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Reaction Mechanism of the PET Degrading Enzyme PETase Studied with DFT/MM Molecular Dynamics Simulations

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Abstract

Polyethylene terephthalate (PET) has been widely used to make disposable bottles, among others, leading to massive PET waste accumulation in the environment. The discovery of the Ideonella sakaiensis PETase and MHETase enzymes, which hydrolyze PET into its constituting monomers, opened the possibility of a promising route for PET biorecycling. We describe an atomistic and thermodynamic interpretation of the catalytic reaction mechanism of PETase using umbrella sampling simulations at the robust PBE/ MM MD level with a large QM region. The reaction mechanism takes place in two stages, acylation and deacylation, each of which occurs through a single, associative, concerted and asynchronous step. Acylation consists of proton transfer from Ser131 to His208, concerted with a nucleophilic attack by Ser131 on the substrate, leading to a tetrahedral transition state, which subsequently results in the release of MHET after the breaking of the ester bond. Deacylation is driven by deprotonation of an active site water molecule by His208, with the resulting

hydroxide attacking the acylated Ser131 intermediate and breaking its bond to the substrate. Subsequently, His208 transfers the water proton to Ser131, with ensuant formation of MHET and enzyme regeneration. The rate-limiting acylation has a free energy barrier of 20.0 kcal·mol-1, consistent with the range of experimental values of 18.0-18.7 kcal·mol-1. Finally, we identify residues whose mutation should increase the enzyme turnover. Specifically, mutation of Asp83, Asp89, and Asp157 by nonpositive residues is expected to decrease the barrier of the rate-limiting step. This work led to the understanding of the catalytic mechanism of PETase and opened the way for additional rational enzyme engineering. © 2021 American Chemical Society.

Author keywords

biodegradation; biorecycling; density functional theory; plastic accumulation; QM/ MM ; umbrella sampling

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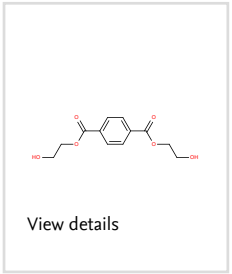
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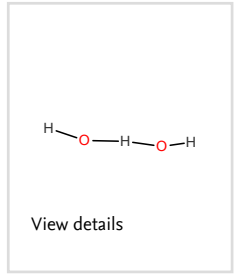
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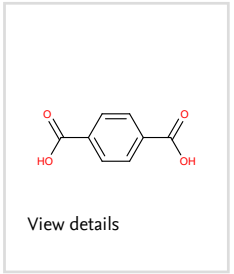
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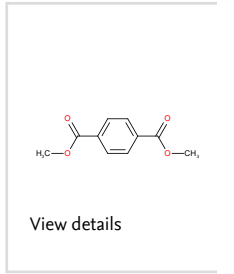
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