Abstract

Plastic waste, including polyurethane (PUR), represents a significant environmental challenge due to its widespread use in various industries and its resistance to natural degradation. As traditional recycling methods for plastics remain inefficient, alternative approaches, such as enzymatic degradation, offer a promising, eco-friendly solution. However, research on enzymes capable of degrading PUR is still limited. This dissertation focuses on investigating the cutinase enzyme from *Thermobifida fusca* (*Tf*Cut2) and its potential for PUR degradation, with Impranil DLN used as a model substrate to explore the enzyme's catalytic activity.

The study employs a combination of computational and experimental methods to identify the molecular determinants involved in substrate binding and catalysis. Molecular docking and molecular dynamics simulations were performed to study the interactions between *Tf*Cut2 and Impranil DLN, identifying key residues that play a role in the enzyme's ability to bind to the PUR substrate. In addition, computational protein design tools were used to engineer mutations aimed at enhancing protein-ligand binding and enzyme's catalytic performance.

Experimental validation of the designed mutants showed that three single-point variants, namely G62A, T61V and T207D, demonstrated significantly increased degradation rates, with G62A achieving more than a twofold improvement in activity over the wild-type enzyme. T207D also showed a marked increase in production yield, further underscoring the potential of rational enzyme engineering. These results suggest that engineering mutations can substantially enhance the catalytic efficiency of *Tf*Cut2 for PUR degradation.

The study highlights the complexities of modelling and experimentally assessing PUR degradation, given the heterogeneity of PUR structures and their degradation products. Despite these challenges, this work establishes a framework for tailoring cutinases for synthetic polymer degradation, providing insights into substrate binding modes, and potential rate-limiting steps. The findings contribute to the broader goal of developing efficient, sustainable solutions for polymer recycling and plastic waste remediation.

Keywords

Plastic biodegradation, Enzymatic degradation, Polyurethane, Cutinase, Enzyme engineering, Molecular modelling, Molecular dynamics, Protein design