Structural basis for enhanced MHET degrading activity of engineered feruloyl esterase

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Abstract

Enzymatic degradation offers a promising solution to the persistent accumulation of synthetic plastics in the environment [1]. Since 2000, numerous Poly(ethylene terephthalate) (PET) hydrolases, or PETases, have been identified. These enzymes cleave the ester bonds of the polymer, producing mono-(2-hydroxyethyl) terephthalate (MHET), as primary degradation product [2]. MHET hydrolases (MHETases) further cleave MHET into terephthalic acid (TPA) and ethylene glycol, enabling their reuse as chemical feedstocks. Ferulic acid esterases (E.C. 3.1.1.73, FAEs) are a class of esterases with biotechnological relevance, as they can hydrolyze the ester linkages between hydroxycinnamic acids and arabinose in the plant cell walls. A FAE from *Fusarium oxysporum* (*Fo*FaeC, PDB ID: 6FAT) [3], belonging to the tannase-like family, is a structural homolog of the bacterial MHETase from *Ideonella sakaiensis* (PDB ID: 6JTT), [4,5]. *Fo*FaeC shows activity on PET oligomers and acts synergistically with PETases to enhance PET degradation [6]. The present study focuses on an *Fo*FaeC variant, *Fo*FaeC_G122S, engineered by structure-guided mutagenesis to mimic the MHETase active site. This variant displays improved catalytic activity against MHET compared to the native enzyme [6]. Its crystal structure in both apo and benzoic acid-bound forms (PBD IDs: 9I50 and 9HUN) were determined at 1.90 and 1.71 Å resolution, respectively. MHET was subsequently docked into the active site of unmodified *Fo*FaeC and G122S variant, followed by molecular dynamics (MD) simulations, to acquire deeper understanding of the observed biochemical findings. The engineered mutation resulted in increased active site flexibility, providing a possible explanation for improved catalytic efficiency. The combined crystallographic and computational analysis provide mechanistic insights into the molecular determinants of PET-active enzymes and their potential for biotechnological applications.

References

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