AR-NW12A, AR-NE3A, BL-5A/2014G537

X-ray Structure of SeMet Derivative of *Clostridium perfringens* Autolysin Catalytic Domain

Shigehiro Kamitori[1,*], Hiromi Yoshida[1], and Eiji Tamai[1,2]
[1]Life Science Research Center and Faculty of Medicine, Kagawa University
1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan
[2]Department of Infectious Disease, College of Pharmaceutical Science Matsuyama University
4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578, Japan

1 Introduction

Gram-positive bacteria possess a thick cell wall (~250 Å) that surrounds their cytoplasma membranes and provides physical protection. The bacterial cell wall is a mesh polymer of peptidoglycans, in which linear glycan backbones consisting of alternating (β1-4) linked *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) are cross-linked by species-specific peptide side chains. Autolysins encoded by bacteria can partially hydrolyze the cell wall peptidoglycan into small sections, to regulate cell division and growth-phase of bacteria. Autolysin *Clostridium perfringens* (Acp) has ten of tandem repeated bacterial Src homology 3 (SH3b) domains at N-terminus as cell wall binding domains [1], and a catalytic domain homologous to glucosaminidase belonging to the glycoside hydrolase 73 (GH73) family at C-terminus. The three-dimensional structure of Acp has been very useful in elucidating their degrading mechanism of the bacterial cell wall. Here we report the X-ray structure of the catalytic domain of SeMet derivative of Acp (SeMet-AcpCD).

2 Experiment

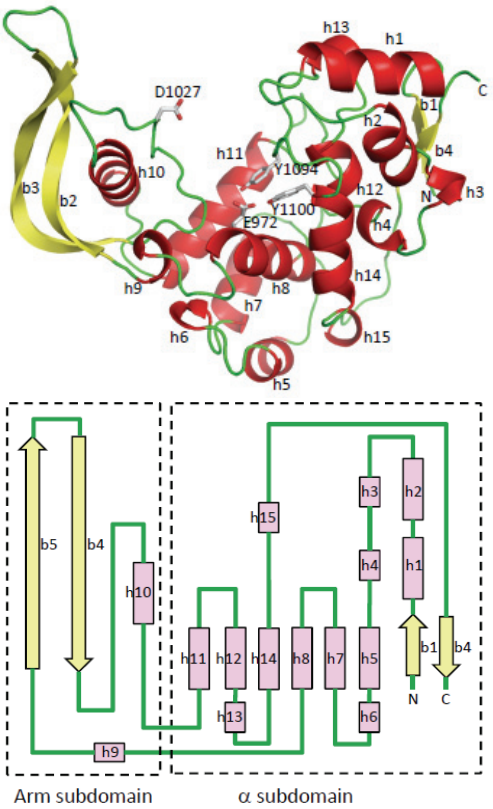
Crystals of SeMet-AcpCD were grown at 293K in a droplet mixed with 1 µl of protein solution (36.6 mg/ml in 5 mM Tris-HCl, pH 7.5) and 1 µl of reservoir solution (50 mM phosphate-citrate pH 4.2, 20 % ethanol, 2.5 % PEG1000) against 50 µl of the reservoir solution, using sitting drop vapor diffusion method. The initial phases of SeMet-AcpCD were obtained by the MAD method using data collected by the ADSC Quantum 210 CCD detector system on the PF-AR NW12A beam line. The structure of SeMet-AcpCD was refined to R-factor of 0.25 (Rfree of 0.30) using 2.28 Å resolution data.

3 Results and Discussion

SeMet-AcpCD consists of two subdomains; α helix bundle subdomain (α subdomain) and a protruding arm-shaped subdomain (arm subdomain), as shown in Fig. 1. There is a deep groove between two subdomains for the substrate binding.

Two acidic residues (E972 and D1027) are disposed to either side of the substrate binding groove, and they are expected to be responsible for the catalytic reaction. However, the distance between them of 13 Å is too far to act as acid/base catalysts in the general acid/base mechanism proposed in many glucoside hydrolase family enzymes, like as hen egg white lysozyme. Tyr1094 and Tyr1100 on the substrate binding surface are strictly conserved in GH73 family enzymes, and they are expected to form stacking interactions with the sugar units of the substrate.

Fig. 1: Overall structure and topology diagram of SeMet-AcpCD.



References

[1] Kamitori, S., Yoshida, H. (2015) Structure-function relationship of bacterial SH3 domains. In: Kurochkina, N (ed) SH Domains: Structure, Mechanisms and Applications, Springer International publishing AG Switzerland, pp. 71-89.

* kamitori@med.kagawa-u.ac.jp