Crystallization and preliminary X-ray crystallographic studies of α-galactosidase I from *Mortierella vinacea*

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α-Galactosidases catalyze the hydrolysis of a galactosyl residue from galactooligosaccharides and galactopolysaccharides. α-Galactosidase I from *Mortierella vinacea* was crystallized in two crystal forms using the hanging-drop vapour-diffusion method. Type 1 crystals belonged to space group $I422$, with unit-cell parameters $a = b = 142.4$, $c = 131.5$ Å, and diffracted to beyond 2.1 Å resolution, while type 2 crystals belonged to space group $P4$, with unit-cell parameters $a = b = 100.9$, $c = 102.7$ Å, and diffracted to beyond 1.6 Å resolution. This enzyme crystallized as a glycoprotein tetramer and the tetrameric structure was located around the crystallographic fourfold axis.

1. Introduction

α-Galactosidase (α-Gal; EC 3.2.1.22) hydrolyzes an α-1,6-linked galactosyl residue from galactooligosaccharides and polymeric galacto-(gluco)mannans and is one of the exoglycosidases. α-Gals are widely distributed in animals, plants and microorganisms. Human α-Gal is known to be a lysosomal exoglycosidase that cleaves the terminal α-galactosyl residue from glycolipids and glycoproteins; mutations in the α-Gal gene cause incomplete degradation of carbohydrates, resulting in lipid defect (Brady *et al.*, 1967; Ioannou *et al.*, 2001). In plants, galactomannan is one of the major storage polysaccharides in seeds and α-Gal is one of the key enzymes in the degradation of cell-wall galactomannan during germination (Reid *et al.*, 1992; Reid, 1995). In the sugar-beet industry, α-Gal has been used to increase the yield of sucrose by eliminating raffinose (Gal$p\alpha1\rightarrow$6Glc$p\alpha1\rightarrow2\beta$Fru$f$), which prevents normal crystallization of beet sugar (Yamane, 1971). Primary structure and hydrophobic cluster analyses have shown that the α-Gals can be classified largely into the glycoside hydrolase families 4, 27 and 36 (Henrissat & Davies, 1997, 2000). α-Gals from eukaryotes have high amino-acid sequence homologies and are generally classified into family 27, whereas prokaryotic α-Gals are grouped into family 36. The hydrolysis reaction of glycoside hydrolase clan D is known to take place by retention of a double-displacement mechanism and the experimentally determined nucleophile of the catalytic residue is an aspartic acid (Hart *et al.*, 2000; Ly *et al.*, 2000). Recently, the crystal structure of rice (*Oryza sativa* L.) α-Gal, which belongs to family 27 of the glycoside hydrolases, has been determined (Fujimoto *et al.*, 2002, 2003).

We have purified and deduced the sequences of α-Gals from *Mortierella vinacea*, *Penicillium purpurogenum*, *Thermus* sp. T2 and rice, and have determined the substrate specificities of each of these enzymes (Shibuya, Kobayashi, Kosamo *et al.*, 1995; Shibuya, Kobayashi, Park *et al.*, 1995; Shibuya *et al.*, 1997, 1998, 1999; Ishiguro *et al.*, 2001; Kim *et al.*, 2002). *M. vinacea* (filamentous fungi) possesses two types of α-Gals, one of which, α-galactosidase I (α-Gal I; DDBJ database accession No. AB018691), consists of 397 amino-acid residues with a molecular weight calculated from the sequence of 44 336 Da, but has molecular weights of 50–56 kDa and 240 kDa when analyzed by SDS–PAGE and gel filtration, respectively (Shibuya *et al.*, 1997). The broadness of the SDS–PAGE band suggests that α-Gal I may be a heterogeneous glycoprotein, resulting from the presence of the sugar chain. In addition, the gel-filtration data suggest that α-Gal I may exist as a tetramer in solution. In the case of *M. vinacea* α-Gal II, the enzyme consists of 376 amino-acid residues with a calculated molecular weight of 41 334 Da and an estimated molecular weight of 51–62 kDa by SDS–PAGE and 60 kDa by gel filtration, suggesting that this enzyme is most likely to exist as a monomeric glycoprotein. The two *M. vinacea* α-Gals have also been found to have different substrate specificities towards galactomannnan, with α-Gal I being specific for the terminal α-galactosyl residue that is linked to the non-reducing-end mannose of mannan (Kaneko *et al.*, 1990), while α-Gal II and rice α-Gal have specificity for the α-galactosyl side chain of galactomannooligosaccharides, as well as for the terminal α-galactosyl residue (Shibuya *et al.*, 1997; Kim *et al.*, 2002). The difference in the substrate specificites of the two enzymes seems to be

caused by insertions in α-Gal I located near the catalytic site. Here, we present preliminary X-ray crystallographic data on the family 27 α-Gal I from *M. vinacea*. The three-dimensional structure of α-Gal I should be helpful in clarifying its substrate-recognition mechanism, as well as its structural identity with rice α-Gal, and contribute to a better understanding of how α-Gals function.

2. Experimental procedures and results

2.1. Crystallization

The *M. vinacea* strain (IFO 6738) was obtained from the Institute for Fermentation, Osaka, Japan. The method of culture of *M. vinacea* and the purification of the enzyme were carried out as described previously (Suzuki *et al.*, 1970; Shibuya *et al.*, 1997). Ammonium sulfate was added to the culture filtrate to 90% saturation and the resulting precipitate was dialyzed against 10 mM sodium phosphate and then subjected to sequential chromatography employing the anion-exchanger DEAE-Sepharose FF (Amersham Biosciences, 2 × 20 cm column) and gel filtration with Sephacryl S-300 HR (Amersham Biosciences, 1.6 × 60 cm column). The purity and homogeneity of the protein were checked by SDS–PAGE. A 10 mg ml^{-1} protein solution was used for initial screening employing a Crystal Screen kit (Hampton Research, USA). A droplet consisting of a mixture of 2.5 µl protein solution and 2.5 µl reservoir solution was equilibrated against 0.5 ml reservoir solution at 293 K. The enzyme crystallized under two different conditions, producing two types of crystals. Plate-shaped type 1 crystals grew in less than a week using a reservoir solution consisting of 0.2 M trisodium citrate dihydrate, 0.1 M Tris buffer pH 8.5 and 30% polyethylene glycol 400. Type 2 crystals, which were bipyramidal in shape, grew in less than a week using a reservoir solution made up of 0.2 M magnesium chloride hexahydrate, 0.1 M HEPES buffer pH 7.5 and 30% polyethylene glycol 400. Reproducing the crystallization conditions on a larger scale gave type 1 crystals of dimensions 0.25 × 0.2 × 0.15 mm and type 2 crystals of 0.3 × 0.3 × 0.3 mm (Fig. 1).

2.2. Data collection

Diffraction data from both native crystals were obtained at beamline BL6A, Photon Factory, Tsukuba, Japan. Crystals of both types were mounted in a quartz glass capillary of 0.3 mm in diameter and then flash-frozen in a nitrogen stream at 100 K. This capillary cryofreezing method improved the mosaicity and the resolution of the data. Diffraction data were collected using an ADSC CCD X-ray detector (ADSC, USA) using a 1° oscillation step over a range of 150° for the type 1 crystal and a 1° oscillation step over a range of 148° for the type 2 crystal (λ = 0.978 Å). All data sets were processed and scaled using the programs *DENZO* and *SCALEPACK* from the *HKL*2000 package (Otwinowski, 1993).

Type 1 α-Gal I crystals belonged to the tetragonal space group *I*422, with unit-cell parameters $a = b = 142.4$, $c = 131.5$ Å, and diffracted to beyond 2.1 Å resolution, while type 2 crystals belonged to the tetragonal space group *P*4, with unit-cell parameters $a = b = 100.9$, $c = 102.7$ Å, and diffracted to beyond 1.6 Å resolution. The data-collection statistics are shown in Table 1. Using the molecular weight of 53 kDa, the V_M value for type 1 crystals was calculated to be 3.2 Å^3 Da^{-1}, assuming the presence of one molecule in the asymmetric unit (a solvent content of approximately 60.7%) and the V_M value of type 2 crystals was calculated to be 2.5 Å^3 Da^{-1}, assuming the presence of two molecules in the asymmetric unit (a solvent content of approximately 49.7%) (Matthews, 1968).

Initial phases were successfully obtained by the molecular-replacement method using the type 1 crystal data, using the structural model of rice α-Gal (PDB code 1uas; 39.3% amino-acid sequence identity; Fujimoto *et al.*, 2003) as a search model with the program *AMoRe* (Navaza, 1994) from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). In addition to the electron density characteristic of protein, density was also observed corresponding to sugar moieties as well as solvent and crystallization reservoir components. This enzyme crystallized as a glycoprotein and the tetrameric structure of this enzyme was located on the crystallographic fourfold axis in both crystals. Several enzymes have been solved in a tetrameric form with this geometry (Varghese *et al.*, 1993; Dreyer & Schulz, 1993; Sintchak *et al.*, 1996). Building and refinement of the structural model are presently under way.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

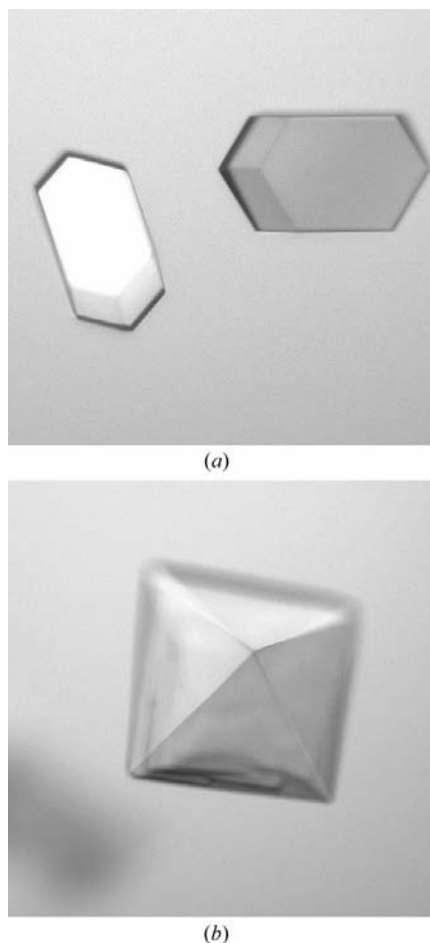|	Crystal type 1	Crystal type 2
Space group	*I*422	*P*4
Unit-cell parameters (Å)	$a = b = 142.4$, $c = 131.5$	$a = b = 100.9$, $c = 102.7$
Wavelength (Å)	0.978	0.978
Resolution (Å)	50.0–2.1 (2.18–2.10)	50.0–1.6 (1.66–1.60)
R_{merge} (%)	7.8 (22.7)	5.2 (22.4)
Completeness (%)	100.0 (100.0)	99.9 (99.6)
Multiplicity	11.9 (11.5)	6.0 (4.1)
Average $I/\sigma(I)$	31.3 (12.3)	31.6 (6.1)
Unique reflections	39683 (3889)	135146 (13430)
Observed reflections	472623	810633

(a)

(b)

Figure 1
Typical crystals of *M. vinacea* α-Gal I in two space groups, (*a*) *I*422, (*b*) *P*4, grown by the hanging-drop vapour-diffusion method.

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