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Crystallization and preliminary X-ray diffraction studies of the glutaminyl cyclase from *Carica papaya* latex

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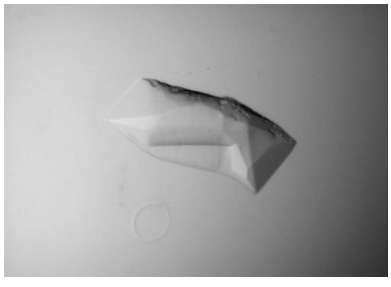

In living systems, the intramolecular cyclization of N-terminal glutamine residues is accomplished by glutaminyl cyclase enzymes (EC 2.3.2.5). While in mammals these enzymes are involved in the synthesis of hormonal and neurotransmitter peptides, the physiological role played by the corresponding plant enzymes still remains to be unravelled. Papaya glutaminyl cyclase (PQC), a 33 kDa enzyme found in the latex of the tropical tree *Carica papaya*, displays an exceptional resistance to chemical and thermal denaturation as well as to proteolysis. In order to elucidate its enzymatic mechanism and to gain insights into the structural determinants underlying its remarkable stability, PQC was isolated from papaya latex, purified and crystallized by the hanging-drop vapour-diffusion method. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 62.82, b = 81.23, c = 108.17 Å and two molecules per asymmetric unit. Diffraction data have been collected at ESRF beamline BM14 and processed to a resolution of 1.7 Å.

1. Introduction

Glutaminyl cyclases (QCs; EC 2.3.2.5) catalyse the conversion of N-terminal glutaminyl residues into pyroglutamic acid with the concomitant liberation of ammonia. QCs are widespread in mammalian neuroendocrine tissues (Sykes *et al.*, 1999; Bockers *et al.*, 1995; Wetsel *et al.*, 1995) and are highly conserved from humans to yeast (Pohl *et al.*, 1991; Song *et al.*, 1994; Bateman *et al.*, 2001). In mammals, this enzyme has been shown to be involved in the post-translation maturation of a number of bioactive secretory peptides and proteins (Busby *et al.*, 1987; Fisher & Spiess, 1987).

However, the first identified QC was isolated from the latex of the tropical plant *Carica papaya* in 1963 (Messer, 1963; Messer & Ottesen, 1965). Since then, putative QCs from other plants have been identified by sequence comparison (Dahl *et al.*, 2000; Azarkan *et al.*, 2002). Unlike mammalian QCs, the physiological function of plant QCs remains unknown (Amrani *et al.*, 1997), although a role in plant defence mechanisms has recently been suggested (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2004). Furthermore, QCs from plants and animals seem to belong to two distinct protein families (Dahl *et al.*, 2000; Schilling *et al.*, 2003) with no sequence homology between them.

The mature papaya glutaminyl cyclase (PQC) is a monomeric glycoprotein of 266 amino acids with a molecular weight of 32 980 Da (Pohl *et al.*, 1991; Zerhouni *et al.*, 1998), exhibiting a remarkable resistance to chemical and thermal denaturation (Zerhouni *et al.*, 1998), as well as to enzymatic proteolysis (Oberg *et al.*, 1998).

In order to elucidate the enzymatic mechanism of PQC and to gain further insights into the structural determinants underlying its exceptional stability, we have initiated the determination of its three-dimensional structure by X-ray crystallography. We report here the crystallization and preliminary X-ray study of the glutaminyl cyclase isolated from *C. papaya* latex. So far, no structure of a glutaminyl cyclase has been reported.

2. Purification

Glutaminyl cyclase was purified from *C. papaya* latex following a procedure derived from that previously reported by Zerhouni *et al.*

(1998) and Azarkan *et al.* (2003, 2004). In order to purify PQC, the soluble fraction of the collected plant latex was fractionated by ion-exchange chromatography on an SP-Sepharose Fast Flow column followed by hydrophobic interaction chromatography on Fractogel EMD Propyl 650 (S). The elution media and chromatographic conditions are described in detail in Azarkan *et al.* (2004). Prior to crystallization, the enzyme was further purified using thiophilic interaction chromatography. The chromatographic support was prepared by activation of Biogel A 0.5m with divinyl sulfone and subsequent treatment with 2-mercaptoethanol (Hansen *et al.*, 1998). The chromatographic conditions in this last purification step are identical to those used to perform the hydrophobic interaction chromatography on Fractogel EMD Propyl 650 (S). The purity of the protein was estimated to be higher than 98% by SDS–PAGE using silver staining.

Prior to crystallization, the protein was exhaustively dialyzed against ultrapure water and concentrated to 31.4 mg ml^{-1} on a 10 000 MWCO Vivaspin 15R concentrator (Sartorius). The protein concentration was determined at 280 nm using a calculated extinction coefficient of 55 190 M^{-1} cm^{-1} (Gill & von Hippel, 1989).

3. Crystallization

All crystallization experiments were performed using the hanging-drop vapour-diffusion method at 293 K. Initial crystals of PQC were grown in 3–4 d from 3 µl drops containing equal volumes of protein (31.4 mg ml^{-1} in water) and reservoir solution consisting of Cryokit (Sigma) solution No. 22. This condition was optimized and crystals suitable for X-ray diffraction studies were grown from 3 µl drops containing equal volumes of protein and reservoir solution [85 m*M* Tris–HCl pH 8.5, 0.17 *M* sodium acetate, 25%(*w*/*v*) PEG 4000 and 15%(*v*/*v*) glycerol] (Fig. 1). Crystals were mounted in cryoloops (Hampton Research) and flash-cooled by direct immersion into liquid nitrogen prior to X-ray diffraction analysis.

4. X-ray analysis

Diffraction data were initially collected in-house with a MAR345 imaging-plate system using Cu $K\alpha$ radiation generated by a Bruker–Nonius FR591 rotating-anode generator equipped with Osmic mirrors. Data were collected in 140 images with a crystal-to-detector distance of 175 mm and 1° oscillation per image. Diffraction data were processed and scaled using the *XDS* suite of programs (Kabsch, 1993). The PQC crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 64.94, b = 83.51, c = 112.75 Å, and diffract at least to 2 Å resolution. The data-collection statistics are summarized in Table 1. Based on the molecular weight of PQC (33 kDa) and space group $P2_12_12_1$, it is assumed that the crystal contains two molecules per asymmetric unit. This assumption gives a V_M value (Matthews, 1968) of 2.1 Å^3 Da^{-1} and a solvent content of 41%.

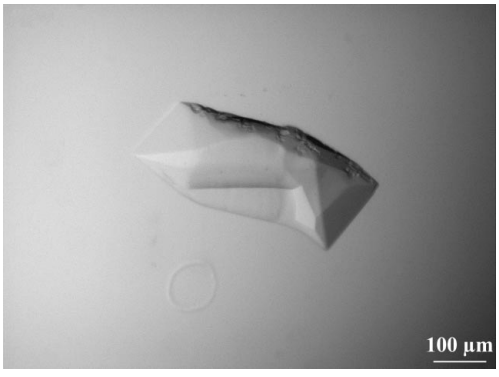

Figure 1
Representative crystal of papaya glutaminyl cyclase.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	Rotating anode	BM14 ESRF
Wavelength (Å)	1.5418	0.953718
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters		
a (Å)	64.94	62.82
b (Å)	83.51	81.23
c (Å)	112.75	108.17
Resolution range (Å)	30.0–2.04 (2.16–2.04)	30.0–1.70 (1.80–1.70)
Observed reflections	205637 (30011)	448581 (71180)
Unique reflections	39369 (6092)	61546 (9753)
R_{merge} (%)	3.7 (13.5)	5.5 (24.0)
Completeness (%)	99.2 (97.2)	99.8 (99.6)
$I/\sigma(I)$	27.1 (11.3)	22.2 (7.4)
$I > 3\sigma(I)$ (%)	91.2 (80.6)	87.4 (66.0)

Higher resolution diffraction data were collected on beamline BM14 at the ESRF. Data were collected in 180 images on a MAR CCD 225 mm detector with a crystal-to-detector distance of 150 mm and an exposure time per frame of 20 s. Intensity data were collected to a resolution of 1.7 Å at 100 K using a wavelength of 0.953718 Å with 1° oscillation per image. Diffraction data were processed and scaled using *XDS*. Data-collection statistics are summarized in Table 1. So far, there is no structure of a homologous protein available for molecular-replacement phasing. Therefore, structure determination by the multiple isomorphous replacement method is currently being undertaken.

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