Development of thermostable β- fructofuranosidase

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Abstract

β-Fructofuranosidase (EC3.2.1.26, β-FFase) from *Arthrobacter* sp. K-1 catalyzes a transfructosyl reaction. From lactose and sucrose, this enzyme can produce lactosucrose which is authorized as the food for specified health uses (FOSHU, Tokuho) by the consumer affairs agency of Japan. Currently, the lactosucrose has been produced by using a batch-reactor system because the thermostability of the β-FFase has not been enough for applying to a flow-reactor system. The industrial process should change to eco-friendly way such as low CO_2 emission, low waste and also low environmental impacts. The aim of our project is to create a hyper-thermostable β-FFase to apply the enzyme to the flow-reactor system. In the previous study, we constructed a thermostable mutant 24Y447P by the random mutagenesis, and partially revealed its thermostabilizing mechanism using fragment molecular orbital (FMO) method on the earth simulator in 2011.

In this time, we have succeeded to develop a novel thermostable mutants designated as 29-3 from the 24Y447P, with 2 amino acid substitutions of K247D and D260P. The half-life period of the 29-3 is surprisingly up to 1.5 times long as 24Y447P at 62℃. MD and FMO simulations suggested that C-terminal region of the β-FFase have an effect on its thermostability. The results were also supported by the C-teminal deletion study that shown GLY533-GLY577 deletion mutant lacking its thermostability. The 29-3 is excellent at lactosucrose productivity that the highest point is marked at 28.8% at 65℃, while the wild type marked at 26.5% at 60℃.

Keywords: Fragment molecular orbital method, Thermostable enzyme, Thermostable mechanism