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, Government of Japan. Currently, the lactosucrose has been produced by using a batch-reactor system because the thermostability of 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 have succeeded to construct a thermostable mutant of the β -FFase, designated as 24Y447P. The half-life periods of wild type and 24Y447P at 60°C are 0.8 days and 15.9 days respectively. The 24Y447P has five amino-acid substitutions in the sequence which may contribute to the thermostability. Mutational analyses revealed that a substitution of 447th amino acid, PHE447PRO, is most important for the increased-up thermostability of 24Y447P. In this study, we applied the fragment molecular orbital method (FMO) in order to simulate the thermostabilising mechanism of PHE447PRO mutation. We found the intermolecular interaction energy of 24Y447P is -7.4 kcal/mol better than the wild type, in terms of around 447th amino acid.

Keywords: Fragment molecular obital method (FMO), Thermostable enzyme, Thermostable mechanism