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Site Direceted Mutagenesis of β-galactosidase from *Lactococcus lactis* ssp. *lactis* 7962

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Abstract

The secondary and the tertiary structure of β-galactosidase from *L. lactis* ssp. *lactis* 7962 were designed by computer simulation program of nnpridict and sybyl ver 6.3. Based on the results, we tried to specifically change amino acids which are thought to be part of the active site. The mutant enzymes with one site substitution runs as follows: Glu-384 was replaced by Gln or Val, His-386 was replaced by Phe, Glu-429 was replaced by Gln, Asn-428 was replaced by Asp, Tyr-475 was replaced by Phe, or Glu-506 was replaced by Asp. The mutant enzymes with the other two substitutions were 384Gln-429Gln and 384Val-429Gln. Studies with site specific mutants of β-galactosidase indicated that Glu-384 and Glu-429 are very important and are probably a ligand to Mg^{++}. Thus, the negatively charged side chains of Glu-384 and Glu-429 is important for divalent cation binding to β-galactosidase. A divalent metal ion, Mg^{++}, is required for maximal activity of native β-galactosidase. Substitution for Tyr-475 or Glu-506 had little effect on the enzyme for Mg^{++}, implying that these two sites are not involved in Mg^{++} binding. Tyr-475 and Glu-506 are conserved in a number of β-galactosidase from difference sources, which sites are supposed to be part of substrate binding site. The enzymes with substitution for Tyr-475 or Glu-506 appear to be totally inactive. The obtained results suggest that Tyr-475 and Glu-506 are important catalytic residues of β-galactosidase.