Data Article

Structural and biochemical data of *Trichoderma harzianum* GH1 β-glucosidases

Renata N. Florindo, Valquiria P. Souza, Hemily S. Mutti, Lívia R. Manzine Margarido, Cesar Camilo, Sandro R. Marana, Igor Polikarpov, Alessandro S. Nascimento



**A R T I C L E  I N F O**

Article history:
Received 6 September 2017
Accepted 20 September 2017
Available online 22 September 2017

**A B S T R A C T**

Here the statistics concerning X-ray data processing and structure refinement are given, together with the substrate preference analysis for ThBgl1 and ThBgl2. Finally, the analysis of the influence of temperature and pH on the activities of both enzymes are shown.

© 2017 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

**Specifications Table**

| Subject area               | Biology                                           |
|----------------------------|---------------------------------------------------|
| More specific subject area | Structural Enzymology                             |
| Type of data               | Table and Figures                                 |
| How data was acquired      | X-ray diffractometer and plate reader             |

DOI of original article: http://dx.doi.org/10.1016/j.nbt.2017.08.012

* Corresponding author at: Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador Saocarlene, 400, Centro, Sao Carlos, SP 13566-590, Brazil.

E-mail address: asnascimento@ifsc.usp.br (A.S. Nascimento).
Data format

Analyzed

Experimental factors

N/A

Experimental features

X-ray data collected from flash frozen single crystals in a home source using CuKα radiation. Enzyme activity data was measured using the synthetic substrate p-nitrophenyl-glucopyranoside.

Data source location

São Carlos, SP, Brazil. -22.008911, -47.897772.

Data accessibility

Structural data is public through the protein data bank (PDB), with access codes 5JBK and 5JBO.

Value of the data

- The data provided in the table shows the quality of the crystal structure used for the analysis of the mechanism of transglycosylation observed in these GH1 β-glucosidases.
- The substrate preference data provided shows that, although the enzymes are very similar, they have marked differences in substrate preference.
- The influence of pH and temperature indicate the optimal conditions for catalysis for the enzymes ThBgl1 and ThBgl2.

1. Data

Three sets of data are shown. First, the statistics and parameters from the X-ray diffraction data processing and structural refinement are given for the crystal structures of the enzymes ThBgl1 and ThBgl2. Second, the preferences for different natural and synthetic substrates are shown for these enzymes and, finally, the influence of pH and temperature on the enzyme activity is provided.

2. Experimental design, materials and methods

The complete description of the methods is found in the associated research article [1]. For the determination of optimum pH and temperatures for enzyme activity, a reaction mixture using the synthetic substrate 4-nitrophenyl-β-D-glucopyranoside (pNPG Fig. 1). Briefly, 50 μL of pNPG (final concentration of 5 mM), 40 μL of 150 mM citrate-phosphate or phosphate buffer at different pHs and 10 μL of enzyme at 0.1 mg/ml were incubated for 5, 10, 15 and 20 minutes at 30 °C. The reaction was

Fig. 1. Influence of pH on ThBgl1 (A) and ThBgl2 (B) activities in pNPG.
stopped by adding 100 μL of Na₂CO₃ 0.5 M and the amount of released products was measured spectrophotometrically at 415 nm. For the determination of optimum temperature, the same reactions were incubated in a temperature range spanning 20 to 70 °C in 5 °C steps (Fig. 2). After 10 minutes, the reaction was stopped by adding 100 μL of Na₂CO₃ 1 M and the amount of released products was measured spectrophotometrically at 405 nm.

Table 1
Data collection and refinement statistics.

| Parameters                        | ThBgl1 | ThBgl2 |
|-----------------------------------|--------|--------|
| PDB code                          | 5JBK   | 5JBO   |
| Wavelength (Å)                    | 1.46   | 1.54   |
| Resolution range (Å)              | 71.92–2.59 (2.69–2.59) | 61.64–1.97 (2.04–1.97) |
| Space Group                       | P 21 21 21 | P 21 21 21 |
| Unit cell                         | 94.9 97.7 106.2 | 57.5 78.1 100.3 |
| Total reflections                  | 31,180 (3015) | 32,663 (3212) |
| Completeness (%)                  | 99.7 (97.6) | 99.9 (100.0) |
| Mean I/sigma(I)                   | 8.3 (2.2) | 8.7 (3.1) |
| Wilson B-factor (Å²)              | 17.46   | 9.23   |
| Rmerge                            | 0.54   | 0.50   |
| Rwork                             | 0.213 (0.271) | 0.1681 (0.2193) |
| Rfree                             | 0.254 (0.308) | 0.2025 (0.2640) |
| Number of non-hydrogen atoms      | 7973   | 4664   |
| Water                             | 498    | 867    |
| Protein residues                  | 930    | 475    |
| RMS (Å)                           | 0.005  | 0.004  |
| RMS (°)                           | 1.06   | 1.03   |
| Ramachandran favoured (%)         | 96     | 97     |
| Ramachandran allowed (%)          | 4      | 3      |
| Ramachandran outliers (%)         | 0      | 0      |
| Clashscore                        | 6.25   | 2.59   |
| Average B-factor                  | 16.40  | 11.80  |
| Macromolecules                    | 16.30  | 9.20   |
| Ligands                           | 16.40  | 0      |
| Solvent                           | 18.10  | 23.20  |

Fig. 2. Temperature influence on ThBgl1 (a) and ThBgl2 (b) activities measured in pNPG.
The substrate preferences for ThBgl1 and ThBgl2 were evaluated using different synthetic substrates: pNPG, 4-nitrophenyl-β-D-xylopyranoside (pPNX), 4-nitrophenyl-α-D-galactopyranoside (pNPGal), 2-nitrophenyl-β-D-galactopyranoside, 4-nitrophenyl-β-D-cellobioside and 4-nitrophenyl-β-D-mannopyranoside (pNPM). All the reactions were tested at 35 °C for ThBgl1 and 40 °C for ThBgl2, using sodium phosphate buffer pH 5.5.

Purified ThBgl1 and ThBgl2 were used for crystallization trials at 25 mg/ml 30 mg/ml, respectively. For ThBgl1 crystals grew in 0.2 M hexahydrated magnesium chloride, 0.1 M HEPES pH 7.5 and 25% PEG3350. For ThBgl2, suitable crystals grew in a solution containing 0.1 MES pH 6.5, 25% PEG 8000. The crystals were flash frozen in a nitrogen stream under 100 K and used for data collection. For ThBgl1 crystal a complete dataset was collected in the MX-2 beamline of the Brazilian Synchrotron Light Source [2]. For ThBgl2 a complete dataset was collected using a Bruker APEX-Duo home source using copper Kα radiation. The scattered intensities were integrated using iMosflm [3] and, after scaling with AIMLESS [4], the structure factors were used for phasing by molecular replacement using the crystal structure of T. reesei GH1 β-glucosidase as the search model [5] and PHASER [6] software. Finally, the crystal structures were refined in iterative cycle of real space manual refinement using Coot [7] and reciprocal space refinement using PHENIX [8] software (Table 1).

Acknowledgements

The authors are indebted with Mariana Lima, João Renato C. Muniz and Maria A.M. Santos.

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2017.09.044.

References

[1] R. Florindo, V.P. Souza, H.S. Mutti, C.M. Camilo, S.R. Marana, I. Polikarpov, A.S. Nascimento, Structural insights into β-glucosidase transglycosylation based on biochemical, structural and computational analysis of two GH1 enzymes from Trichoderma harzianum, N. Biotechnol. Submitted (n.d.).

[2] B.G. Guimaraes, L. Sanfelici, R.T. Neuenschwander, F. Rodrigues, W.C. Grizolli, M.A. Raulik, J.R. Piton, B.C. Meyer, A.S. Nascimento, I. Polikarpov, The MX2 macromolecular crystallography beamline: a wiggler X-ray source at the LNLS, J. Synchrotron Radiat. 16 (2009) 69–75. http://dx.doi.org/10.1107/S0909049508034870.

[3] H.R. Powell, O. Johnson, A.G.W. Leslie, Autoindexing diffraction images with iMosflm, Acta Crystallogr. Sect. D Biol. Crystallogr. 69 (2013) 1195–1203. http://dx.doi.org/10.1107/S0907444912048524.

[4] P.R. Evans, An introduction to data reduction: space-group determination, scaling and intensity statistics, Acta Crystallogr. Sect. D-Biol. Crystallogr. 67 (2011) 282–292. http://dx.doi.org/10.1107/s090744491003982x.

[5] W.Y. Jeng, N.C. Wang, M.H. Lin, C.T. Lin, Y.C. Liaw, W.J. Chang, C.I. Liu, P.H. Liang, A.H.J. Wang, Structural and functional analysis of three β-glucosidases from bacterium Clostridium cellulovorans, fungus Trichoderma reesei and termite Neotermes koshunensis, J. Struct. Biol. 173 (2011) 46–56. http://dx.doi.org/10.1016/j.jsb.2010.07.008.

[6] A.J. McCoy, R.W. Grosse-Kunstleve, P.D. Adams, M.D. Winn, L.C. Storoni, R.J. Read, Phaser crystallographic software, J. Appl. Crystallogr. 40 (2007) 658–674. http://dx.doi.org/10.1107/s0021889907021206.

[7] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta Crystallogr. Sect. D-Biol. Crystallogr. 60 (2004) 2126–2132. http://dx.doi.org/10.1107/s0907444904019158.

[8] P.V. Afonine, R.W. Grosse-Kunstleve, N. Echols, J.J. Headd, N.W. Moriarty, M. Mustyakimov, T.C. Terwilliger, A. Urzhumtsev, P.H. Zwart, P.D. Adams, Towards automated crystallographic structure refinement with phenix.refine, Acta Crystallogr. Sect. D-Biol. Crystallogr. 68 (2012) 352–367. http://dx.doi.org/10.1107/s0907444912001308.