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Improvement of Amylomaltase from *Thermus aquaticus* by Random and Saturation Mutageneses

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Amylomaltase (EC 2.4.1.25) from *Thermus aquaticus* catalyzes an intramolecular transglycosylation of α-1,4 glucan and produces cycloamylose, which is a cyclic α-1,4 glucan with a degree of polymerization of 22 and higher. The amylomaltase has weak but significant hydrolytic activity together with its major transglycosylation activity, which consequently decreases the yield of cycloamylose. To diminish the hydrolytic activity of this enzyme, random mutations are introduced into the gene coding for this enzyme. In the random mutagensesis experiment, it is suggested that tyrosine 54 (Y54), far away from the catalytic site, was involved in hydrolytic activity. In order to investigate the function of Y54, we have performed saturating mutagenesis at Y54 within the amylomaltase and examined the properties of the mutated enzymes. The reaction specificities of the mutated enzymes were surprisingly changed by only one amino acid replacement at Y54. Y54G mutated enzyme had higher cyclization activity in addition to the lower hydrolytic activity. These mutated enzymes also provided useful information to gain further understanding for the activity and the specificity of this enzyme.

Key words: amylomaltase, protein engineering, cycloamylose, cyclic glucan, *Thermus aquaticus*

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