Rational design strategy to optimize the catalytic performance of glucose oxidase to achieve environmentally sustainable development

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Introduction

Glucose oxidase (GOx) is a kind of oxidoreductase, which uses molecular oxygen as electron acceptor to catalyze glucose to produce gluconic acid (GA) and hydrogen peroxide (H_2O_2). It has the advantages of substrate specificity, strong catalytic activity and low environmental pollution, and is widely used in food processing, oral hygiene, chemical industry, and medical diagnosis. GOX can be used in the bleaching process of the textile industry to reduce the environmental pollution and sewage problems caused by the traditional bleaching process. In the feed industry, GOx is an effective feed additive to replace antibiotics, which can avoid environmental problems caused by antibiotic resistance and drug residues, and achieve sustainable development of the environment.

Methods

Based on computer simulation, through the rational design of FAD-binding energy optimization, enhancement of hydrophobic core and introduction of hydrogen bonding network, site-specific mutation of GODA was carried out, and Pic-9γ carrier was connected and electrically transferred into GS115 yeast cells. After screening of converters, enzyme solution was obtained, enzymatic properties were determined, and the mutant with improved thermal stability was obtained.

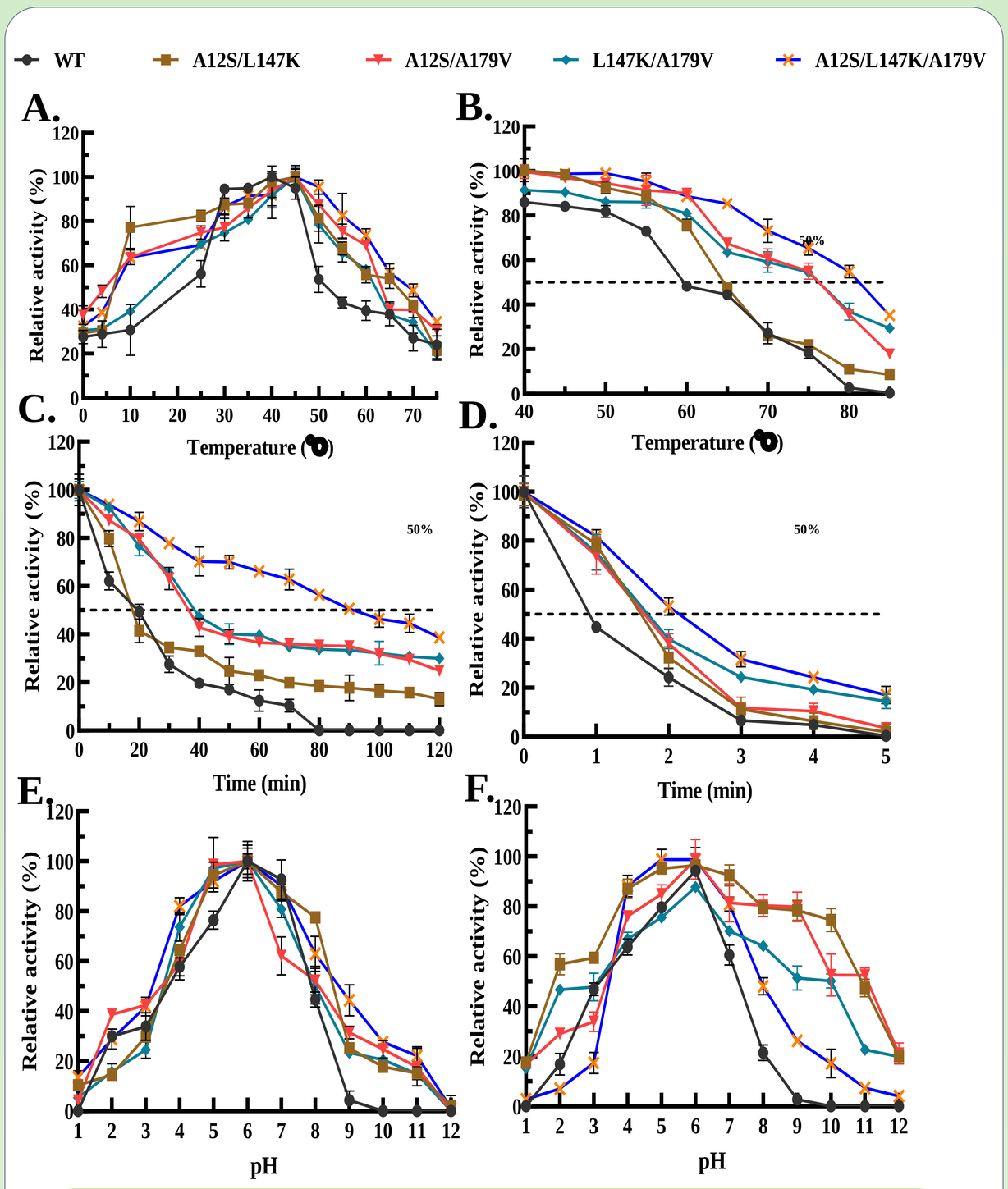
Results & Discussion

Combined the dominant mutants, and the results showed that the T_{50} value of the wild type was 60℃, and the A12S/L147K/A179V was 21℃ higher than that of WT. At 70℃, the $t_{1/2}$ of the dominant combination mutant was 68min, 4.5 times longer than that of WT (20 min). At 75℃, the $t_{1/2}$ of the combination mutation was 40min, 2.2 times longer than that of WT (7min). The three-point combination mutation showed better thermal stability, showing the addition of thermal stability between point mutations.

A12S/L147K/A179V also shows better alkali stability in alkaline environments (pH9-10), which is more conducive to its important role in alkaline production environments and improve production efficiency.

In order to explore the molecular mechanism related to the improvement of thermal stability of dominant mutant A12S/L147K/A179V, MD simulation of 30ns was performed on wild type and mutant at 300K. Root mean square fluctuation (RMSF) values showed that L147K and A179V mutations reduced the flexibility of the associated residues, anwd reduced RMSF values in the flexible region enhanced the rigidity of the enzyme protein, thereby improving its stability. At the same time, the lower Rg value of A12S/L147K/A179V also proves that the enzyme protein has a lower expansion degree and a stronger stability.

The WT and the three-point combination mutation trajectories in the equilibrium state of molecular dynamics simulation were analyzed for protein secondary structure. Compared with WT, the random curl in three-point combination mutation is reduced, and more B-angle is generated. B-angle has strong stability and rigidity, and is one of the important structural units of protein structure. More B-angle is generated in three-point combination mutation, which enhances the stability of protein to a certain extent.



(A) The optimum temperature for the wild type and its mutants; (B) T_{50} of the wild type and its mutants;(C) Half-life $t_{1/2}$ of the wild type and its mutants at 70℃; (D) Half-life $t_{1/2}$ of wild-type GODA and its mutants at 75℃(E) The optimum pH of the wild type and its mutants; (F) pH stability of the wild type and its mutants

Conclusion

Based on computer rational design and molecular dynamics simulation, we improved the catalytic efficiency and thermal stability of glucose oxidase, thus laying a theoretical foundation for the molecular improvement of glucose oxidase. In addition, these findings provide additional options for industrial production,, mitigating potential environmental risks associated with the production process, and have important implications for achieving environmental sustainability.

Acknowledgement

This Start-up Funding from Jiangsu University of Science and Technology.

References

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