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

Crystallization and preliminary X-ray analysis of α-xylosidase from *Escherichia coli*

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Received 2 September 2004
Accepted 15 December 2004
Online 8 January 2005

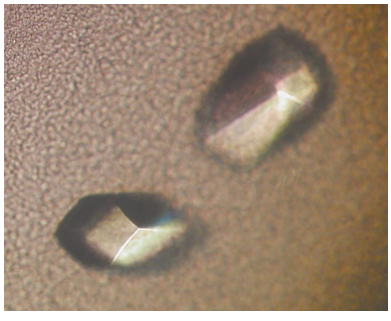
Glycoside hydrolases have been implicated in many biological processes. To date, they have been classified into 93 glycoside hydrolase (GH) families based on amino-acid sequence similarity. α-Xylosidase from *Escherichia coli* belongs to GH family 31 and catalyzes the release of α-xylose from the non-reducing terminal side of α-xyloside. Single crystals of α-xylosidase have been grown by vapour diffusion at 293 K from 10%(*w*/*v*) PEG 20K, 2%(*v*/*v*) 2-propanol, 2%(*v*/*v*) glycerol and 0.1 *M* 2-morpholinoethanesulfonic acid pH 5.5. These crystals belong to space group $P2_12_12_1$ and X-ray diffraction data were collected to a resolution of 2.75 Å. Crystals of selenomethionyl-substituted α-xylosidase were also obtained, which diffracted to at least 3.0 Å. Based on the value of V_M, the asymmetric unit in these crystals was assumed to contain six molecules.

1. Introduction

α-Xylosidase catalyzes the release of α-xylose from the non-reducing terminal side of α-xyloside. α-Xylosidase activity was recently detected from the product of the *yicI* gene in *Escherichia coli* (Okuyama *et al.*, 2004). *Escherichia coli* α-xylosidase (EcXe) is an enzyme of 772 amino-acid residues (relative molecular mass M_r = 88 079). EcXe exhibits catalytic activity against α-xylosyl fluoride, isoprimeverose (6-*O*-α-xylopyranosyl-glucopyranose) and α-xyloside in xyloglucan oligosaccharides. However, unlike other α-xylosidases (nasturtium, *Arabidopsis thaliana* and *Sulfolobus solfataricus* α-xylosidases), which also show α-glucosidase activity (Crombie *et al.*, 2002; Moracci *et al.*, 2000; Sampedro *et al.*, 2001), EcXe does not show this activity.

Based on sequence similarity, EcXe has been assigned to glycoside hydrolase (GH) family 31 (Henrissat, 1991; Henrissat & Bairoch, 1993, 1996). The enzymes classified as belonging to GH family 31 include the family II α-glucosidases (EC 3.2.1.20), glucoamylases (EC 3.2.1.3), sucrase-isomaltases (EC 3.2.1.48 and EC 3.2.1.10), α-xylosidases (EC 3.2.1.–), α-glucan lyases (EC 4.2.2.13) and isomaltosyltransferases (EC 2.4.1.–). A previous study of *Schizosaccharomyces pombe* α-glucosidase belonging to the same family indicated that three acidic residues corresponding to Asp416, Glu419 and Asp482 of EcXe are candidates for the catalytic residues. These residues are located in the highly conserved sequences 'region A' and 'region B' (Okuyama *et al.*, 2004). However, no structural information is available with regard to GH family 31.

As a step towards understanding the correlation between three-dimensional structure and properties, we report the crystallization and preliminary X-ray crystallographic analysis of EcXe.



2. Protein preparation and crystallization

Recombinant EcXe was expressed and purified according to the procedure reported previously (Okuyama *et al.*, 2004) with slight modifications. Fractions containing EcXe were collected, dialyzed against 2-morpholinoethanesulfonic acid (MES) buffer (10 m*M*, pH 6.0) and concentrated by ultrafiltration using Amicon-Ultra 30 000 MWCO (Amicon Inc.) to a final concentration of 10 mg ml^{-1}. The purity of the protein was analyzed by MALDI–TOF mass spectrometry (Voyager DE-PRO, PerSeptive Biosystems). Initial crystal-

Table 1
Data collection and statistics.

Values in parentheses are for the highest resolution shell.

		SeMet		
Native		Peak	Edge	Remote
Beamline	BL41XU, SPring-8	BL41XU, SPring-8		
Detector	MAR CCD	MAR CCD		
Exposure time (s)	8	10		
Camera distance (mm)	180	185		
Rotation angle per frame (°)	0.6	1.0		
Overall rotation angle (°)	180	200		
Wavelength (Å)	0.9796	0.9793	0.9795	0.9700
Space group	$P2_12_12_1$	$P2_12_12_1$		
Unit-cell parameters (Å)	a = 161.2, b = 174.9, c = 211.2	a = 120.9, b = 162.0, c = 295.6		
Resolution (Å)	35–2.75 (2.90–2.75)	50–3.0 (3.13–3.00)		
No. of unique reflections	154560 (22428)	116698 (13819)	116646 (13818)	116780 (13,809)
Completeness (%)	99.8 (99.8)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Redundancy	4.8 (4.4)	5.2 (5.5)	4.6 (4.8)	4.9 (5.2)
Average $I/\sigma(I)$	6.8 (2.1)	8.5 (2.1)	8.4 (2.4)	8.5 (2.1)
R_{sym}†	0.109 (0.410)	0.175 (0.535)	0.091 (0.338)	0.091 (0.373)

† $R_{sym} = \sum_h \sum_j |\langle I\rangle_h - I_{h,j}| / \sum_h \sum_j I_{h,j}$, where $\langle I\rangle_h$ is the mean intensity of symmetry-equivalent reflections.

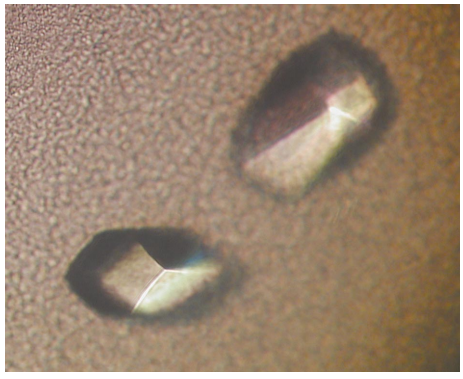


Figure 1
Crystals of EcXe of approximate dimensions 80 × 80 × 200 µm.

lization trials were performed by the sitting-drop vapour-diffusion method in 96-well plates at 293 K using a series of crystallization kits (Hampton Research and deCode Genetics). Each drop was prepared by mixing 1 µl protein solution prepared as described above with the same volume of reservoir solution. The first crystals were obtained with the reservoir composition 0.1 *M* citric acid pH 5.0, 10.0%(*w*/*v*) PEG 6000. As they only showed diffraction to approximately 6 Å they were not further used. A second round of searching for good-quality crystals was conducted using the hanging-drop vapour-diffusion method in 24-well plates. Each drop contained 2.0 µl protein solution (10–13 mg ml^{-1}) and an equal volume of reservoir solution.

After optimization of crystallization conditions by varying the pH, protein concentration, precipitants and additives, well ordered crystals were obtained in the presence of 7%(*w*/*v*) PEG 20K, 2%(*v*/*v*) 2-propanol, 2%(*v*/*v*) glycerol and 0.1 *M* MES pH 5.5 (Fig. 1). The final protein concentration in the drop was 25 mg ml^{-1}. Crystals of selenomethionyl-substituted α-xylosidase were also obtained under the same conditions.

3. Data collection and preliminary X-ray analysis

The data sets of both native and selenomethionyl-substituted crystals were collected at beamline BL41XU of SPring-8 using a MAR CCD detector. Prior to diffraction data collection, crystals were cryo-protected by transfer into reservoir solution containing 25%(*w*/*v*) 2-methyl-2,4-pentanediol (MPD) in 4 min. Based on the fluorescence spectrum at the the Se *K* edge, multiwavelength anomalous diffraction (MAD) data of selenomethionyl-substituted crystals were collected at three wavelengths corresponding to the maximum f'' (0.9793 Å, peak), the minimum f' (0.9795 Å, edge) and a reference point (0.9700 Å, remote). The data sets were processed with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Details of data collection and processing are given in Table 1. Based on the values of the Matthews coefficient (V_M; Matthews, 1968; 2.97 Å^3 Da^{-1}, $V_{solvent}$ = 58.2% for the native crystal and 2.74 Å^3 Da^{-1}, $V_{solvent}$ = 55.2% for the selenomethionyl-substituted crystal), the asymmetric unit of each crystal was estimated to contain six protomers. This observation was in agreement with the results of gel-filtration chromatography, suggesting that EcXe is present as a hexameric form in solution (Okuyama *et al.*, 2004). Structural analyses are currently under way in our laboratory.

We thank M. Kawamoto and H. Sakai of the Japan Synchrotron Radiation Research Institute (JASRI) for their kind help in data collection on beamline BL41XU at SPring-8. This work was supported in part by a Grant-in-Aid for Scientific Research and a research grant from the National Project on Protein Structural and Functional Analyses from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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