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Preliminary X-ray analysis of *Escherichia coli* K12 YgjK protein, which is homologous to processing α-glucosidase I

Takashi TONOZUKA*, Akiko UECHI, Masahiro MIZUNO, Kazuhiro ICHIKAWA, Atsushi NISHIKAWA, Yoshiyuki SAKANO
Department of Applied Biological Science, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

Introduction

Processing α-glucosidase I (EC. 3.2.1.106) specifically hydrolyzes the terminal α1,2-glucosidic linkage of $Glc_3Man_9GlcNAc_2$, which is identified as an oligosaccharide precursor of *N*-linked glycoproteins forming in eukaryotes. In glycosyl hydrolases (GH), the classification by Coutinho and Henrissat is widely used (http://afmb.cnrs-mrs.fr/CAZY/). In this classification system, processing α-glucosidase I falls into the GH family 63 with no homology to any other glycosidase whose three-dimensional structure has been determined. No three-dimensional structures of this enzyme have been reported yet, probably because the construction of heterologous expression system for eukaryotic proteins is generally more difficult than that for bacterial proteins. Interestingly, many bacteria have been reported to possess genes for proteins homologous to the GH family 63 glucosidases, although their physiological roles are not known. Here, we report the crystallization and preliminary X-ray analysis of *Escherichia coli* YgjK protein, whose primary structure is homologous to GH family 63 glucosidases.

Materials and Methods

Cloned YgjK was prepared from E. coli BL21(DE3) cells harboring the plasmid pYgjK-SIG. The cells were grown at 30 °C on 600 ml of Luria-Bertani (LB) medium containing ampicillin (50 μg/ml), and then induced with IPTG to a final concentration of 0.1 mM. The cells were harvested by centrifugation, and the enzyme was purified using a HiPrep 16/10 Phenyl FF column and a Q-Sepharose 16/10 HP anion-exchange column. The purified protein was detected as a single band on SDS-PAGE. Crystals were grown by the hanging-drop vapor-diffusion method using polyethylene glycol 8000. To perform data collection at a cryogenic temperature, the crystal was transferred to the reservoir solution, which was used as the cryoprotectant solution in this study, and then immediately flash-frozen in a stream of nitrogen gas at 100 K. Diffraction data were collected at the PF-AR NW-12 beam line, and the data set was processed with the program DPS.

Results and Discussion

The preliminary crystallographic data obtained under cryo-conditions are monoclinic, and one full set of intensity data at 1.8 Å resolution has been collected. Data collection statistics and crystal data were summarized in Table 1. A solvent content of 42 % (V_M = 2.1 $Å^3$ Da^{-1}) was calculated using the program Matthews_coeff from the CCP4 suite of software. This indicates that the crystals are expected to contain two molecules per asymmetric unit. We are searching for heavy-atom derivatives for determination of the structure.

Table 1: Data-collection statistics. Reproduced, with permission, from reference [1].

Wavelength (Å)	1.0
Temperature (K)	100
Resolution range (Å)	29.5 - 1.8
No. of observed reflections	930373
No. of unique reflections	131055
Completeness (%)	98.9 (97.8)[2]
R_{merge}[1]	0.088 (0.207)[2]
$I/\sigma(I)$	5.8 (3.4)[2]
Space group	$P2_1$
Cell dimension	
a (Å)	88.5
b (Å)	137.1
c (Å)	60.9
β (º)	98.1
Cell volume ($Å^3$)	731062

[1] $R_{merge} = \Sigma\Sigma \mid I_i - \langle I \rangle \mid / \Sigma \langle I \rangle$.
[2] The values for the highest resolution shell are given in parentheses (1.89 – 1.80 Å).

References

[1] T. Tonozuka et al., Acta Cryst., D60, 1284-1285 (2004).

*tonozuka@cc.tuat.ac.jp