STUDIES ON IMMOBILIZATION OF α—AMYLASE OF CYANOGEN BROMIDE ACTIVATED SEPHADEX G 200

K. Wimalasena, A. B. Attygalle and K. Balasubramaniam
(*Department of Biochemistry, University of Colombo*)

α—Amylase was immobilized by the coupling of α amylase to Cyanogen Bromide activated Sephadex G-200. On activation of Sephadex by Cyanogen Bromide 80% contraction by volume was observed. The activity of α amylase free and coupled were measured in Starch—Phosphate buffer (pH 6.9) using Dinitro salicylic acid reagent. The protein was determined by the Kjeldhal method. It was found that the activity of the coupled enzyme was about 4% although 10.56% of the protein was coupled. The immobilized α-amylase was most stable to heat (45° C) and hydrogen ions (pH 7.0 and 8.3) when compared with the soluble α-amylase. Since the activity of the solid enzyme was much less (≏4%) than the original enzyme, present work is concentrated on the study of the factors affecting on the activity of the solid enzyme such as steric hinderance and multiple attachment of the enzyme to the matrix. We hope that the steric hinderance problem could be overcome by introducing a long chain hydrocarbon spacer arm in between the matrix and the enzyme, and that the multiple attachment of the enzyme to the matrix could be decreased by the controlled Cyanogen Bromide activation of Sephadex before enzyme coupling.