

## Kinetic Analysis and Identification of Essential Catalytic Residues of $\alpha$ -L-Arabinofuranosidase from the Hyperthermophile *Thermotoga maritima*

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### Abstract

$\alpha$ -L-Arabinofuranosidase ( $\alpha$ -L-AFase, EC 3.2.1.55) catalyzes the hydrolysis of  $\alpha$ -1,2 or  $\alpha$ -1,3 and  $\alpha$ -1,5 L-arabinofuranosidic bonds in hemicelluloses such as arabinoxylan, arabinan and other arabinose-containing polysaccharides. A gene encoding  $\alpha$ -L-AFase was isolated from the hyperthermophilic microorganism, *Thermotoga maritima*. The enzyme for ABFB gene (*abfB*) was expressed in *E. coli* and the enzyme properties were characterized(1). In order to identify critical amino acid residues, the ABFB amino acid sequence was aligned with other glucosyltransferase sequences, and three amino acid residues were selected for site-directed mutagenesis experiments. Glu-26, Glu-172, and Glu-281 were independently replaced with alanine, aspartic acid and glutamine, respectively. Replacement of those residues caused decrease of  $K_{cat}$  by a factor of between  $10^3$  and  $10^4$  and this fact indicated that all of these residues were important for catalytic activity. The kinetic analysis and structural comparison with AFase of *Geobacillus stearothermophilus*(2) revealed that Glu172 and Glu281 played a role as the acid/base and nucleophile residues, respectively, and Glu26 as a substrate binding site.

### References

1. Yoon, H -S., I. Keum, N. S. Han, and C. H. Kim, Molecular cloning and characterization of a gene encoding  $\alpha$ -L-arabinofuranosidase form *Thermotoga maritima* (2004), Food Sci. Biotechnol. 13, 244-247.
2. Hovel, K., D. Shallom, K. Niefind, T. Baasov, G. Shoham, Y. Shoham, and D. Schomburg, Crystallization and preliminary X-ray analysis of a family 51  $\alpha$ -L-arabinofuranosidase from *Geobacillus stearothermophilus* T-6 (2003), Acta Crystallogr. D., 59, 913-915.