X06SA/2021G104

Structural basis for enhancement of enzyme activity and stability of PET degrading cutinase 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 is a member of the lipase family, and certain cutinase or cutinase-like enzymes have been reported to degrade polyethylene terephthalate (PET), a type of persistent plastic. A cutinase derived from the thermophilic bacterium *Saccharomonospora viridis* AHK190 (Cut190) exhibits high thermostability and enzymatic activity, making it a promising candidate for recycling waste plastic through enzymatic treatment.

Previously, we have shown that Cut190 retains its activity at temperatures above PET's glass transition temperature, which is around 60-65 °C. This high thermostability is advantageous for the efficient degradation of PET. Additionally, we have found that calcium ions significantly influence the thermostability and enzymatic activity of Cut190. The crystal structures of both the calcium-bound and calcium-free forms of Cut190 have revealed that the calcium binding site is distant from the active site and that calcium ions act as allosteric effectors, regulating the open/closed conformational transition at the active site. We have also reported two small aliphatic substrate bound states of Cut190 [1], elucidating the mechanism of the enzymatic activity regulated by calcium binding and dissociating.

In this study, we have successfully solved structures of complexes with molecules having a ring structure, which are similar to PET (-like) substrate with an aromatic ring. The obtained structures allow us to discuss more detailed mechanism of the recognition mode for PET degrading activity of Cut190.

2 Experiment

A mutant of Cut190 (Cut190*SS_S176A) was overexpressed in *Escherichia coli* and purified as described previously [2]. Crystals of the complex of the enzyme and 2-(N-morpholino)ethanesulfonic acid (MES) were obtained in crystallization buffer containing 0.1 M MES monohydrate pH 6.5, 0.2Mammonium sulfate, and 30%(w/v) PEGMME 5,000. The crystals were flash-frozen using liquid nitrogen, before X-ray diffraction experiments.

Collected diffraction data were automatically processed by PReMo [3]. Manual data processing using XDS [4] was performed as necessary. Phases were determined by the molecular replacement method using PHASER [5]. Several cycles of manual model rebuilding and refinement were performed by using Coot [6] and PHENIX [7], respectively.

3 Results and Discussion

The structure of the MES complex (Fig. 1) was determined at 2.6 Å resolution. The morpholine group is situated among the aromatic residues Phe106 and Trp201. This mode of accommodation by a ring-shaped molecule is very similar to what we observed in the structure of the Cut190*SS complex with dioxane, as we previously reported [2]. Conversely, a sulfo group is recognized in the oxyanion hole formed by the main chain amines of Phe106 and Met177 through hydrogen bonds. Additionally, the side chain of His254, part of the canonical catalytic triad, also forms a hydrogen bond with the sulfo group. These observations strongly suggest that the bound MES molecule mimics the tetrahedral intermediate during enzymatic catalysis.

These results encourage further research to synthesize PET-like compounds containing sulfo or phosphonate group that will mimic tetrahedral intermediates and to analyze the complexed structures.

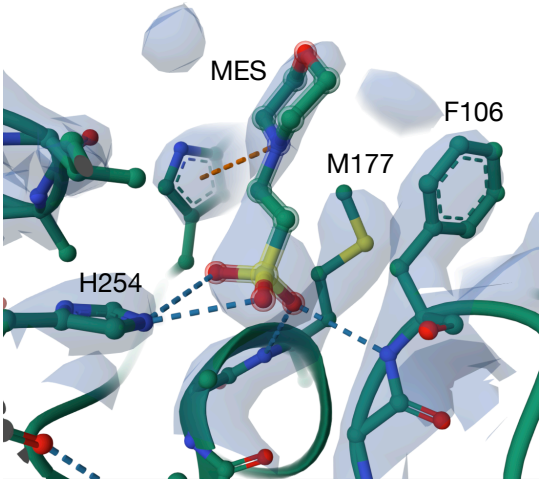

Fig. 1: Closeup view of MES binding site.

References

[1] N. Numoto *et al*., *Biochemistry* **57,** *5289* (2018).
[2] M. Emori *et al*., Proteins **89,** 502 (2021).
[3] Y. Yamada *et al*., *J. Phys. Conf. Ser.* **425,** 012017 (2013).
[4] W. Kabsh, *Acta Cryst.* **D66**, 125 (2010).
[5] A. J. McCoy *et al*., *J. Appl. Crystallogr.* **40**, 658 (2007).
[6] P. Emsley *et al., Acta Cryst.* **D66**, 486 (2010).
[7] P. D. Adams *et al., Acta Cryst.* **D66**, 213 (2010).

* numoto.str@mri.tmd.ac.jp
(Present E-mail: numoto.csb@okayama-u.ac.jp)