Biological Science

6A, 17A, NW12A/2008G013

Crystal structure of *Escherichia coli* YgjK, an enzyme homologous to eukaryotic processing α-glucosidase I

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Introduction

Proteins belonging to the glycoside hydrolase family 63 (GH63) are found in bacteria, archaea, and eukaryotes. The eukaryotic GH63 proteins have been well documented as processing α-glucosidase I, although their three-dimensional structures have not yet been reported. Processing α-glucosidase I specifically hydrolyzes the terminal α-1,2-glucoside linkage of $Glc_3Man_9GlcNAc_2$, an oligosaccharide precursor of N-linked glycoproteins. Many bacteria and archaea have been reported to possess genes for proteins that are homologous to processing α-glucosidase I classified into GH63. These organisms, however, do not produce the eukaryotic-type N-linked oligosaccharides, and the physiological roles of the bacterial proteins are not known. Here we report the crystal structure of YgjK, a bacterial GH63 enzyme.

Materials and Methods

The crystals were grown at 20 ºC using the hanging drop, vapor diffusion method, in which 1.0 μL of 10 mg/mL SeMet YgjK solution in 20 mM Tris-HCl buffer (pH 7.5) was mixed with an equal volume of a crystallization reservoir solution containing 20% (w/w) polyethylene glycol 8000 and 0.6 M magnesium chloride in 100 mM Tris-HCl buffer (pH 5.8). To perform data collection at cryogenic temperatures, the crystals were immersed in a cryo-protectant solution consisting of the well solution with the addition of glycerol to a final concentration of 20% (w/v)[1]. The diffraction data of SeMet YgjK and YgjK-monosaccharide complexes were collected at the beamlines of PF BL-6A, PF BL-17A, and PF-AR NW12.

The program SOLVE was used for the phase calculation of the SAD data set. Automatic model building was done using RESOLVE. A rough model of YgjK was obtained, and was further built using ARP/wARP. Manual adjustment and rebuilding of the model were carried out using the program Xfit in the XtalView package. The refinement was performed with the program CNS.

Results and Discussion

YgjK consists of an N-domain, A-domain, and a linker region[2] (Fig. 1). The N-domain is structurally homologous with bacterial GH15 enzymes, glucoamylase and glucodextranase. The A-domain, which forms an $(\alpha/\alpha)_6$-barrel, also showed homology with enzymes belonging to clans GH-G (GH37 and GH63), GH-L (GH15 and GH65) and GH-L-like (GH94). The structures of the $(\alpha/\alpha)_6$-barrel of YgjK and those of clan GH-L enzymes showed relatively good matches, and Asp501 and Glu727 of YgjK may act as the catalytic acid and base, respectively, based on the structural comparison. YgjK showed the highest activity for the α-1,3-glucosidic linkage of nigerose but also hydrolyzed trehalose, kojibiose, and maltooligosaccahrides from maltose to maltoheptaose, although these activities were approximately 100-fold lower than that for nigerose. These findings suggest that YgjK is a glucosidase with a relaxed specificity for sugars.

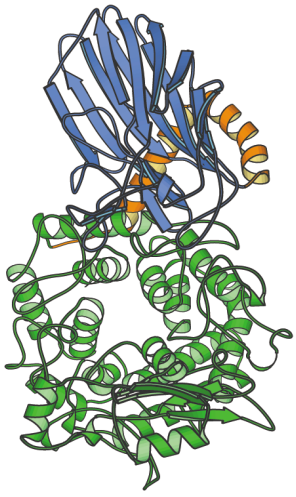

Fig. 1. Structure of YgjK. N- and A-domains, and the linker region are shown in blue, green and orange, respectively.

References

[1] T. Tonozuka et al., Acta Crystallogr. Sect. D 60, 1284 (2004).
[2] Y. Kurakata et al., J. Mol. Biol. 381, 116 (2008).

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