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Molecular Engineering of Lytic Polysaccharide Monooxygenase TthLPMO9G: Rational Design of Mutations and evaluation of Substrate Recognition, Mode of Action, and Reducing Agent Specificity

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PURPOSE OF THE ABSTRACT

Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that play a crucial role in the oxidative degradation of recalcitrant carbohydrate polymers. Study of their activity provides insights into the biological mechanisms employed in nature for the polysaccharide degradation. Introducing point mutations is a common way to elucidate the catalytic activity of enzymes and shed light on the importance of targeted amino acids. In this study, three LPMOs from the thermophilic fungus Thermothelomyces thermophila, including the wild-type TthLPMO9G and two mutants generated following a rational mutation approach, H140A and S28A, were evaluated for their activity on cellulose, the specificity towards reducing agents with either O2 or H2O2 as co-substrate, as well as the stability against temperature and oxidative stress. Notably, the H140A mutant exhibited a weakened catalytic activity towards cellulose, although its stability and its monooxygenase and peroxygenase reactivity in the absence of substrate remained unaltered, which highlights the importance of His in this position for substrate recognition and catalysis. The S28A mutant exhibited a different pattern of soluble products released when acting on cellulose, which implies an altered mode of action compared to the wild-type enzyme when reduced by specific electron donors. The results showed that mutation in specific amino acids has a significant effect on the substrate recognition and activity of TthLPMO9G on cellulose, as well as the type of the produced oxidized oligosaccharides.

FIGURES

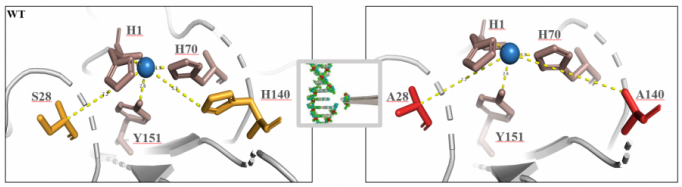

FIGURE 1

Comparison of wild-type TthLPMO9G and two point mutants.

The wild-type TthLPMO9G protein is shown in gray and the amino acids of interest are shown in orange color, while the H140A and S28A mutants are shown in red, respectively. The H140A mutation was introduced at position 140, which is located next to the a

FIGURE 2

KEYWORDS

lytic polysaccharide monooxygenases | point mutations | oxidoreductases | cellulose

BIBLIOGRAPHY

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