Crystal structure of chitosanase from a novel bacterium *Matsuebacter chitosanotabidus* 3001

Yutaka YORINAGA[1], Taiki AMAKATA[1], Makoto KAWAMUKAI[1], Hideyuki MATSUDA[1], Kensaku HAMADA[*2]

[1]Faculty of Life and Environmental Science, [2]Interdisciplinary Faculty of Science and Engineering, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan

Introduction

Chitosanase (EC 3.2.1.132) catalyzes the hydrolysis of glycosidic bonds of chitosan in an end fashion, producing chitosan oligosaccarides. The enzyme are produced by many organisms, including actinomycetes, fungi, plants and bacteria. Bacteria chitosanase are important for maintenance of the ecological balance. The extracellular chitosanase (34,000 M_r) from a novel gram-negative bacterium *Matsuebacter chitosanotabidus* 3001 (ChoA) is most active on 90 % deacetylated chitosan, but this dose not hydrolyze chitin, cellulose and their derivatives [1].

Experimental and Results

The crystals of ChoA adding 6xHis Tag obtained under the same condition by the sitting-drop vapor-diffusion method belong to ether the orthorhombic space group $P2_12_12_1$ with unit-cell parameters a = 52.13, b = 56.40, c = 207.35 Å, Z = 8 nor the tetragonal space group $P4_32_12$ (or $P4_12_12$) with unit-cell parameters a = b = 54.27, c = 202.62 Å and Z = 8. The diffraction data of native, Hg- and Pt- derivative crystals were collected at 100 K using the synchrotron radiation source on BL-6A and BL-18B stations, Photon Factory. Detectors used were Sakabe's Waissenberg camera and ADSC CCD camera at BL-6A and ADSC Quantum 4R CCD detector at BL-18B. The wavelength of synchrotron X-ray was 1.000 Å, and cryo The data from Sakabe's Waissenberg camera were processed using the program DENZO and SCALEPACK [2], and those from ADSC CCD camera were processed using the program MOSFLM [3] and SCALA from CCP4. A diffraction data from native crystal belonging space group $P2_12_12_1$ were collected up to 2.2 Å resolution at Beamline BL24A at Spring-8. Since there are two molecules in the asymmetric unit for $P2_12_12$ crystals, a self-rotation function was calculated with 4 Å resolution data. The function suggest the presence of non-crystallographic twofold axis nearly parallel to the diagonal line of a and b axes. The positions of Pt-binding sites and those of Hg-binding sites was located by the isomorphous difference Patterson function with choPt2 and choHg2 data, respectively. However, the MIR phasing power was too weak to make a MIR density map.

For tetragonal crystals, the isomorphous difference Patterson map calculated with various resolutions did not allowed us to locate heavy atom.

The Br derivative was prepared by soaking the crystals for ca 30 seconds in the reserver containing 0.5 M sodium bromide and 25 % trehalose. The diffraction data were collected on BL-18B with the wavelength of 0.9198 Å (the maximum of the fluorescence spectrum, f" maximum), and were processed using MOSFLM and SCALA. The anomalous difference Patterson map too noisy to find Br positins.

Now, crystals of SeMet substituted ChoA are available for the phasing performed using MAD.

References

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* hamadak@cis.shimane-u.ac.jp

Table 1. Data collection statistics

Crystal form	choNa3	choHg1	choHg2	choPt1	choPt2
Crystal System	tetragonal	orthorhombic	orthorhombic	tetragonal	orthorhombic
Derivative	Native	2mM $HgCl_2$	2mM $Hg(OOCCH_3)_2$	10mM K_2PtCl_4	10mM K_2PtCl_4
Cryo buffer	reserver	30% trehalose	30% trehalose	30% trehalose	reserver
Oscillation angle (°C)	1.0	1.0	1.0	1.0	1.0
Total oscillation range (°C)	90.0	90.0	90.0	90.0	90.0
Crystal to IP distance (mm)	150	160	200	200	230
Exposure time (sec)	90	120	60	30	60
Resolution limit (Å)	2.3	2.0	2.3	3.3	2.0
Completeness (%)	98.7	92.2	83.8	77.8	83.8
Rmerge (%)	6.8	6.8	5.1	5.7	5.2