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Crystallization and preliminary crystallographic analysis of porcine acylaminoacyl peptidase

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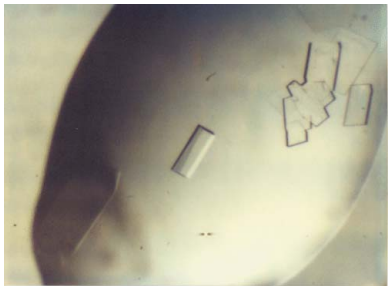

Acylaminoacyl peptidase (also known as acylamino-acid-releasing enzyme or acylpeptide hydrolase; EC 3.4.19.1) is an unusual member of the prolyl oligopeptidase family catalysing the hydrolysis of an *N*-acylated peptide to an acylamino acid and a peptide with a free N-terminus. Acylaminoacyl peptidase purified from porcine liver has been crystallized in mother liquor containing 0.1 *M* Tris–HCl pH 7.0, 10%(*w*/*v*) polyethylene glycol 8000, 50 m*M* $MgCl_2$ and 1%(*w*/*v*) CHAPS using the hanging-drop vapour-diffusion technique. A full data set to 3.4 Å resolution was collected at ESRF beamline ID14-4 and space group *C*222 was assigned, with unit-cell parameters a = 84.8, b = 421.1, c = 212.0 Å and four molecules in the asymmetric unit.

1. Introduction

Acylaminoacyl peptidase (also known as acylamino-acid-releasing enzyme or acylpeptide hydrolase; EC 3.4.19.1) is a member of the prolyl oligopeptidase family, which is a subfamily of the serine proteases. Prolyl oligopeptidases bear little sequence resemblance to classic serine proteases such as trypsin and subtilisin except for a consensus motif Gly-*X*-Ser-*X*-Gly that contains the active-site serine (Brenner, 1988). As for all serine oligopeptidases, acylaminoacyl peptidase has an active site consisting of three catalytically competent residues, serine, histidine and aspartic acid (Polgár, 1992; Rawlings *et al.*, 1991; Jones *et al.*, 1994), in this case Ser587, Asp675 and His707. Oligopeptidases select oligopeptide substrates that are comprised of not more than about 30 amino-acid residues. Acylaminoacyl peptidase (AAP) is unique in the prolyl peptidase family owing to its substrate preference. It is a cytoplasmic exopeptidase catalysing the hydrolysis of an N-acylated peptide to an acylamino acid and a peptide with a free N-terminus (Tsunasawa *et al.*, 1975).

The biological role of acylaminoacyl peptidase is still unclear; however, it may have a role in moderating concentrations of acetylated peptides such as α-melanocyte-stimulating hormone (Jones & Manning, 1988). The absence of human AAP function has also been correlated with cell proliferation in small-cell lung carcinomas and renal carcinomas (Erlandsson *et al.*, 1991; Naylor *et al.*, 1989), but the potential role of the enzyme in the malignant state of these cell lines has not been established. More recently, acylaminoacyl peptidase in porcine brain has been shown to be a more sensitive target for organophosphorus compounds than acetylcholinesterase (Richards & Lees, 2002; Duysen *et al.*, 2001) and hence may be a potential site for cognitive-enhancing compounds (Richards *et al.*, 2000).

The crystal structure of prolyl oligopeptidase, an enzyme implicated in the metabolism of neuropeptides (Polgár, 2002), shows a peptidase domain with an α/β-hydrolase fold and a regulatory seven-bladed β-propeller (Fülöp *et al.*, 1998). The unusual structure of the β-propeller allows an explanation of how large structured peptides and proteins are excluded from the central cavity and are thus protected from accidental degradation. Crystal structures of another member of the prolyl oligopeptidase family, dipeptidyl peptidase IV, show that it is a dimer with an eight-bladed propeller (Engel *et al.*, 2003; Hiramatsu *et al.*, 2003; Oefner *et al.*, 2003; Rasmussen *et al.*, 2003; Thoma *et al.*, 2003). In contrast, mammalian acylaminoacyl peptidase is tetrameric. Feese *et al.* (1993) obtained suitable crystals

of the human enzyme (92% identity to the porcine enzyme), but no structure determination followed this work. Most recently, Bartlam *et al.* (2004) reported the crystal structure of acylaminoacyl peptidase from the thermophilic archeon *Aeropyrum pernix* K1. They showed it to have a seven-bladed propeller and to be dimeric. This enzyme is only distantly related to its mammalian counterpart, sharing only 20–27% sequence identity and differing in oligomeric state.

In this paper, we report the crystallization and preliminary X-ray diffraction studies of porcine liver acylaminoacyl peptidase (M_r = 81 239 per subunit) in the anticipation that the structure will provide a mammalian model for this enzyme and will provide useful insights into the tetrameric assembly of the subunits.

2. Materials and methods

2.1. Preparation of porcine liver acylaminoacyl peptidase

Acylaminoacyl peptidase from porcine liver was purified as previously described by Kiss *et al.* (2004). The enzyme was concentrated to 10 mg ml^{-1} for crystallization purposes and was stored in aliquots at 253 K until required.

2.2. Crystallization of acylaminoacyl peptidase

Initial crystallization trials were performed with screens from Hampton and Emerald Biostructures Inc. using the hanging-drop vapour-diffusion technique. Fine needles were obtained with condition No. 43 from Wizard Screen 2 from Emerald Biostructures Inc. [0.1 *M* Tris–HCl pH 7.0, 10%(*w*/*v*) PEG 8000 and 0.2 *M* $MgCl_2$] using 1 µl protein solution at 10 mg ml^{-1} (in 20 m*M* MES pH 6.5, 1 m*M* DTE) mixed with an equal volume of mother liquor. After optimization, crystals grew after 1 d at 291 K in mother liquor consisting of 0.1 *M* Tris–HCl pH 7.0, 10%(*w*/*v*) PEG 8000, 50 m*M* $MgCl_2$ and 1–2%(*w*/*v*) CHAPS (Fig. 1). Improved crystals were obtained by microseeding. A drop consisting of 1 µl acylaminoacyl peptidase and 1 µl mother liquor was mixed and immediately streaked with crushed acylaminoacyl peptidase crystals. Large crystals grew within several hours of setup.

2.3. Cryoprotection and chemical cross-linking

Exhaustive cryoprotection trials using various substances including oils, salts, sugars and polymers failed to find a suitable cryoprotectant for the acylaminoacyl peptidase crystals. Techniques adopted included soaks of various duration, the progressive addition of cryoprotectant over time and flash-freezing in liquid propane. As these were unsuccessful, a chemical cross-linking approach was used to stabilize the crystals prior to cryoprotection.

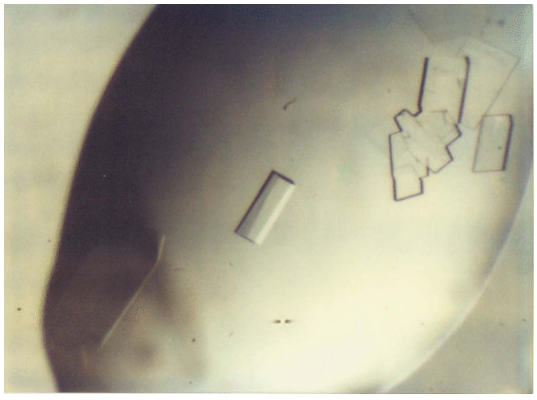

Figure 1
Photograph of an acylaminoacyl peptidase crystal; the largest dimension is 0.3 mm.

Table 1
Data-collection and processing statistics.

Values in parentheses refer to the highest resolution shell.

Synchrotron-radiation source	ESRF ID14-4
Detector	ADSC Q4
Wavelength (Å)	0.976
Space group	*C*222
Unit-cell parameters (Å)	a = 84.8, b = 421.1, c = 212.0
Molecules per AU	4
Matthews coefficient (Å^3 Da^{-1})	2.91
Solvent convent (%)	58
Resolution range (Å)	58–3.4 (3.52–3.40)
Total observations	317717
Unique reflections	98002
Average $I/\sigma(I)$	5.6 (1.6)
R_{merge}† (%)	0.183 (0.428)
Completeness (%)	97.3(95.7)

† $R_{merge} = \sum_j \sum_h |I_{h,j} - \langle I_h \rangle| / \sum_j \sum_h \langle I_h \rangle$, where $I_{h,j}$ is the jth observation of reflection h and $\langle I_h \rangle$ is the mean intensity of that reflection.

Acylaminoacyl peptidase crystals were cross-linked according to the method of Lusty (1999). A cover slip with a crystallization drop containing a crystal of acylaminoacyl peptidase was incubated for 20 min over a well containing 0.5 ml mother liquor and a microbridge with 2 µl of 25%(*v*/*v*) glutaraldehyde pH 3.0 placed in the well. The crystals were immediately cryoprotected by dipping in mother liquor containing 20% ethylene glycol for 2–3 s and were immediately frozen in an N_2 cryostream (Oxford Cryosystems).

2.4. X-ray diffraction analysis

A data set was collected from a native crystal at beamline ID14-4 at the ESRF, Grenoble to a resolution of 3.4 Å. Initially crystals diffracted to about 3 Å, but the resolution limit deteriorated after a few degrees of exposure. A typical diffraction image is shown in Fig. 2.

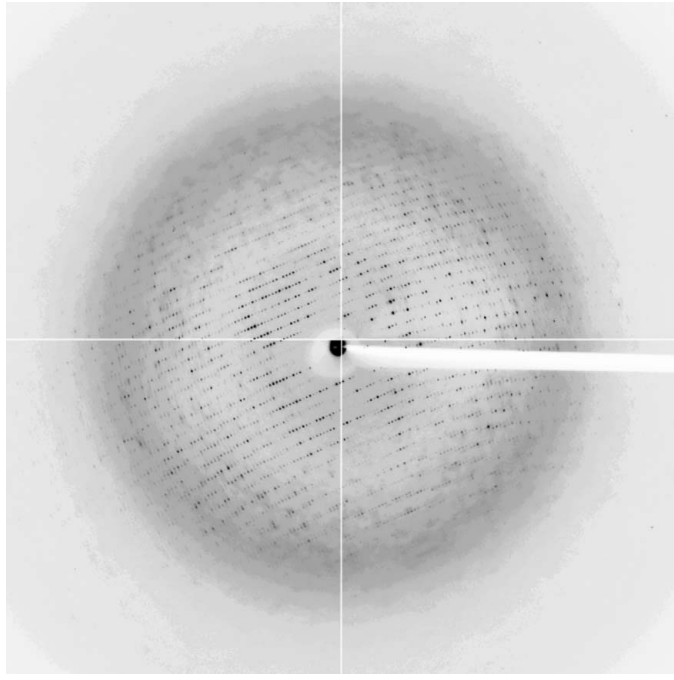

Figure 2
A typical diffraction image of an acylaminoacyl crystal collected at beamline ID14-4 at the ESRF using an ADSC Q4 CCD detector. The oscillation range was 1°, the resolution at the edge is 2.4 Å and the diffraction limit is about 3 Å.

All data were indexed, integrated and scaled using the *HKL* suite of programs (Otwinowski & Minor, 1997). The crystals belong to the orthorhombic space group *C*222. The Matthews probability calculation suggests four molecules (one tetramer or two half-tetramers) in the asymmetric unit, with a V_M value of 2.91 Å^3 Da^{-1}, corresponding to a solvent content of 58%. Data-collection and processing statistics are shown in Table 1. A self-rotation function did not reveal the presence of a non-crystallographic rotational axis. Porcine acylaminoacyl peptidase shares only 20% sequence identity with the enzyme from the thermophilic archeon *A. pernix* (Bartlam *et al.*, 2004) and low sequence identity coupled with the four molecules in the asymmetric unit resulted in the unsurprising failure of molecular replacement using the above coordinates. A search for heavy-atom derivatives is in progress.

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