

Alkaline active maltohexaose forming α -amylase from *Bacillus halodurans* LBK 34.,

Abstract:

The gene encoding Amy 34, a maltohexaose-forming α -amylase from *Bacillus halodurans* LBK 34 isolated from Lake Bogoria, Kenya, was cloned and sequenced. The mature peptide consists of 958 amino acids with a theoretical molecular weight of 107.2 kDa and pI 4.41, respectively. The gene was expressed in *Escherichia coli* and the recombinant enzyme purified to homogeneity by a combination of metal chelate affinity and size exclusion chromatography. The pure enzyme exhibited optimum activity at 60 °C and pH 10.5–11.5. The enzyme retained over 60% activity after incubation at 55 °C for 4 h and was most stable at pH 9.0. Complete inhibition of enzyme activity was observed in presence of 5 mM Cu²⁺, Fe²⁺, Fe³⁺, Mn²⁺ and 5 mM EDTA. The enzyme displayed 80% of its original activity in presence of 1% (w/v) SDS and was stable in presence of up to 5 mM DTT. Maltohexaose (G6) was the main initial product of starch hydrolysis while other products formed were G4 > G2 > G5 > G3 and G1. The main end product of the enzyme's action on amylose, amylopectin and maltodextrin is maltotetraose. Amy 34 could not hydrolyse pullulan, α and β -cyclodextrin but could hydrolyse γ -cyclodextrin to produce glucose, maltose and maltotetraose. Maltotetraose was the smallest α -(1–4) linked maltooligosaccharide that could be hydrolysed by the enzyme.