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Crystallization and preliminary X-ray analysis of arabinofuranosidase C from *Aspergillus niger* strain 3M43

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Abstract

Crystals of arabinofuranosidase C purified from *Aspergillus niger* strain 3M43 have been obtained by vapour diffusion. The crystal belongs to the space group $P2_1$ with cell parameters $a = 44.28$, $b = 71.99$, $c = 45.27$ Å and $\beta = 105.98°$ with one molecule in the asymmetric unit. The X-ray diffraction pattern of these crystals extends to at least 2.20 Å resolution with the use of synchrotron radiation. These crystals are stable on exposure to radiation and are suitable for structure determination.

1. Introduction

α-L-arabinofuranosidases (Abf's) (E.C. 3.2.1.55) are exo-type hydrolases which liberate L-arabinose from oligo or polysaccharides which contain α-L-linked arabinofuranosyl residues at the non-reducing ends. Examples of such oligo\polysaccharides include L-arabinosides of low molecular weight, polymeric arabinan containing (1,3) and or (1,5) α-L linkages, L-arabinoxylan and L-arabinogalactan. This activity is known to be produced extracellularly in most cases by plants, bacteria and fungi (Kaji, 1984).

Aspergillus niger is an example of a saprophytic hyphal fungus capable of degrading branched chain oligo or polysaccharides to liberate L-arabinose. This process is reported to involve two distinct Abf's, AbfA (83 kDa) (Flipphi, Heuvel, van der Veen, Visser & Graaff, 1993) and AbfB (67 kDa) (Flipphi, Visser, van der Veen & Graaff, 1993) and an *endo*-1-5-α-L-arabinase (AbnA) (Voragen, Rombouts, Searle-van Leeuwen, Schols & Pilnik, 1987; Rombouts *et al.*, 1988; van der Veen, Flipphi, Voragen & Visser, 1993). A fourth L-araban degrading enzyme α-L-arabinofuranoside C (AbfC) has now been isolated, purified and crystallized from *Aspergillus niger* strain 3M43. The near complete sequence data of this protein (to be published) indicates an approximate molecular weight of 32 kDa which agrees with the mass determined by laser desorption spectrometry (32270±50 Da) and sodium dodecyl sulfate (SDS) gel electrophoresis (33 kDa). The partial protein sequence of AbfC suggests that it has a single homologue in the sequence database, the catalytic domain of xylanase C (*XYNC*) an arabinofuranosidase from *Pseudomonas fluorescens* (Kellet *et al.*, 1990), with which it shares 35% amino-acid identity.

2. Materials and methods

2.1. *Strain and growth media*

Aspergillus niger strain 3M43 was employed in this study. A semi-synthetic growth medium (pH 6.50) containing wheat bran and sugar beet pulp as the carbon source was used for the cultivation. Following fermentation (303 K) the medium was harvested and any solid material removed *via* filtration.

2.2. *Assay*

The enzyme activity of AbfC was measured on the basis of release of reducing sugars from various substrates. These included arabinoxylan from wheat, oat or larch, arabinogalactates (branched or debranched), arabinan or sugar beet pectin. For routine analysis 1% sugar beet pectin was utilized as substrate.

The assay mixture containing 1% polymeric substrate in 50 m*M* sodium acetate buffer pH 4.80 and 10 µl of enzyme at an appropriate concentration was incubated at 313 K for one hour or more depending on the substrate. The products of hydrolysis (arabinose, xylose, xylobiose and xylotriose) were then analysed by high-performance liquid chromatography using a Dionex anion-exchange column or thin-layer chromatography by spotting onto silica plates.

2.3. *Protein purification*

The culture supernatant was concentrated prior to loading onto an ion-exchange column. The column used was a Q Sepharose (25 × 100 mm) high-performance grade (Pharmacia) equilibriated with 20 m*M* Tris–HCl pH 7.50. Elution of the protein was achieved by running a gradient of 0–500 m*M* sodium chloride (NaCl) and all fractions of the eluate were collected. The AbfC was eluted at 130–150 m*M* NaCl.

To remove salt from the protein the fractions containing AbfC were combined and loaded onto a G-25 Sepharose superfine column (500 × 200 mm) (Pharmacia) and eluted with distilled water. After desalting the enzyme was concentrated using High-Trap spin columns (Pharmacia) and loaded onto a Superdex 50 (50 × 600 mm) gel-filtration column. It was then eluted with 0.2 *M* sodium phosphate buffer pH 7.0 containing 0.2 *M* NaCl and all fractions of eluate were collected. Fractions containing AbfC activity were combined and desalted as described above and then loaded onto a hydrophobic interaction column (16 × 100 mm) *i.e.* phenylsepharose high-performance (Pharmacia) equilibriated with 50 m*M* phosphate buffer pH 6.0, containing 1.5 *M* ammonium sulfate. Finally the protein was eluted using a gradient from 1.5–0.0 *M* ammonium sulfate. All the fractions were collected and all those containing AbfC activity were pooled.

2.4. *Protein purity*

The purity was checked by SDS polyacrylamide gel electrophoresis using the Phast gel system (Pharmacia) and visualized by silver staining.

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2.5. *Crystallization*

The enzyme was dialysed into 20 m*M* Tris–HCl pH 7.0 containing 2 m*M* sodium azide and then concentrated in a 10 ml stirred cell concentrator (Amicon Co.) fitted with a 10 kDa cut-off membrane (Flowgen). Using the conventional hanging-drop method over 1 ml resevoirs in Linbro trays different crystallization conditions were surveyed by the protocol of Jancarik & Kim (1991), using both crystal screens I and II supplied by Hampton Research. Assuming 1 mg ml^{-1} gives an OD_{280} of 1.0, an estimated protein concentration of 10.0 mg ml^{-1} was used to set up droplets of 2 µl of protein and 2 µl of reservoir. Crystals grew from several droplets of the initial screen where polyethylene glycol (PEG) was the precipitant. After refinement of the crystallization conditions the best crystals were obtained at 291 K from hanging drops containing 4 µl of protein at 32.8 mg ml^{-1} in 20 m*M* Tris–HCl, 2 m*M* sodium azide pH 7.0 mixed with 2 µl of reservoir solution which consisted of 23–28% PEG 2000, 3350 or 4000, 0.1 *M* Tris–HCl pH 8.50 and 0.1 *M* magnesium chloride. One of these crystals was selected for data collection. The dimensions of the crystal used were 1.0 × 0.5 × 0.1 mm (see Fig. 1).

3. Results and discussion

Data extending to 2.2 Å resolution were collected at the LURE-DCI synchrotron (Orsay, France) Station D41 operating at a wavelength of 1.386 Å and using an imaging-plate system with a diameter of 180 mm (MAR Research, Hamburg). A total of 40 frames of 2° oscillations were measured using a crystal-to-detector distance of 169 mm. Owing to time limitations, 26 frames were collected, the crystal moved through 20°, and a further 14 frames collected in order to obtain sufficient data to characterize the crystal.

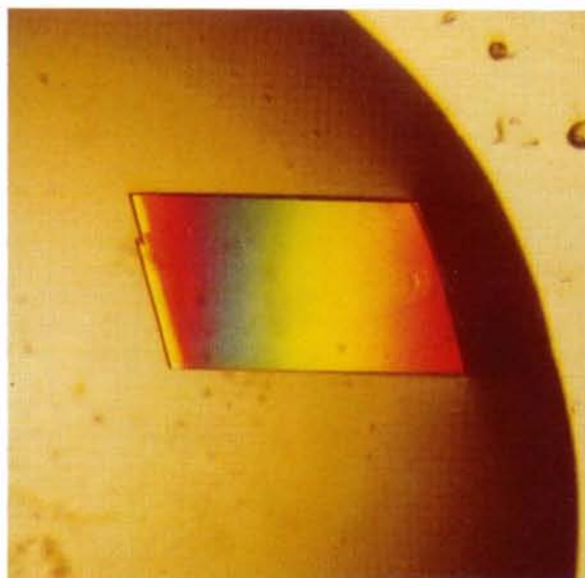

Fig. 1. A micrograph of a single crystal of AbfC under polarized light. Cell dimensions are 1.0 × 0.5 × 0.1 mm.

The images were processed with *DENZO* (Otwinowski, 1993). *DENZO*, in conjunction with the display program *XDISPLAYF* (Minor, 1993), was used to autoindex an image. Autoindexing gave a convincing primitive monoclinic cell (unit-cell distortion of 0.14%, compared with 0.64% for the next best choice of a centred monoclinic cell). The space group was determined as $P2_1$ with unit-cell dimensions $a = 44.28$, $b = 71.99$, $c = 45.27$ Å and $\beta = 105.98°$. Assuming one protein molecule of molecular mass 32 kDa in the asymmetric unit, the value of the crystal packing parameter V_m is 2.17 $Å^3$ Da^{-1}, well within the accepted range for proteins (Matthews, 1968). The data were scaled and merged using the program *SCALEPACK* from the *HKL* program suite (Otwinowski, 1993) to yield 12 973 independent reflections, 92.8% of a complete data set to 2.20 Å resolution, from a total of 29 167 useable measurements with an overall merging *R* factor on intensity of 0.064 for 8 125 reflections measured more than once. The data set was 83.7% complete in the last resolution shell of 2.24–2.20 Å.

We have shown we can obtain useful 2.2 Å resolution data from crystals of arabinofuranosidase C. Glycosyl hydrolases have been classified into families based on amino-acid sequence homology (Henrissat, 1991). According to this classification, AbfA belongs to family 51, AbfB to family 54 and AbnA to family 43 (Henrissat & Bairoch, 1996). The homologue of AbfC, XYNC is currently unclassified. This is the first report of a crystallographic characterization of an arabinofuranosidase, possibly from a new family. A search for heavy-atom derivatives is in progress.

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