IMMOBILIZATION OF AMYLOGLUCOSIDASE ON DEAE-CELLULOSE

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The iso-electric pH of amyloglucosidase, as determined by test tube method using DEAE-Cellulose and buffer of varying pH, was 3.8. At pH 7.0 about 95% of the added enzyme activity was absorbed. Available capacity of DEAE-Cellulose was determined batchwisely, by taking 1g of resin in different test tubes with increasing amounts of enzyme and allowing these to equilibrate at pH 7.0 (0.01M phosphate buffer) for 8 hrs. The available capacity was 5300 active units/g of resin (1 unit = 1mg glucose, produced/min) and protein adsorbed was 260mg/g of resin. When the adsorption capacity of DEAE-Cellulose for amyloglucosidase was determined in a packed column by adding successive equal amounts of the enzyme at pH 7.0 with equilibration time of 10 minutes, the maximum adsorption of amyloglucosidase was found to be 174 active units (9mg protein)/2.5g of resin which is equivalent to 1.3% of available capacity of the resin. When the enzyme was eluted at pH 3.0 from the column the eluate had 8mg of protein and the activity was 106 units. Hence for commercial purpose batchwise adsorption is preferable.

There is an ion exchange equilibrium between an ion exchange resin (stationary phase) and the mobile electrolyte phase. The effective distribution co-efficient is defined as the enzyme activity in mobile electrolyte phase to the enzyme activity adsorbed in resin. The effective distribution co-efficient was 0.164 and this effective distribution co-efficient remained constant up to 62% of available capacity of resin. Above this limit, the effective distribution co-effcient was increased as shown by increased amount of enzyme activity in the mobile phase. When only 0.2% of the available capacity of the resin was used (by adsorbing 54 units of enzyme to 5g resin at pH 7.0) the amount of enzyme that leaked (described from resin) into 165ml eluate of 0.01m phosphate buffer pH 7.0 was 26.4% (of the adsorbed enzyme). Cross-linking with 0.2% of glutaraldehyde to prevent the leaking of the adsorbed enzyme, reduced the activity by 85%.