Purification, immobilization and high stabilization of a β-glucosidase from *Aspergillus japonicus* by ionic adsorption

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A β-glucosidase from *Aspergillus japonicus* was produced by submerged fermentation using sugar cane bagasse in nature as carbon source, at 30 ºC, for 72 hours. The dialyzed crude extract containing the active β-glucosidase was applied to chromatographic columns, in three successive steps (DEAE-fractogel, MANAE- agarose and octyl-sepharose). The enzyme migrated as a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the molecular weight of the enzyme was estimated to be 114 kDa. The β-glucosidase activity assay was determined using p-Nitrophenyl-β-D-glucopyranoside (β-pNPG) as substrate, in a reaction time of ten minutes. The purified enzyme was immobilized by ionic interaction on MANAE agarose and DEAE-celulose. Soluble β-glucosidase presented a half-life of 20 min, at 60ºC, while the MANAE-agarose and DEAE-celulose derivatives presented a half-life of 25 and 48 hours respectively. The optima pH for soluble β-glucosidase, MANAE-agarose and DEAE-cellulose derivatives was 4.5. The optima temperature for both derivatives and soluble enzyme was 65ºC. The amino acid sequence determined by mass spectrometry demonstrated a similar structure for the β-glucosidase of *Aspergillus niger* and *A. kawachii*. The Km values for soluble enzyme, MANAE-agarose and DEAE-cellulose derivatives using β-pNPG as substrate were 0.4, 1.6 and 0.96 mg/mL, respectively. The Vmax values were 24, 25.8 and 13.6 U/mg protein for the soluble enzyme, MANAE-agarose and DEAE-cellulose derivatives, respectively. Because of these characteristics, the MANAE-agarose and DEAE-cellulose derivatives can be successfully applied in processes which involve high temperatures.

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