Construction of Thermostable Enzymes with Protein Engineering

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Abstract

For the industrial application of enzymes, high catalytic activity and thermostability are essential factors. To date, the thermostability of many enzymes has been enhanced using protein engineering method in order to confer properties suitable for industrial use. Random mutagenesis has been widely employed as a representative strategy for improving enzyme thermostability. In recent years, AI-based and computational design methods have also been applied to protein engineering; however, their success rates remain relatively low. In comparison with such semi–trial-and-error approaches, rational protein design based on structural models and experimental structural data has been developing as a powerful and efficient strategy for constructing thermostable enzymes within a short time.

In this study, we report how enzyme thermostability can be improved through the introduction of multiple mutations. The sequence shuffling method employed in this work enabled the generation of thermostable mutant enzymes without reducing their specific activity. Furthermore, the sequence shuffling method is not only an effective method for screening mutation candidates but also a useful approach for elucidating structural differences among catalytically homologous enzymes derived from different species. Finally, based on the structural analyses of the wild-type enzyme and the thermostable mutants, we discuss the molecular mechanisms of individual mutations and provide new insights into their thermostabilizing effects.

Keywords

Thermostable, Thermophile, X-ray crystallography, Protein engineering, Bioinformatics.