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Site-Directed Mutagenesis of Tryptophan 622 of *Thermoactinomyces vulgaris* R-47 Glucoamylase: pH Optima and Activities of Five Mutants

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In *Aspergillus awamori* glucoamylase, the optimal pH has been reported to increase to maintain activity by a mutation of Ser411 which forms a hydrogen-bond with a catalytic base (Fang and Ford, *Protein Eng.*, 11, 383-388 (1998)). Most glucoamylases have either Ser or Gly at this position, whereas only *Thermoactinomyces vulgaris* R-47 glucoamylase (TGA) and two putative glucoamylases have Trp. We focused on Trp622 in TGA and examined the pH optima of five mutants, W622C, W622D, W622G, W622H and W622S. The pH optima of these mutants were 6.2-6.8, which was identical to or slightly lower than that of the wild-type enzyme. However, the activities of these mutants at pH optima decreased to 4.3-52% of that of wild-type enzyme. From these results and information on the crystal structures of glucoamylases, Trp622 in TGA is suggested to be an important residue for substrate binding rather than for determination of optimal pH.

Key words: glucoamylase, optimal pH, catalytic base

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