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Biochemical and Catalytic Analysis of Computationally Designed Hyperthermostable Bacterial DyP-type Peroxidases

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PURPOSE OF THE ABSTRACT

DyP-type peroxidases (DyPs) are microbial enzymes that catalyze the oxidation of a wide range of substrates, including lignin-derived compounds and metals, such as Mn2+ and Fe2+ and have enormous biotechnological potential in biorefineries. Nonetheless, several questions on the molecular basis of enzyme function and stability remain unanswered. Protein stability is a complex, multi-variable equation, and tackling it with rational single-point mutations or through iterative rounds of random mutagenesis can be time and energy-consuming. We have applied computational tools to design hyperthermostable variants of PpDyP and have produced and characterized the variants suggested by the web servers PROSS (dsPpDyP_1), with 29 mutations, and FireProt (dsPpDyP_2), with 21 mutations. Both enzymes exhibit remarkable improvements, i) shifting the optimum activity temperature by 35-40 °C and ii) exhibiting a melting temperature of 75-85 °C, circa 10-20 °C higher than wild-type, without jeopardizing the enzyme activity. Moreover, the mutations inserted in the dsPpDyP_1 variant abolished the temperature-induced aggregation of its tertiary structure in a 20 to 100 °C temperature ramp. To assess the mechanisms behind the improved thermostability, DNA shuffling of genes of variants vs. wild-type gene was performed, and libraries of enzyme chimeras were screened to select variants maintaining the hyperthermostable phenotype while showing a reduced mutational load. The biochemical and kinetic analysis of a subset of variants hypothetically containing only neutral or beneficial mutations is presented and discussed.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Protein Stability | Protein Engineering | Computational Engineering | Biocatalysis

BIBLIOGRAPHY