Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray study of β-mannosidase from *Trichoderma reesei*

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Received 18 November 1999
Accepted 20 December 1999

β-Mannosidase from *Trichoderma reesei*, a 105 kDa glycoprotein, has been crystallized. The crystals belong to the space group $P4_12_12$ or $P4_32_12$, with unit-cell dimensions $a = b = 165.86$, $c = 122.46$ Å, and diffract beyond 2.75 Å resolution. X-ray diffraction data were collected from a frozen crystal on a synchrotron X-ray source.

1. Introduction

β-Mannosidase is an exoglycosidase that catalyzes hydrolysis of non-reducing residues of β-D-mannose in β-D-mannosides. The enzyme has been isolated from plants (Houston *et al.*, 1974; Li & Lee, 1972), fungi (Bouquelet *et al.*, 1978; Neustroev *et al.*, 1992; Kulminskaya *et al.*, 1999), animals (Iwasaki *et al.*, 1989; Cavanagh *et al.*, 1985) and bacteria (Akino *et al.*, 1988; Oda *et al.*, 1993), including thermophilic and alkalophilic microorganisms. The complete human (Alkhayat *et al.*, 1998), caprine (Leipprandt *et al.*, 1996), bovine (Chen *et al.*, 1995) and *Aspergillus aculeatus* (Takada *et al.*, 1999) β-mannosidase cDNAs have been sequenced, revealing 75% identity between the first three sequences (Alkhayat *et al.*, 1998). The lysosomal degradation of N-glycoproteins is the most important process in which β-mannosidase participates. Glycoprotein degradation occurs predominantly within cell lysosomes by the sequential action of endo- and exoglycosidases. β-Mannosidase cleaves mannosidic bonds in the core part of N-linked glycans attached to glycoproteins (Scriver *et al.*, 1989).

In man and ruminants, a deficiency of β-mannosidase leads to an autosomal recessive inherited lysosomal storage disease (Watts & Gibbs, 1986) called β-mannosidosis. The disease is characterized by storage and excretion of the disaccharide Man-β(1→4)-GlcNAc, the trisaccharide Man-β(1→4)-GlcNAc-β(1→4)-GlcNAc and other more complex undegraded oligosaccharides in various tissues (Percheron *et al.*, 1992). The disorder was first described in Nubian goats (Jones & Dawson, 1981) and later in humans (Cooper & Sardharwalla, 1986; Wenger *et al.*, 1986) and Saler cattle (Bryan *et al.*, 1990). In man, clinical manifestations are heterogeneous and include mental retardation, peripheral neuropathy, skeletal abnormalities and others (Rodriguez-Serna *et al.*, 1996). A theoretical study of structural and functional features of bovine β-mannosidase (Chen *et al.*, 1995) and another four enzymes implicated in lysosomal storage diseases, based on a family classification of glycosidases (EC 3.2.1.*x*), was performed using the hydrophobic cluster analysis method (Durand *et al.*, 1997). Some likely preserved features of the catalytic domains were deduced, despite the low levels of sequence identity. Another observed pathological variation of the enzyme, which manifests itself in a significantly decreased serum activity in 64 diabetic patients, remains to be investigated (Bernard *et al.*, 1985).

Fungi and bacteria able to degrade hemicellulose also secrete β-mannosidase. This β-mannosidase hydrolyzes β(1→4)-D-mannosyl groups from manno-oligosaccharides and mannose-containing glycopepetides produced from the hemicellulose pulp by endoenzymes (Kuhad *et al.*, 1997). In seeds that have galactomannans as storage carbohydrates, the enzyme converts manno-oligosaccharides to monosaccharides (Dey & del Campillo, 1984). Some enzymes have a substrate-binding site in addition to the catalytic site, which may modulate enzymatic action, possibly within a multidomain structure (Kuhad *et al.*, 1997; Neustroev *et al.*, 1993; Voet & Voet, 1995).

A non-catalytic galactomannan-binding site was established in β-mannosidase from *T. reesei* on the base of kinetic studies (Kulminskaya *et al.*, 1999). Knowledge of the three-dimensional structure of the enzyme will help in the understanding of the structural basis of its activity and function.

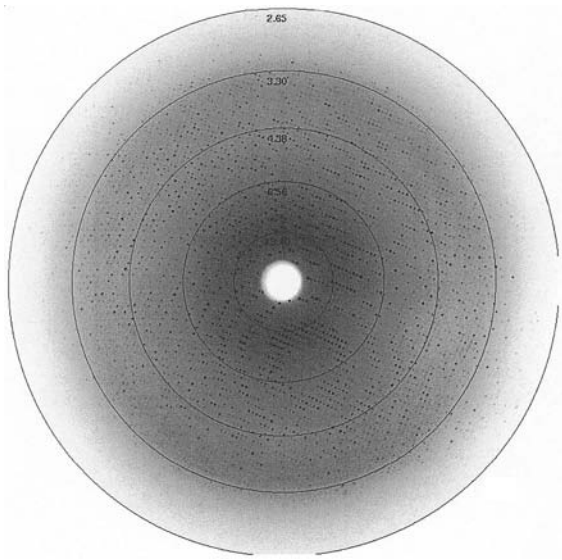

Figure 1
A typical diffraction pattern from the data set reported above. The crystal-to-detector distance was set to give a resolution limit of 2.65 Å at the outer edge of the image. Other resolution rings were drawn.

Table 1
Data-collection statistics.

Values in parentheses refer to the last resolution shell.

Number of images	115
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell dimensions at 80 K (Å)	
$a = b$	165.86
c	122.46
Resolution range (Å)	20.00–2.75
Last shell (Å)	2.81–2.75
Number of measured reflections	373335 (17061)
Number of unique reflections	44389 (2693)
Redundancy	8.41 (6.34)
R_{sym}† (%)	0.214 (0.697)
Completeness (%)	99.1 (91.6)
$\langle I/\sigma(I)\rangle$	12.80 (2.13)

† $R_{sym} = \sum(I - \langle I\rangle)/\sum I$.

2. Crystallization and data collection

β-Mannosidase was purified to homogeneity from a culture filtrate of *T. reesei*, as described by Kulminskaya *et al.* (1999). Initial crystallization conditions were screened by the sparse-matrix method (Jancarik & Kim, 1991) using the macromolecular crystallization reagent kits I and II (Hampton Research). In each trial, a hanging drop of 1 µl of protein solution (10 mg ml^{-1} in water) mixed with 1 µl of precipitant solution was equilibrated against a reservoir containing 500 µl of precipitant solution. Small clusters of needles grew at room temperature in precipitant solution 12 [30%(*v*/*v*) PEG 400, 0.1 *M* $CdCl_2$, 0.1 *M* sodium acetate pH 4.6] from the Hampton reagent kit II in one week. The experiment was repeated at 277 K and small crystals were obtained from a drop containing protein solution, precipitant solution and water in a 2:1:1 ratio. Further optimization at 291 K including pH refinement (McPherson, 1995) led to the following conditions. A reservoir solution containing 26% PEG 400, 0.13 *M* $CdCl_2$ and 0.1 *M* sodium acetate pH 4.7 was prepared. A 2 µl drop of the protein solution was mixed with 1 µl of reservoir solution plus 1 µl of water. After equilibration, well formed bipyramidal crystals of dimensions 0.5 × 0.4 × 0.3 mm grew in 3–15 d. The crystals obtained diffracted poorly and to low resolution. In spite of the fact that the crystals showed good morphology and size, they were rapidly damaged when exposed to the X-ray beam, resisting a few images only. The best resolution of the room-temperature data set was limited to 3.5 Å. Cryo-crystallographic techniques (Garman & Schneider, 1997) were employed to overcome radiation damage. Crystallization conditions allowed flash-freezing of the crystal in a gaseous nitrogen stream (Oxford Cryosystems) without further modifications of the mother liquor. Several diffraction data sets were collected. Data were collected on a 345 mm MAR Research imaging-plate detector at the LNLS Protein Crystallography beamline (Polikarpov, Oliva *et al.*, 1998; Polikarpov, Perles *et al.*, 1998) by the oscillation method from a single native crystal at 80 ± 1 K (Fig. 1). The wavelength was 1.38 Å and the crystal-to-detector distance was 205 mm, with an oscillation range of 1° and an exposure time of ∼15 min per image.

3. Results and discussion

Data were processed using the *DENZO* and *SCALEPACK* packages (Otwinowski & Minor, 1997). Results of data processing are summarized in Table 1. Flash-freezing significantly improved the crystal's resistance to X-rays. This allowed a higher X-ray dose per image. An improvement in the $I/\sigma(I)$ ratio along with a high redundancy led to better statistics and a higher resolution cutoff. We aim to solve this structure by the multiple isomorphous replacement method. Screening for heavy-atom derivatives is under way.

This work was supported by grants 98/06761-1 and 99/03387-4 from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, and by grant 97-04-50035 from the Russian Foundation for Basic Research (RFBR), Russia. RA and IP gratefully acknowledge the financial support from FAPESP and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

References

Akino, T., Nakamura, N. & Horikoshi, K. (1988). *Agric. Biol. Chem. Tokyo*, **52**(6), 1459–1464.
Alkhayat, A. H., Kraemer, S. A., Leipprandt, J. R., Macek, M., Kleijer, W. J. & Friderici, K. H. (1998). *Hum. Mol. Genet.* **7**(1), 75–83.
Bernard, M., Brochet, C. & Percheron, F. (1985). *Clin. Chim. Acta*, **152**(1/2), 171–174.
Bouquelet, S., Spik, G. & Montreuil, J. (1978). *Biochim. Biophys. Acta*, **522**, 521–530.
Bryan, L., Schmutz, S., Hodges, S. D., Snyder, F. F. (1990). *Biochem. Biophys. Res. Commun.* **173**(2), 491–495.
Cavanagh, K. T., Fisher, R. A., Legler, G., Herrchen, M., Jones, M. Z., Julich, E., Sewell Alger, R. P., Sinnott, M. L. & Wilkinson, F. E. (1985). *Enzyme*, **34**(2), 75–82.
Chen, H., Leipprandt, J. R., Traviss, C. E., Sopher, B. L., Jones, M. Z., Cavanagh, K. T. & Friderici, K. H. (1995). *J. Biol. Chem.* **270**(8), 3841–3848.
Cooper, A. & Sardharwalla, I. B. (1986). *N. Engl. J. Med.* **315**(19), 1231.
Dey, P. M. & del Campillo, E. (1984). *Adv. Enzymol. Relat. Areas Mol. Biol.* **56**, 141–249.
Durand, P., Lehn, P., Callebaut, I., Fabrega, S., Henrissat, B. & Mornon, J. P. (1997). *Glycobiology*, **7**(2), 277–284.
Garman, E. F. & Schneider, T. R. (1997). *J. Appl. Cryst.* **30**, 211–237.
Houston, C. W., Latimer, S. B. & Mitchell, E. D. (1974). *Biochim. Biophys. Acta*, **370**(1), 276–282.
Iwasaki, Y., Tsuji, A., Omura, K. & Suzuki, Y. (1989). *J. Biochem. (Tokyo)*, **106** (2), 331–335.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jones, M. Z. & Dawson, G. (1981). *J. Biol. Chem.* **256**(10), 5185–5188.
Kuhad, R. C., Singh, A. & Eriksson, K. L. (1997). *Biochem. Eng. Biotechnol.* **57**, 45–125.
Kulminskaya, A. A., Eneiskaya, E. V., Isaeva-Ivanova, L. S., Savel'ev, A. N., Sidorenko, I. A., Shabalin, K. A., Golubev, A. M. & Neustroev, K. N. (1999). *Enzyme Microb. Technol.* **25**(3–5), 372–377.
Leipprandt, J. R., Kraemer, S. A., Haithcock, B. E., Chen, H., Dyme, J. L., Cavanagh, K. T., Friderici, K. H. & Jones, M. Z. (1996). *Genomics*, **37**(1), 51–56.
Li, Y. T. & Lee, Y. C. (1972). *J. Biol. Chem.* **247**, 3677–3683.
McPherson, A. (1995). *J. Appl. Cryst.* **28**, 362–365.
Neustroev, K. N., Krylov, A. S., Firsov, L. M., Abroskina, O. N. & Khorlin, A. Y. (1992). *Biochemistry (Moscow)*, **56**(8), 982–986.
Neustroev, K. N., Valter, S. N., Timchenko, N. V., Firsov, L. M., Golubev, A. M. & Khokhlov, S. E. (1993). *Biochem. Mol. Biol. Int.* **30**(1), 115–120.
Oda, Y., Komaki, T. & Tonomura, K. (1993). *Food Microbiol.* **10**(4), 353–358.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Percheron, F., Foglietti, M. J., Bernard, M. & Ricard, B. (1992). *Biochimie*, **74** (1), 5–11.
Polikarpov, I., Oliva, G., Castellano, E. E., Garratt, R. C., Arruda, P., Leite, A. & Craievich, A. (1998). *Nucl. Instrum. Methods A*, **405**, 159–164.
Polikarpov, I., Perles, L. A., de Oliveira, R. T., Oliva, G., Castellano, E. E., Garratt, R. C. & Craievich, A. (1998). *J. Synchrotron Rad.* **5**(2), 72–76.
Rodriguez-Serna, M., Botella-Estrada, R. M. D., Chabas, A., Coll, M., Oliver, V., Febrer, M. & Aliaga, A. (1996). *Arch. Dermatol.* **132**(10), 1219–1222.
Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (1989). *The Metabolic Basis of Inherited Disease*, Vol. II, 6th ed. New York: McGraw–Hill, Inc.
Takada, G., Kawaguchi, T., Kaga, T., Sumitani, J. & Arai, M. (1999). *Biosci. Biotechnol. Biochem.* **63**(1), 206–209.
Voet, D. & Voet, J. (1995). *Biochemistry*, 2nd ed. New York: John Wiley & Sons, Inc.
Watts, R. W. E. & Gibbs, D. A. (1986). *Lysosomal Storage Diseases – Biochemical and Clinical Aspects.* Philadelphia: Taylor & Francis, Inc.
Wenger, D. A., Sujanski, E., Fennessey, P. V. & Thompson, J. N. (1986). *N. Engl. J. Med.* **315**(19), 1201–1205.