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## Engineering *Thermus* Maltogenic Amylase with Improved Thermostability: Probing the Role of the Conserved Calcium Binding Site in Cyclodextrin-degrading Enzymes

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*Thermus* maltogenic amylase (ThMA), one of the cyclodextrin (CD)-degrading enzymes, is expected to have a calcium-binding site (Ca<sup>2+</sup> site) based on multiple sequence alignments. In spite of the presumption of the Ca<sup>2+</sup> site in ThMA, however, its thermostability is independent of calcium ions. In order to investigate the effect of calcium ions on thermostability, two mutations (Ile152Asn and Ser153Asn) were introduced into Ca<sup>2+</sup> sites of a thermostabilized ThMA mutant (ThMA-DM2) using site-directed mutagenesis. The resultant mutant (ThMA-DM2-Ca) showed highly improved thermostability in the presence of calcium ions. The relative hydrolysis activity of ThMA-DM2-Ca also increased in comparison with that of ThMA-DM2 whereas transglycosylation activity was not affected by substitutions. The result confirmed that the residues located in the Ca<sup>2+</sup> site (Ile152 and Ser153) played critical role in stabilizing CD-degrading enzymes.

**Key words:** calcium binding site, cyclodextrin-degrading enzymes, mutagenesis, thermostability, *Thermus* maltogenic amylase

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