BL-1A, -5A, -17A, AR-NW12A/2017G514

Crystal structure analysis of the substrate-binding complexes of PET degrading enzyme Cut190

Nobutaka NUMOTO[1,*]
[1] Medical Research Institute, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima Bunkyo-ku, Tokyo, 113-8510, Japan

1 Introduction

Cutinase belongs to the lipase family, and some of the cutinase or cutinase-like enzyme have been reported that it can degrade polyethylene terephthalate (PET), which is a representative of persistent plastics. A cutinase from a thermophilic isolate, *Saccharomonospora viridis* AHK190 (Cut190) show high thermostability and enzymatic activity, and therefore it would provide attractive solution for recycling of waste plastic by the enzymatic treatment.

We have previously reported that Cut190 retain the activity at a temperature higher than the glass transition temperature of PET of approximately 60–65 °C. This high thermostability is indispensable for efficient degradation of PET. We have also characterized remarkable nature of Cut190 that calcium ions regulate the thermostability and enzymatic activity. Crystal structures of calcium bound and free form of Cut190 [1] have demonstrated that calcium binding site is far from the active site and the calcium ion regulates open/closed conformational transition at the active site as an allosteric effector.

In this study, we used improved mutant of Cut190 S226P/R228S, whose thermostability and enzymatic activity are enhanced. We introduced additional inactive substitution at the active Ser residue as S176A, for preparing the substrate complexes of Cut190. We have successfully solved two small substrate bound states of Cut190 S176A/S226P/R228S [2]. The obtained structures allow us to discuss more detailed mechanism of the enzymatic activity regulated by calcium binding and dissociating.

2 Experiment

Cut190 S176A/S226P/R228S mutant was overexpressed in *Escherichia coli* and purified as described previously. Crystallization was performed by the hanging-drop vapor-diffusion method at 20ºC. Prior to data collection, the crystals were soaked in cryo-protectant solutions containing 20% (v/v) glycerol and 10-100 mM substrates of monoethyl succinate (Et-succinate) and monoethyl adipate (Et-adipate), along with their respective reservoir buffers and flash-frozen using liquid nitrogen.

X-ray diffraction experiments were performed at the beamlines BL-1A, -5A, -17A, and NW12A at PF and PF-AR, KEK. All data were processed and scaled using XDS [3]. The initial phases were determined by the molecular replacement method using the program PHASER [4]. Several cycles of manual model rebuilding by using COOT [5] and refinement by using PHENIX [6] were performed.

3 Results and Discussion

We obtained the crystals suitable for the substrates soaking with the buffer containing 100 mM MES (pH 6.5), 10 mM zinc sulfate heptahydrate, and 25% (v/v) PEG monomethyl ether 550.

The structure of Et-succinate bound form was determined at 1.34 Å resolution, and clearly indicate that the enzyme release calcium ion and conformational change allow to accommodate the substrate tightly, thereby it is assumed as a pre-reaction state (Fig. 1, left). In contrast, Et-adipate bound form was determined at 1.4 Å resolution, and is assumed to be a post-reaction state (Fig. 1, right) because the carboxyl group of the bound substrate is located proximally to the active site, mimicking just after cleavage at the ester group of the substrates.

We propose the novel enzymatic cycle of Cut190 [2]. First, the calcium ion at the allosteric site temporarily dissociates and Cut190 shifts to the pre-reaction state when the substrate is bound at the active site. Next, the enzymatic reaction proceeds to cleave the ester group, and the calcium ion is bound again after the cleavage of the substrate. The re-binding of Ca causes the structural changes to promote product elimination and new substrate binding.

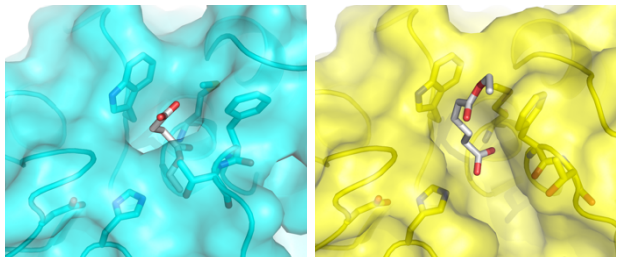

Fig. 1: Structures of pre-reaction (cyan) and post-reaction (yellow) states of Cut190 S176A/S226P/R228S.

References

[1] T. Miyakawa *et al*., *Appl*. *Microbiol*. *Biotechnol*. **99,** 4297 (2015).
[2] N. Numoto *et al*., *Biochemistry* **57,** *5289* (2018).
[3] W. Kabsh, *Acta Cryst*. **D66**, 125 (2010).
[4] A. J. McCoy *et al*., *J*. *Appl*. *Crystallogr*. **40**, 658 (2007).
[5] P. Emsley *et al*., *Acta Cryst*. **D66**, 486 (2010).
[6] P. D. Adams *et al*., *Acta Cryst*. **D66**, 213 (2010).

* numoto.str@mri.tmd.ac.jp