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

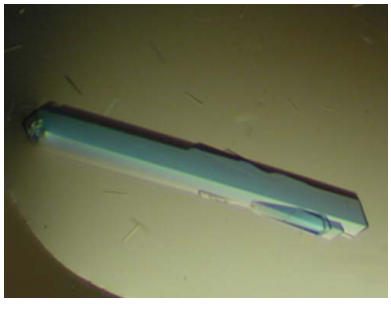
Crystallization and preliminary X-ray analysis of an arabinoxylan arabinofuranohydrolase from *Bacillus subtilis*

Elien Vandermarliere,[a]* Tine M. Bourgois,[b] Steven Van Campenhout,[b] Sergei V. Strelkov,[a] Guido Volckaert,[b] Jan A. Delcour,[c] Christophe M. Courtin[c] and Anja Rabijns[a]

[a]Laboratory for Biocrystallography, Katholieke Universiteit Leuven, Herestraat 49, O&N II bus 822, 3000 Leuven, Belgium, [b]Laboratory of Gene Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 21, 3001 Leuven, Belgium, and [c]Laboratory of Food Chemistry and Biochemistry, Department of Molecular and Microbial Systems, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20 bus 2463, 3001 Leuven, Belgium

Correspondence e-mail: elien.vandermarliere@pharm.kuleuven.be

Received 3 July 2007
Accepted 10 July 2007



Arabinoxylan arabinofuranohydrolases (AXH) are α-L-arabinofuranosidases (EC 3.2.1.55) that specifically hydrolyse the glycosidic bond between arabinofuranosyl substituents and xylopyranosyl residues from arabinoxylan, hence their name. In this study, the crystallization and preliminary X-ray analysis of the AXH from *Bacillus subtilis*, a glycoside hydrolase belonging to family 43, is described. Purified recombinant AXH crystallized in the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 68.7, b = 73.7, c = 106.5 Å. X-ray diffraction data were collected to a resolution of 1.55 Å.

1. Introduction

Arabinoxylan is one of the hemicelluloses found in the cell walls of plants. It is composed of a homopolymeric linear backbone of β-1,4-linked D-xylopyranosyl units which can be O2- and/or O3-substituted with L-arabinofuranosyl units (Jeffries, 1994). Complete arabinoxylan breakdown requires a variety of cooperatively acting enzymes. Hydrolysis of the backbone into xylo-oligosaccharides is mainly performed by endo-1,4-β-xylanases (EC 3.2.1.8). The xylo-oligosaccharides formed are then further degraded to xyloses by β-xylosidases (EC 3.2.1.37). Arabinose substituents are hydrolyzed by α-L-arabinofuranosidases (EC 3.2.1.55; Henrissat *et al.*, 1998).

The latter enzymes can be classified into two types according to their substrate specificity: types A and B. Type A arabinofuranosidases are only active against small substrates, while type B arabinofuranosidases are active against both oligomeric and polymeric substrates. Some type B arabinofuranosidases specifically cleave arabinofuranosyl units from arabinoxylan only and hence are termed arabinoxylan arabinofuranohydrolases (AXHs; Pitson *et al.*, 1996). AXHs can be further divided in two or possibly three groups. The AXH-m group only release arabinose from monosubstituted xylose residues, while the AXH-d group only release arabinose from doubly substituted xylose residues (Van Laere *et al.*, 1997, 1999). Ferré and coworkers suggested that AXH from barley malt releases arabinose from both single and double-substituted xylose residues and thus can be classified as AXH-md (Ferré *et al.*, 2000).

Recently, XynD from *Bacillus subtilis* subspecies *subtilis* ATCC 6051, which was previously predicted to be a member of glycoside hydrolase family 43 displaying endoxylanase activity supplemented with arabinofuranosidase co-activity, has been characterized as an arabinoxylan arabinofuranohydrolase that cleaves arabinose units from O2- or O3-monosubstituted xylose residues, *i.e.* as an AXH-m2,3 (Bourgois *et al.*, 2007).

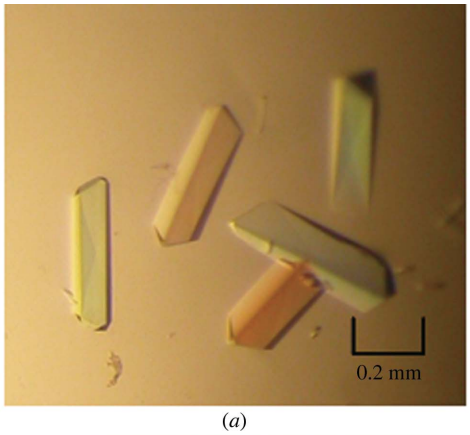
Glycoside hydrolases (GH) of family 43 have a catalytic domain consisting of a five-bladed β-propeller analogous to tachylectin (Beisel *et al.*, 1999), as was first observed for *Cellvibrio japonicus* α-L-arabinanase 43A (Arb43A; Nurizzo *et al.*, 2002). The active site is located in a V-shaped groove across the face of the propeller that is flanked by binding subsites. Although Arb43A has no associated carbohydrate-binding module (CBM), unlike other GH family 43 members, *B. subtilis* AXH has been proposed to contain a CBM which belongs to CBM family 6 (CBM-6; Bourgois *et al.*, 2007). Members of CBM-6 display a β-sandwich fold (Czjzek *et al.*, 2001).

Within GH family 43, structures are presently available of a β-xylosidase and an arabinanase, both of which are in complex with their substrate (Brüx *et al.*, 2006; Nurizzo *et al.*, 2002), but no structure of a GH family 43 arabinofuranosidase has yet been determined (http://www.cazy.org). Here, we describe the crystallization and preliminary X-ray analysis of the GH family 43 arabinoxylan arabinofuranohydrolase from *B. subtilis* soaked with xylotriose in order to gain structural insight in the binding of substrate by the enzyme.

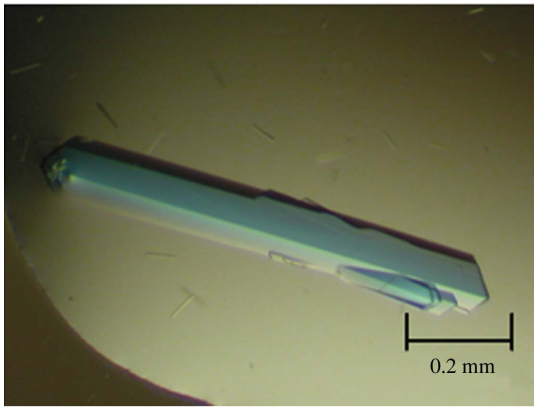
2. Experimental procedures

2.1. Crystallization

Recombinant AXH from *B. subtilis* was expressed and purified as described previously (Bourgois *et al.*, 2007). Purified AXH was concentrated to 10 mg ml^{-1} in 25 m*M* sodium acetate pH 5.0 using a Microcon centrifugal filter device (Millipore, Billerica, MA, USA). The final concentration was determined by absorbance measurements at 280 nm. All crystallization experiments were set up manually and performed at 277 K using the hanging-drop vapour-diffusion method (Unge, 1999). Initial crystallization conditions were screened using the commercially available Structure Screens 1 and 2 (Hampton Research, CA, USA) with drops formed by mixing equal volumes (1 µl) of protein and precipitant solution, which were equilibrated against 700 µl precipitant solution (Jancarik & Kim, 1991). Initial crystals were grown from drops containing 4.0 *M* sodium formate.



(*a*)



(*b*)

Figure 1
(*a*) Crystals of AXH under polarized light grown in 4.0 *M* sodium formate and 1.0 *M* lithium chloride. Average crystal dimensions are approximately 300 × 30 × 30 µm. (*b*) An AXH crystal under polarized light grown in 4.0 *M* sodium formate and 0.5 *M* sodium fluoride. The crystal dimensions are 1.4 × 0.1 × 0.1 mm

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.0788
Space group	$P2_12_12_1$
Unit-cell parameters	
a (Å)	68.7
b (Å)	73.7
c (Å)	106.5
Resolution (Å)	50–1.55 (1.58–1.55)
Reflections	
Total	258631
Unique	76939 (3868)
Completeness (%)	98.1 (99.9)
Mean $I/\sigma(I)$	14.0 (3.9)
Multiplicity	3.4
$R_{\rm merge}$† (%)	8.3 (32.2)

† $R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$.

2.2. Data collection

Prior to data collection, the crystals were transferred briefly into a cryoprotectant composed of 4.0 *M* sodium formate supplemented with 30%(*v*/*v*) glycerol and a saturated concentration of xylotriose (Megazyme, Bray, Ireland) and flash-cooled in liquid nitrogen using a cryo-loop. Diffraction data were collected using a charge-coupled device camera (MAR CCD 165 mm) at beamline BW7a of DESY, EMBL Hamburg, Germany at cryogenic temperature (Oxford Cryosystems Cryostreams, Oxford, England). A total of 180 frames of data were collected with an oscillation angle of 0.5° and an exposure dose of 1500 kHz for each image. The crystal-to-detector distance was 90 mm. The diffraction images were visualized using *XDisplayF*, processed using *DENZO* and scaled and merged using *SCALEPACK* from the *HKL* suite of programs (Otwinowski & Minor, 1997).

3. Results and discussion

Initially, the growth of thick needle-like crystals was observed in 4.0 *M* sodium formate. Refinement of this condition using the Additive Screen (Hampton Research) resulted in several conditions in which rod-like crystals of varying size (Fig. 1) could be grown (Cudney *et al.*, 1994). For this, drops were formed by mixing 1.25 µl protein solution with 1 µl precipitant solution and 0.25 µl additive solution and were equilibrated against 700 µl precipitant solution. Useful additives were 1.0 *M* lithium chloride, 30%(*w*/*v*) sucrose, 0.5 *M* sodium fluoride, 30%(*w*/*v*) dimethyl sulfoxide and 0.1 *M* L-cysteine. All crystals diffracted in the resolution range 2.4–1.5 Å.

Crystals of approximately 1.4 × 0.1 × 0.1 mm in size grown in 4.0 *M* sodium formate and 1.0 *M* lithium chloride were used for data collection (Table 1) with a synchrotron source. Prior to data collection, these crystals were soaked in a supersaturated solution of xylotriose. They belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 68.7$, $b = 73.7$, $c = 106.5$ Å, and diffract to a resolution of 1.55 Å. Assuming the presence of one monomer per asymmetric unit, the calculated $V_{\rm M}$ value (Matthews, 1968) and solvent content are 2.57 Å^3 Da^{-1} and 52.2%, respectively, which are within the normal range of values observed for soluble protein crystals. Trials to solve the structure *via* molecular replacement are ongoing.

We thank the staff of the EMBL/DESY Hamburg Outstation for the provision of synchrotron facilities, skilful technical assistance and

financial support through the I3 contract with the European Commission for support of access for external users. This work was funded by the Flemish IWT (Instituut voor de aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen, SBO project funding), the Flemish FWO (Fonds voor Wetenschappelijk Onderzoek Vlaanderen, postdoctoral fellowship to AR) and the 'Bijzonder Onderzoeksfonds K. U. Leuven' (postdoctoral fellowship to SVC).

References

Beisel, H., Kawabata, S., Iwanaga, S., Huber, R. & Bode, W. (1999). *EMBO J.* **18**, 2313–2322.
Bourgois, T. M., Van Craeyveld, V., Van Campenhout, S., Courtin, C. M., Delcour, J. A., Robben, J. & Volckaert, G. (2007). *Appl. Microbiol. Biotechnol.* **75**, 1309–1317.
Brüx, C., Ben-David, A., Shallom-Shezifi, D., Leon, M., Niefind, K., Shoham, G., Shoham, Y. & Schomburg, D. (2006). *J. Mol. Biol.* **359**, 97–109.
Cudney, B., Patel, S., Weisgraber, K. & Newhouse, Y. (1994). *Acta Cryst.* D**50**, 414–423.
Czjzek, M., Bolan, D. N., Mosbah, A., Allouch, J., Fontes, C. M. & Ferreira, L. M. (2001). *J. Biol. Chem.* **276**, 48580–48587.
Ferré, H., Broberg, A., Duus, J. Ø. & Thomson, K. K. (2000). *Eur. J. Biochem.* **267**, 6633–6641.
Henrissat, B., Teeri, T. T. & Warren, R. A. J. (1998). *FEBS Lett.* **425**, 352–354.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jeffries, T. W. (1994). *Biochemistry of Microbial Degradation*, edited by C. Ratledge, pp. 233–277. Dordrecht: Kluwer Academic Publishers.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nurizzo, D., Turkenburg, J. P., Charnock, S. J., Roberts, S. M., Dodson, E. J., McKie, V. A., Taylor, E. J., Gilbert, H. J. & Davies, G. J. (2002). *Nature Struct. Biol.* **9**, 665–668.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pitson, S. M., Voragen, A. G. J. & Beldman, G. (1996). *FEBS Lett.* **398**, 7–11.
Unge, T. (1999). *Protein Crystallization: Techniques, Strategies and Tips*, edited by T. M. Bergfors, pp. 9–17. La Jolla, CA, USA: International University Line.
Van Laere, K. M. J., Beldman, G. & Voragen, A. G. J. (1997). *Appl. Microbiol. Biotechnol.* **47**, 231–235.
Van Laere, K. M. J., Voragen, C. H. J., Kroef, T., van den Broek, L. A. M., Beldman, G. & Voragen, A. G. J. (1999). *Appl. Microbiol. Biotechnol.* **51**, 606–613.