Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of GlcNAcα1,4Gal-releasing endo-β-galactosidase from *Clostridium perfringens*

Lu Deng,[a] Zhi-Jie Liu,[b] Hisashi Ashida,[c] Su-Chen Li,[c] Yu-Teh Li,[c] Peter Horanyi,[b] Wolfram Tempel,[b] John Rose[b] and Bi-Cheng Wang[b]*

[a]Department of Chemistry, University of Georgia, Athens, GA 30602, USA, [b]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA, and [c]Department of Biochemistry, Tulane University Health Sciences Center School of Medicine, New Orleans, Louisiana 70112, USA

Correspondence e-mail: wang@bcl1.bmb.uga.edu

Received 13 October 2003
Accepted 15 December 2003

The unique clostridial endo-β-galactosidase (Endo-β-Gal$_{GnGa}$) capable of releasing the disaccharide GlcNAcα1,4Gal from O-glycans expressed in the gastric gland mucous cell-type mucin has been crystallized. The crystal belongs to space group $P6_3$, with unit-cell parameters a = 160.4, c = 86.1 Å. Under cryocooled conditions and using a synchrotron X-ray source, the crystals diffract to 1.82 Å resolution. The asymmetric unit contains two or three molecules.

1. Introduction

Clostridium perfringens is known to cause a wide variety of diseases in humans and animals (Smith, 1979; Niilo, 1980; Sterne, 1981). A unique endoglycosidase named GlcNAcα1,4Gal-releasing endo-β-galactosidase (Endo-β-Gal$_{GnGa}$) isolated from *C. perfringens* was found to specifically release the disaccharide GlcNAcα1,4Gal from O-glycans expressed in the gastric gland mucous cell-type mucin. This enzyme hydrolyzes the endo-β-galactosyl linkage not only in the GlcNAcα1,4Galβ1,4GlcNAc sequence, but also in GlcNAcα1,4Galβ1,3GalNAcαSer/Thr. The strict specificity for Endo-β-Gal$_{GnGa}$ to release the disaccharide GlcNAcα1,4Gal distinguishes this enzyme from the other three types of reported endo-β-galactosidases (Ashida *et al.*, 2001). The primary sequence study shows that this enzyme consists of 420 amino-acid residues, including a 17-residue signal peptide at the N-terminus. It was also found to contain the E*X*D*X*(*X*)E sequence (Glu168–Glu173) that has been identified as the catalytic motif of the retaining glycoside hydrolase families 16 and 7. Based on site-directed mutagenesis, Glu168 and Glu173 were found to be essential for Endo-β-Gal$_{GnGa}$ activity (Ashida *et al.*, 2002). The roles of catalytic nucleophile and general acid/base, respectively, are highly likely for these two residues in the catalytic pathway, based on the sequence-homology study of the catalytic motif. The existence of such an unusual endo-β-galactosidase in *C. perfringens* is very intriguing. This unique endo-β-galactosidase should become useful for studying the structure and biological function of glycoconjugates containing the GlcNAcα1,4Gal epitope.

In this paper, we describe the crystallization and preliminary X-ray diffraction data of Endo-β-Gal$_{GnGa}$. Further analysis of the crystal structure of this novel enzyme may reveal its functional domain and provide insights into its reaction mechanism.

2. Materials and methods

The pure recombinant enzyme was prepared according to the previously described procedure (Ashida *et al.*, 2002). For crystallization trials, Endo-β-Gal$_{GnGa}$ was concentrated to 16.7 mg ml^{-1} in 0.1 *M* ammonium acetate pH 6.0. Crystallization conditions were screened using the sparse-matrix sampling method (Jancarik & Kim, 1991) with reagents from both Hampton Research and Emerald Biostructures. The modified microbatch method (Chayen *et al.*, 1990; D'Arcy *et al.*, 1996) was used by mixing equal volumes (0.5 µl) of the protein and the screening solutions in the wells of a Nunc HLA plate. The HLA plate was then sealed with 4 ml of a combination of silicon and paraffin oil (5:5). Two hits were found with small crystals, but only one of them gave decent X-ray diffraction. After optimization, the best conditions for crystallization were found to be 42% ammonium sulfate, 2% PEG 400 in 0.1 *M* Na HEPES pH 7.4. Beautiful crystals (Fig. 1) with maximum dimensions of about 0.3 × 0.3 × 0.2 mm were obtained after incubating at 277 K for 2 d.

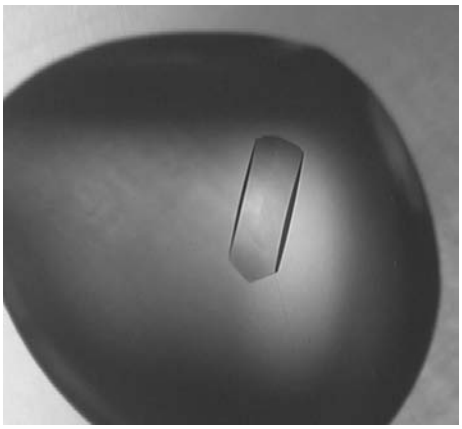

Figure 1
Native crystal of GlcNAcα1,4Gal-releasing endo-β-galactosidase from *C. perfringens* with dimensions of 0.15 × 0.15 × 0.4 mm.

 DOI: 10.1107/S0907444903028919

Table 1
Data-processing statistics.

Values for the outer shell are in parentheses.

Resolution range (Å)	50–1.82 (1.90–1.82)
Unique reflections	107796 (13186)
Completeness (%)	95.8 (94.4)
$I/\sigma(I)$	25.1 (8.1)
$R_{\rm merge}$† (%)	5.8 (21.8)

† $R_{\rm merge} = \sum_{hkl}[\sum_i(|I_{hkl,i} - \langle I_{hkl}\rangle|)]/\sum_{hkl,i}\langle I_{hkl}\rangle$, where $I_{hkl,i}$ is the intensity of an individual measurement of the reflection with the Miller indices h, k and l and $\langle I_{hkl}\rangle$ is the mean intensity of that reflection.

3. Results and discussion

Similar-shaped Endo-β-Gal$_{\rm GnGa}$ crystals were obtained by growing crystals at both 291 and 277 K under the same crystallization conditions. However, crystals produced at 291 K gave very weak X-ray diffraction, indicating that the enzyme crystal is sensitive to temperature and prefers to grow at low temperature. Fully grown crystals were soaked with artificial mother liquor (42% ammonium sulfate, 2% PEG 400, 0.1 *M* Na HEPES pH 7.4 and 15% glycerol) for 1 min prior to flash-freezing (Hope, 1988) and data collection. Preliminary diffraction data were collected at beamline 22-ID in the facilities of the South East Regional Collaborative Access team (SER-CAT) at the Advanced Photon Source, using a MAR Research 165 mm CCD detector and 0.98 Å wavelength X-rays. The crystal diffracted to 1.82 Å resolution. Data processing was carried out using *HKL*2000 (Otwinowski & Minor, 1997). The data-processing statistics are given in Table 1. Systematic absences suggest that the crystal belongs to space group $P6_3$, with unit-cell parameters a = 160.4, c = 86.1 Å. Assuming one molecule per asymmetric unit, the Matthews coefficient (Matthews, 1968) was calculated to be 6.48 Å^3 Da^{-1}, indicating there to be two or three molecules per asymmetric unit, corresponding to 60 or 40% solvent content, respectively. Structure determination is currently in progress.

We thank the support from the National Institutes of Health, the University of Georgia Research Foundation and Georgia Research Alliance. Data were collected at Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. W-31-109-Eng-38.

References

Ashida, H., Anderson, K., Nakayama, J., Maskos, K., Chou, C.-W., Cole, R. B., Li, S.-C. & Li, Y.-T. (2001). *J. Biol. Chem.* **276**, 28226–28232.
Ashida, H., Maskos, K., Li, S.-C. & Li, Y.-T. (2002). *Biochemistry*, **41**, 2388–2395.
Chayen, N. E., Shaw Stewart, P. D., Meader, D. L. & Blow, D. M. (1990). *J. Appl. Cryst.* **23**, 297–302.
D'Arcy, A., Elmore, C., Stihle, M. & Johnston, J. E. (1996). *J. Cryst. Growth*, **168**, 175–180.
Hope, H. (1988). *Acta Cryst.* B**44**, 22–26.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Niilo, L. (1980). *Can. Vet. J.* **21**, 141–148.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Smith, L. D. (1979). *Rev. Infect. Dis.* **1**, 254–262.
Sterne, M. (1981). *Br. Vet. J.* **137**, 443–454.