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Crystal structure of a novel goose-type lysozyme with chitinase activity from *Ralstonia* sp. A-471

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Chitin is one of the most abundant biopolymer in nature. At least 10 gigatons (1×10^{13} kg) of chitin are synthesized each year, and a large majority of them are discarded. However, chitin can be a significant biological resource because *N*-acetyl-chitooligosaccharides and chitooligosaccharides, products of hydrolysis of chitin, have various biological functions including antibacterial activity and antitumor activity. Recently, we have cloned the gene encoding goose-type (G-type) lysozyme with chitinase activity from thermophilic strain *Ralstonia* sp. A-471 (Ra-ChiC)[1]. Ra-ChiC is composed of chitin binding domain, linker region, and catalytic domain. Although catalytic residue of G-type lysozyme is conserved in the catalytic domain of Ra-ChiC, the enzyme does not share significant sequence similarity with G-type lysozyme (less than 20% sequence identity). In addition, enzymatic assays showed that Ra-ChiC has only chitinase activity; in other words, it does not have lysozyme activity. It has also been shown that Ra-ChiC has notable features; that is, it is stable over a pH range 5.0-10.0 and shows maximum activity at 55 °C. Thus, Ra-ChiC would be useful for industrial applications. In this study, we aim to reveal how Ra-ChiC catalyzes the hydrolysis of chitin and why Ra-ChiC exhibit chitinase activity instead of lysozyme activity.

We expressed and purified the recombinant Ra-ChiC. Crystallization experiments were subsequently carried out, and single crystals of Ra-ChiC were obtained as a result of optimization of crystallization condition. We also crystallized SeMet-substituted Ra-ChiC, and the structure was determined by MAD phasing. We succeeded in determination of the crystal structure of whole of the catalytic domain and a large part of the linker region of Ra-ChiC (Fig. 1). From the structure, it appeared that the catalytic domain is composed of 7 α-helices, and 5 of them adopt similar conformation with G-type lysozyme. However, two aspartic acid residues which would involve in the catalytic activity of G-type lysozyme are not conserved in Ra-ChiC, while Glu141 corresponding to the catalytic residue of G-type lysozyme is structurally conserved. Our result suggests that these differences would result in different functions between chitinase activity and lysozyme activity.

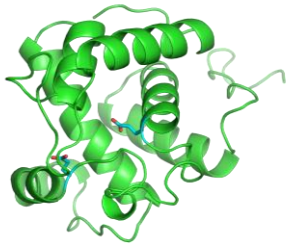

Fig. 1 Overall structure of Ra-ChiC

Reference

[1] Ueda, M *et al*, (2009) *Appl. Microbiol. Biotechnol.*, 81, 1077-1085