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KINETIC STUDIES ON SOLUBLE AND IMMOBILIZED ALPHA AMYLASE AND GLUCOAMYLASE

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Abstract: Alpha amylase and glucoamylase were coupled seperately to Sepharose–4B which was activated by an electrophilic method (by using cyanogen bromide). The kinetic properties of these enzymes in immobilized form were compared with those of their soluble enzymes. The Km values of soluble and immobilized alpha amylase for starch were 0.89% (W/V) and 1.33% (W/V) respectively at pH 6.9 and at 40^{0}C and the Km values of soluble and immobilized glucoamylase for starch were 0.25% (W/V) and 0.72% (W/V) respectively at pH 4.8 and at 50^{0}C. The optimum pH for soluble alpha amylase compared with that of the immobilized enzyme shifted from 6.9 to 6.5 in 0.02M phosphate buffer while in the case of glucoamylase the pH optimum in 0.01M acetate buffer increased from 4.8 to 5.2 on immobilization. There was no shift in optimum phosphate concentration (0.02M) for both soluble and immobilized alpha amylase at pH 6.9 and at 40^{0}C and likewise no shift in optimum acetate concentration (0.01M) for soluble and immobilized glucoamylase at pH 4.8 and at 50^{0}C. The optimum temperature for the activities of soluble alpha amylase compared with the immobilized enzyme assayed in 0.02M phosphate buffer (pH 6.9) shifted from 45^{0}C to 50^{0}C and that of glucoamylase assayed in 0.01M acetate buffer (pH 4.8) shifted from 55^{0}C to 58^{0}C.

1. Introduction

Enzymes of industrial or analytical use could have increased applicability, if they could be immobilized while still in an active state. Many studies have been reported on the preparation of immobilized enzymes.[2,3,6,7,8] Great interest in the use of water insoluble enzymes has been shown in industry. These enzymes can be readily recovered from the reaction mixture resulting in a considerable saving in process costs by repeatedly using the enzymes.[9]

Many products are currently produced from starch through the use of enzymes, whether soluble or immobilized. Both alpha amylase (1,4—α—D glucan glucanohydrolase; E.C.3.2.1.1) and glucoamylase (1,4—α—D glucan glucohydrolase; E.C.3.2.1.3) are important industrial enzymes. These are used in large scale for liquefaction and saccharification of starch.

Immobilized enzymes may exhibit slightly altered chemical and physical properties.[6,8] Therefore while designing chemical reactors for utilizing immobilized enzymes, certain parameters such as catalyst size, liquid flow—rate, temperature, pH, ionic strength, substrate concentration etc.,must be carefully controlled in order to yield optimum conversion.

Our earlier study was undertaken to develop a functional immobilized alpha amylase and glucoamylase.[1] This paper compares the kinetic proper-

ties of the soluble and immobilized forms of alpha amylase and glucoamylase. A knowledge of the kinetic parameters could be helpful in the design of reactors for continuous production of liquid glucose from soluble starch.

2. Materials and Methods

2.1 Materials

Alpha amylase (Hog pancreas) was obtained from Sigma Chemical Company, U.S.A. crude glucoamylase *(Aspergillus niger)* was from Ceylon Institute of Scientific and Industrial Research (C.I.S.I.R.), Sri Lanka, Sepharose—4B was from Pharmacia Fine Chemicals, and starch (soluble) was from BDH, UK. Cyanogen bromide was prepared in our laboratory.[1]

2.2 Immobilization of Alpha Amylase and Glucoamylase to Sepharose — 4B

In the preparation of both the immobilized alpha amylase and glucoamylase the soluble enzymes used had a specific activity of 906 u/mg protein and 370 u/mg protein respectively. They were prepared by electrophilic method.[1]

In the comparative studies equal amounts of soluble and immobilized enzyme proteins (0.96 mg protein as determined by micro—Kjeldhal method) were used.

The reducing equivalents produced by the action of alpha amylase and glucoamylase on starch were measured in terms of maltose and glucose respectively.[4]

One unit of alpha amylase will liberate 1μg of maltose from starch in 3 min at pH 6.9 and at 40^{0}C. One unit of glucoamylase will liberate 1μg of glucose from starch in 10 min at pH 4.8 and at 50^{0}C.

2.3 Kinetic Properties of Soluble and Immobilized Alpha Amylase and Glucoamylase

The Michaelis constants (Km) of both soluble and immobilized alpha amylase and glucoamylase were estimated by varying the concentrations of starch from 0.1% (W/V) to 2.0% (W/V) in 0.02M phosphate buffer (pH 6.9) at 40^{0}C and in 0.01M acetate buffer (pH 4.8) at 50^{0}C respectively.

The effect of pH on the activities of alpha amylase and glucoamylase was studied by altering the pH from 5.6 to 8.0 of 0.02M phosphate buffer at 40^{0}C and from 3.6 to 6.0 of 0.01M acetate buffer at 50^{0}C respectively.

Phosphate buffer (pH 6.9) in the range of 0.01 to 0.12M for a alpha amylase at 40^{0}C and acetate buffer (pH 4.8) in the range of 0.004 to 0.05M for glucoamylase at 50^{0}C were used to study the effect of ionic strengths on these enzymes.

The temperature optimum for alpha amylase in 0.02M phosphate buffer (pH 6.9) and glucoamylase in 0.01M acetate buffer (pH 4.8) were studied in the range of 4^0C to 80^0C.

The percentage activity was calculated in all cases (Figures 1 & 2 ; Table I, II & III) for both alpha amylase and glucoamylase as the activity at a particular condition / the maximum activity obtained in that set of experimental conditions x 100.

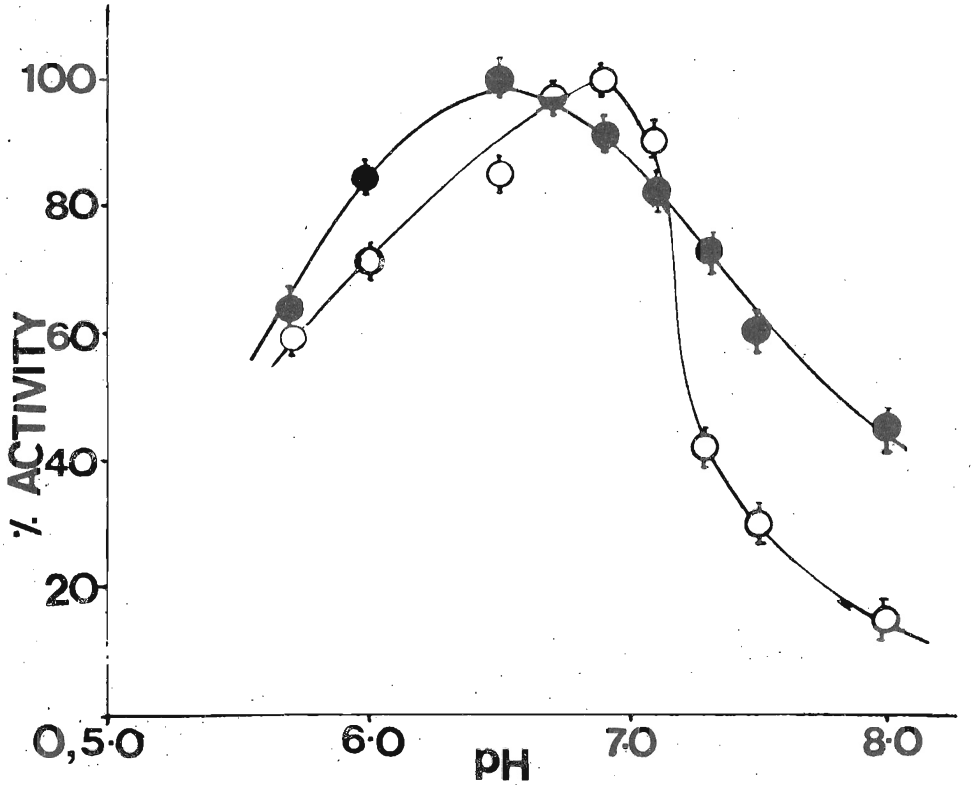

Figure 1. Effect of pH on soluble and immobilized alpha amylase activity in 0.02M phosphate buffer at 40^0C.

—O—O—Soluble enzyme —●—●—Immobilized enzyme.

(Each point is the mean of 4 experiments carried out in duplicates and bars indicate the standard deviations).

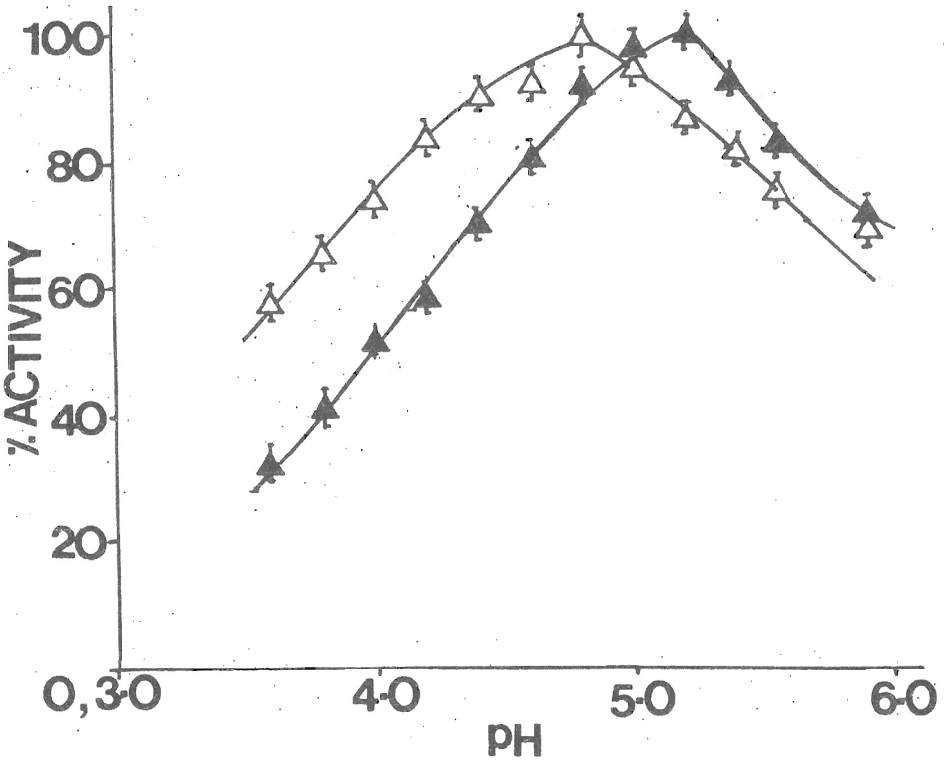

Figure 2. Effect of pH on soluble and immobilized glucoamylase activity in 0.01M acetate buffer at 50^0C.

—△—△— Soluble enzyme —▲—▲— Immobilized enzymes.

(Each point is the mean of 4 experiments carried out in duplicates and bars indicate the standard deviations).

3. Results and Discussion

The Km values of soluble and immobilized alpha amylase for starch (calculated from Lineweaver — Burk plots) were 0.89% and 1.33% respectively and that of soluble and immobilized glucoamylase for starch were 0.25% and 0.72% respectively. Krakowick *et. al.*[6] have immobilized glucoamylase (type 150, Novo) on aluminium oxide and on aluminium oxide activated by $TiCl_4$. The Km values of soluble glucoamylase, glucoamylase immobilized to aluminium oxide and glucoamylase coupled to activated aluminium oxide for starch were found to be 0.5%, 6.69% and 28.15% respectively. The increase in Km value of immobilized enzymes to that of the soluble enzyme may be due to the limitations of diffusion caused by unstirred layer which

surrounds the water insoluble particles. This causes a decrease in the affinity or an increase in the Km of the enzyme for the substrate.

The optimum pH values for soluble and immobilized alpha amylase were 6.9 and 6.5 in 0.02M phosphate buffer (Figure 1) and for soluble and immobilized glucoamylase were 4.8 and 5.2 in 0.01M acetate buffer (Figure 2). Purified glucoamylase from *Aspergillus niger* showed the pH optimum at 4.3 with relatively high activity in the pH range between 3.6 and 4.6.[5] But in our experiment the crude enzyme used showed a pH optimum of 4.8 which could be due to the presence of isoenzymes. Wykes *et. al.*[8] showed that the optimum pH values for soluble and CM— cellulose bound alpha amylase were 6.0 and 6.5 respectively. In that study CM—cellulose which has a negatively charged matrix was used while in this study the matrix (sepharose—4B) was neutral and hence, the change in pH optimum could not be due to the charge effect of the matrix. It is possibly due to the changes in conformation of the enzymes resulting from immobilization.

The concentration of phosphate buffer had a marked effect on soluble alpha amylase compared to the immobilized enzyme (Table 1). Although the soluble and the immobilized alpha amylase showed optimal activity at 0.02M phosphate concentration, the immobilized alpha amylase had considerably higher activities than the soluble alpha amylase at all other phosphate concentrations. Likewise the immobilized glucoamylase at all acetate concentrations except at the optimal 0.01M acetate concentration (Table 2).

Table 1. Effect of phosphate concentration on soluble and immobilized alpha amylase activities at pH 6.9 and at 40°C.

Concentration of phosphate (mM)	% Activity of alpha amylase	
	Soluble	Immobilized
10	56	95
20	100	100
30	60	95
40	45	90
50	36	75
70	31	67
100	25	64
120	24	61

Mean value of 3 experiments as described in section 2.3.
Absolute activities for soluble and immobilized alpha amylase were 870 u/ml and 1430 u/ml respectively.

Table 2. Effect of acetate concentration on soluble and immobilized glucoamylase activities at pH 4.8 and at 50°C.

Concentration of acetate (mM)	% Activity of glucoamylase	
	Soluble	Immobilized
4	77	90
6	90	96
8	99	98
10	100	100
20	90	97
30	81	94
40	74	87
50	66	83

Mean values of 3 experiments as described in section 2.3.

Absolute activities of soluble and immobilized glucoamylase were 355 u/ml and 390 u/ml respectively.

The optimum temperature for soluble and immobilized alpha amylase were 45°C and 50°C respectively and that of soluble and immobilized glucoamylase were 55°C and 58°C (Table 3) showing that immobilization had increased the optimum temperature of both enzymes slightly.

Table 3. Effect of temperature on the activities of soluble and immobilized alpha amylase (in 0.02M phosphate buffer pH 6.9) and glucoamylase (in 0.01M acetate buffer pH 4.8).

Temperature (^{0}C)	% Activity			
	Alpha amylase		Glucoamylase	
	Soluble	Immobilized	Soluble	Immobilized
04	36	29	23	52
30	57	56	43	61
40	87	78	55	68
45	100	87	70	75
50	79	100	80	83
55	55	77	100	95
58	—	—	97	100
60	35	56	95	97
70	25	41	48	70
80	20	26	14	50

Mean values of 3 experiments as described in section 2.3.
Absolute activities of soluble and immobilized alpha amylase were 1000 u/ml and 1833 u/ml respectively and that for glucoamylase were 444 u/ml and 470 u/ml respectively.

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