Crystallography

5A, NE3A, NW12A/2009G589

Crystal Structure of Goose-Type Lysozyme with Chitinase Activity from *Ralstonia* sp. A-471

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Introduction

Chitinase from *Ralstonia* sp. A-471 (Ra-ChiC) composed of a chitin binding domain (residues 36-80) and a catalytic domain (residues 103-252) belongs to glycoside hydrolase (GH) family 23 as well as goose-type (G-type) lysozymes. Although Ra-ChiC has a sequence similarity in its catalytic domain to G-type lysozymes, our previous catalytic analyses have shown that Ra-ChiC does not exhibit lysozyme activity but does exhibit only chitinase activity [1]. In addition, catalytic activity of Ra-ChiC toward *N*-acetylglucosamine (GlcNAc) hexasaccharide has shown different phenomenon from that of G-type lysozymes with respect to the cleavage site of substrate. To obtain structural basis of substrate recognition and catalytic mechanisms of Ra-ChiC, we have determined the crystal structures of Ra-ChiC catalytic domain in the absence and presence of GlcNAc residues.

X-ray diffraction studies and structure determinations

Since full-length Ra-ChiC had not crystallized despite screening above 2000 crystallization conditions, we have constructed a deletion mutant, the catalytic domain with part of an interdomain linker of Ra-ChiC (Ra-ChiC$_{89\text{-}252}$), and thus successfully obtained the crystals [2]. The crystals belonged to the space group $P6_{1(5)}22$ with unit-cell parameters a = b = 100Å, c = 243Å and diffracted beyond 2.0 Å resolution at beamline BL5A. MR analysis was attempted using several coordinates, however, no search model gave a significant solution for phase determination. Therefore, four data sets using SeMet labeled crystals were collected on and around the selenium K absorption edge at beamline NW12A. The sugar free structure of Ra-ChiC$_{89\text{-}252}$ (Ra-ChiC$_{free}$) was refined to R_{cryst} / R_{free} of 0.196 / 0.244 at 1.9 Å resolution using MAD phase.

GlcNAc-binding Ra-ChiC$_{89\text{-}252}$ crystals were obtained by soaking (Ra-ChiC$_{soak}$) and co-crystallization (Ra-ChiC$_{co\text{-}cry}$) methods. The data sets were collected at beamline NE3A. The present models of the Ra-ChiC$_{soak}$ and Ra-ChiC$_{co\text{-}cry}$ were refined at resolutions of 2.3 Å and 1.5 Å, and to an R_{cryst} / R_{free} of 0.217 / 0.265 and 0.161 / 0.192, respectively.

Crystal structure of Ra-ChiC$_{89\text{-}252}$

Overall structure of the catalytic domain of Ra-ChiC is composed of six α-helices and one 3_{10} helix. Significant conformational difference was not observed between the Ra-ChiC$_{free}$ structure and the GlcNAc-binding structures. In the Ra-ChiC$_{soak}$ structure, two GlcNAc disaccharide residues are observed in -3 to -2 and +1 to +2 subsites in one of the four molecules in the asymmetric unit. Comparison of the Ra-ChiC$_{soak}$ structure with Atlantic cod G-type lysozyme (gLYS: PDB ID = 3GXR) showed similar overall structure with RMSD value of 1.45 Å over corresponding 100 Cα atoms (Left figure). However, the numbers of sugar-binding subsites are different between them; i.e. Ra-ChiC catalytic domain has five subsites at a maximum (core subsites should be three), while gLYS or G-type lysozymes have six subsites. In addition, the substrate-binding site of Ra-ChiC catalytic domain is covered with long loop region comprising Gly217-Leu233 (shown in magenta in left figure) and forms narrow tunnel-shaped cavity (Right figure), whereas G-type lysozymes have substrate-binding cleft exposing to solvent. These differences in substrate-binding site architectures should result in different catalytic activity between Ra-ChiC and G-type lysozymes.

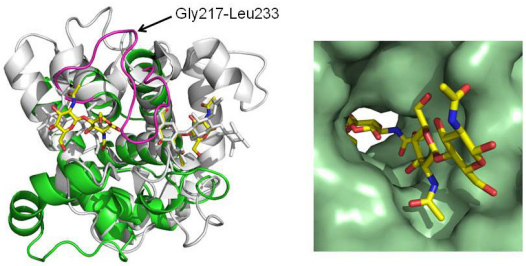

Figure.
Left: Superposition of the Ra-ChiC$_{soak}$ (colored) and gLYS (gray, PDB code; 3GXR) structures.
Right: close-up view of tunnel-shaped substrate-binding site of Ra-ChiC.

References

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