ONLINE ISSN : 1880-7291
PRINT ISSN : 1344-7882

Journal of Applied Glycoscience
Vol. 51 (2004) , No. 2 pp.161-167

[PDF (497K)] [References]

Reaction Mechanism Based on X-ray Crystallography at Atomic Resolution of Endopolygalacutronase I from Fungus *Stereum purpureum*

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(Received January 14, 2004)

Three crystal structures of endopolygalacturonase I (EndoPG I) from *Stereum purpureum* have been determined in this study: the unliganded EndoPG I, the binary and ternary complexes of EndoPG I with galacturonate. Consequently, the structural basis for substrate binding and the catalytic mechanism of EndoPG I have been elucidated by X-ray crystallography. Crystals of deglycosylated EndoPG Ia have been obtained using PEG4000 as precipitate with the hanging-drop vapor diffusion method. The crystal belongs to space group *P*1, with unit-cell parameters *a*=37.26 Å, *b*=46.34 Å , *c*=52.05 Å, α=67.17°, β=72.44°, γ=68.90°. The crystal diffracts to ultra-high (0.96 Å) resolution using synchrotron radiation of SPring-8. Crystal structures of EndoPG I were determined by the multiple wavelength anomalous dispersion (MAD) method. For MAD phasing, three wavelength data sets of K_2PtCl_4 derivative crystal were collected at SPring-8. The structure model was refined anisotropically with SHELXL-97, with an *R* factor of 11.4% and an R_{free} factor of 14.0% at 0.96 Å resolution. The enzyme folds into a right-handed parallel β-helix with 10 complete turns. The crystal structures of its binary complex with one D-galacturonate and its ternary complex with two D-galacturonates were also determined to identify the substrate binding site at 1.0 and 1.15 Å resolutions, respectively. In the binary complex, one β-D-galactopyranuronate, Gal*p*A, was found in the reducing end side of Asp153, Asp173 and Asp174, which are considered as candidates of catalytic residues.

This reveals that the position of Gal*p*A is the +1 subsite, thus proving the strong affinity of the +1 subsite expected from the bond cleavage frequency on oligo-galacturonates. In the ternary complex, an additional β-D-galactofuranuronate was found in the -1 subsite. In both subsites, the recognition of the galacturonate carboxy group is important in galacturonate binding. In the +1 subsite, the carboxy group interacts with three basic residues, His195, Arg226 and Lys228, which were conserved in all endopolygalacturonases. In the -1 subsite, the unique non-prolyl *cis*-peptide bond is believed to be involved in binding the carboxy group of the substrate. Based on the structures of Gal*f*A and Gal*p*A bound in the ternary complex, a structural model of the di-galacturonic acid part of the substrate molecule bound in both the -1 and +1 subsites across from the catalytic residues was constructed. The di-galacturonate model structure sheds light on the catalytic mechanism. Asp173 is at the appropriate position to be a proton donor to the fissile glycosidic bond. Asp153 or Asp174 seems to act as a general base to abstract a proton from the nucleophilic water.

Key words: X-ray crystallography, atomic resolution, *Stereum purpureum*, endopolygalacutronase I

To cite this article:

Tetsuya Shimizu, Toru Nakatsu, Kazuo Miyairi, Toshikatsu Okuno and Hiroaki Kato: Reaction Mechanism Based on X-ray Crystallography at Atomic Resolution of Endopolygalacutronase I from Fungus *Stereum purpureum* . *J. Appl. Glycosci.*, **51**, 161-167 (2004) .

JOI JST.JSTAGE/jag/51.161