DESIGN OF ENGINEERED ACTIVE ZYMOGEN OF MICROBIAL TRANSGLUTAMINASE

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Microbial transglutaminase (MTG) has shown to be a powerful biocatalytic glue for site-specific crosslinking of a range of biomolecules and synthetic molecules, those handled with an MTG-reactive moiety. The preparation of active recombinant MTG requires the posttranslational proteolytic digestion of propeptide working as an intramolecular chaperon to assist the correct folding of MTG zymogen in the biosynthesis. Herein, we propose an engineered active zymogen of MTG (EAZY-MTG) that is expressed as soluble form in the host E. coli cytosol and exhibits the crosslinking activity without limited proteolysis. Based on the 3D structure of MTG zymogen, saturated mutagenesis of K10 or Y12 in propeptide domain leads to generate several active zymogen mutants. In particular, K10D/Y12G mutant exhibited the catalytic activity comparable with a mature form. However, the expression level was low possibly due to the reduction of chaperone activity and/or the promiscuous substrate specificity of MTG, which is potentially harmful to the host cells. By contrast, soluble K10R/Y12A mutant was expressed in the host cytosol and exhibited unique substrate-dependent reactivity toward peptidyl substrates. Our proof-of-concept study provides insights into the design of a new biocatalyst by using the zymogen as a scaffold and will convey a potential route to the high-throughput screening of MTG mutants for bioconjugation applications.

Figure 1 – Schematic illustration of the preparation of recombinant active mutants from MTG zymogen (i) by limited proteolysis by thrombin or (ii) designing mutated propeptide to modulate the interaction with the active site.