Comparison of kinetic properties of crude and purified α-amylase from *Bacillus licheniformis* ATCC 6346 with commercial amylase from *Bacillus licheniformis*

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Thermostable α-amylases are generally used for industrial applications. Kinetic properties of crude and purified extra-cellular thermo-stable α-amylase from *Bacillus licheniformis* ATCC 6346 were compared with commercial (Termamyl^R, NOVO industries from Denmark) α-amylase from *Bacillus licheniformis*. The influence of incubation time on the production of reducing sugar from starch ($20gl^{-1}$) was studied for 60min at pH 7.0 and 85°C. Commercial and crude α-amylases showed zero order kinetics for 10min while purified α-amylase showed zero order kinetics for 8min. The activities of crude, purified and commercial α-amylases were measured at different temperatures ranging from 40 to 95°C and the optimum temperature for the activities of crude and purified enzymes was 85°C while that for the commercial enzyme was 90°C. The optimum pH was 7.0 for the crude, purified and commercial enzymes at 85°C with starch ($20gl^{-1}$). Michaelis constants for crude, purified and commercial enzymes to soluble starch were 0.47, 1.42 and 0.71 gdl^{-1} respectively at pH 7.0 and at 85°C. When the crude enzyme was pre-incubated at 85°C and at pH 7.0, it lost 40% of its initial activity at 10min while the purified enzyme lost 75% of its initial activity at 10min and the commercial enzyme did not lose activity at 10min. When the crude and purified α-amylases were pre-incubated with 1 mM Ca^{2+}, 100% of initial enzyme activities were retained at 60min at 85°C and pH 7.0. Thus Ca^{2+} stabilizes the α-amylase from *Bacillus licheniformis* ATCC 6346. Substrate specificity for crude and purified enzymes were carried out. Crude and purified enzymes showed 119, 77.7 & 20.3 and 107,60, & 20% of relative activities respectively with amylose, amylopectin, and maltose when compared to soluble starch at 85°C and pH 7.0. Both crude and purified enzymes showed no activity with cellulose, sucrose and pullulan. Therefore substrate specificity indicated, that both purified and crude α-amylases were able to hydrolyse mainly starch, amylose and amylopectin.

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