SLASS 1984

IMMOBILIZATION AND KINETIC STUDIES OF α - AMYLASE AND GLUCOAMYLASE

V.Arasaratnam and K.Balasubramaniam.
Department of Biochemistry, Faculty of Medicine, University of Jaffna,

α-Amylase and glucoamylase were coupled to Sepharose - 4 B which was activated by electrophilic and nucleophilic methods using cyanogen bromide. Cyanogen bromide concentration was directly proportional to the concentration of the enzyme protein coupled. When 120 mg CNBr /g gel was used for activation by electrophilic and nucleophilic methods, the protein coupled was 70% and 19% respectively. The coupling was linear up to 10 min and reached maximum by 0.5 hr. When the enzyme concentration added for coupling increased from 1 - 1000 µg, the % activity of the immobilized enzyme decreased and it was inversely proportional to the log of enzyme concentration added.

The apparent Km of the immobilized α-amylase and glucoamylase for starch was 1.33% and 0.72% respectively. The pH optimum of soluble α-amylase compared with that of the immobilized enzyme shifted from 6.9 to 6.5 in 0.02M phosphate buffer. The shift in pH optima for glucoamylase was from 4.8 to 5.2 when 0.1M acetate buffer was used.

The temperature optimum of immobilized α-amylase compared with the soluble enzyme shifted from 45°C to 50°C and that for glucoamylase was from 55°C to 58°C. In the temperature range

from 30°C to 60°C the immobilized α-amylase was most stable at 50°C whereas the soluble enzyme was most stable at 45°C indicating that the immobilized enzyme has a slightly higher temperature stability. The glucoamylase, both soluble and immobilized, were most stable at 4°C than at higher temperatures.

Work is in progress to study the optimal conditions for the hydrolysis of starch.