5)
Average $I/\sigma(I)$	7.2 (2.5)
Mosaic spread (°)	0.51

3. Results and discussion

Crystals of *Er. carotovora* L-asparaginase belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 78.0, b = 112.3, c = 78.7 Å, β = 101.9°. Assuming the presence of four molecules (34 kDa each) in the crystallographic asymmetric unit, the Matthews coefficient $V_{\rm M}$ (Matthews, 1968) is 2.48 Å^3 Da^{-1}, corresponding to a solvent content of 50.0%. This is in agreement with the presence of homotetramers, as found in the crystals of other asparaginases. Different combinations of salts and PEG 3350 were used to obtain crystals of *Er. carotovora* asparaginase that were suitable for crystallographic analysis. Of the salts tried, the use of NaF provided the best crystals for further crystallographic studies. The crystal was sensitive to X-rays, as reflected by the relatively high overall $R_{\rm merge}$ (12.9%). In order to collect a complete data set, the crystal was translated twice to overcome the deterioration of the diffraction arising from radiation damage.

Crystal structure determination has been pursued by molecular replacement with the program *AMoRe* (Navaza & Saludjian, 1997) using a polyalanine model of *Er. chrysanthemi* L-asparaginase (PDB code 1hfw). Data in the 8.0–3.5 Å resolution range were used for the rotation and translation searches. The top solution from the rotation function was used in the translation search to locate the first of the four molecules. A correlation coefficient of 59.1% and an R factor of 40.0% were obtained when all four molecules were positioned. Inspection of crystal-packing contacts revealed no major clashes. Rigid-body refinement in *CNS* v.1.1 (Brünger *et al.*, 1998) followed by calculation of an averaged electron-density map with the program *DM* (Collaborative Computational Project, Number 4, 1994) was used to place side chains. Alternate cycles of manual rebuilding and refinement with *CNS* v.1.1 have resulted in a current $R_{\rm cryst}$ and $R_{\rm free}$ (with 5% of the reflections put aside) of 21.2 and 24.4%, respectively. Inspection of the difference Fourier electron-density map has shown the presence of an L-glutamate moiety in the active site of each

subunit. Further refinement and placement of water molecules and the ligands are in progress. As a next step, a higher resolution data set will be collected using synchrotron radiation. Determination of the crystal structure of *Er. carotovora* L-asparaginase will provide suggestions for protein-engineering efforts and the design of mutants for future biochemical and structural studies.

Financial support from the Academy of Finland, Sigrid Jusélius Foundation and the Russian Fund for Basic Research is greatly acknowledged.

References

Aghaiypour, K., Wlodawer, A. & Lubkowski, J. (2001). *Biochemistry*, **40**, 5655–5664.
Borek, D., Michalska, K., Brzezinski, K., Kisiel, A., Podkowinski, J., Bonthron, D. T., Krowarsch, D., Otlewski, J. & Jaskólski, M. (2004). *Eur. J. Biochem.* **271**, 3215–3226.
Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, N., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Duval, M., Suciu, S., Ferster, A., Rialland, X., Nelken, B., Lutz, P., Benoit, Y., Robert, A., Marel, A.-M., Vilmer, E., Otten, J. & Philippe, N. (2002). *Blood*, **99**, 2734–2739.
Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.
Kozak, M., Borek, D., Janowski, R. & Jaskólski, M. (2002). *Acta Cryst.* D**58**, 130–132.
Krasotkina, J., Borisova, A. A., Gevaziev, Y. V. & Sokolov, N. N. (2004). *Biotechnol. Appl. Biochem.* **39**, 215–221.
Lubkowski, J., Dauter, M., Aghaiypour, K., Wlodawer, A. & Dauter, Z. (2003). *Acta Cryst.* D**59**, 84–92.
Lubkowski, J., Palm, G. J., Gilliland, G. L., Derst, C. & Rohm, K.-H. (1996). *Eur. J. Biochem.* **241**, 201–207.
Lubkowski, J., Wlodawer, A., Annon, H. L., Copeland, T. D. & Swain, A. L. (1994). *Biochemistry*, **33**, 10257–10265.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. & Saludjian, P. (1997). *Methods Enzymol.* **276**, 581–584.
Sanches, M., Barbosa, J. A. R. G., de Oliveira, R. T., Abrahão Neto, J. A. & Polikarpov, I. (2003). *Acta Cryst.* D**59**, 416–422.
Swain, A. L., Jaskólski, M., Housset, D., Rao, J. K. M. & Wlodawer, A. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 1474–1478.