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Research Article

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Immobilization of porcine pancreas lipase onto bristles of plastic brush: Kinetic properties

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ABSTRACT

Lipase is an important enzyme, which hydrolyzes fat into fatty acid, diglycerides, monoglycerides and glycerol. The substrate specificity of lipase is often crucial to their application for analytical and industrial purposes. In the present investigation, commercially available porcine pancreas lipase has been immobilized on to plastic brush through covalent bonding using glutaraldehyde. The immobilized lipase retained about 50 % of the initial activity of soluble enzyme and the conjugation yield was 0.04mg/cm^2. The optimum pH of lipase enzyme was decreased from pH 7.5 to 6.5 after immobilization; which might be due to the loss of- NH_2 groups from the enzyme surface upon conjugation to brush since glutaraldehyde coupling involves the -NH_2 groups of enzyme for covalent bonding. The immobilized lipase showed maximum activity at 37°C which is higher than that of the free enzyme (35°C). This indicates an increase in the thermal stability of enzyme after immobilization. The time of incubation for the maximum activity of immobilized enzyme was increased from 20 min to 30 min. The olive oil (substrate) concentration required for the maximum activity or saturation of lipase was decreased from 50 mM to 40mM after immobilization. When immobilized lipase was employed along with the aqueous solution of commercially available detergents (2g/100ml) for the removal of oil stain from cotton clothes, it gave better washing compared to that of detergents alone.

Keywords: Lipase, Immobilization, Glutaraldehyde, Brush.

INTRODUCTION

Enzymes are used in various industries such as pharmaceutical and agrochemical industries, polymer synthesis and personal care products and cosmetic [1]. Immobilization of enzyme provides its reuse and thus, gaining its importance [2]. Immobilization of enzymes has been done using different kind of supports with different applications [8-10]. Immobilization of lipase from Candida rugosa on Y-Fe_2O_3 magnetic nanoparticle through covalent coupling for long term stabling, on chitosan with activation of hydroxyl groups, on pH sensitive support by covalent biniding for enantioselective hydrolysis of ketroprofen ester has been done [4-6]. In laundry enzymes like protease, lipase plays an important role. Lipase hydrolyses emulsified triglycerides of long chain fatty acid by acting on ester bonds [7]. The Lipases can remove the fat stain more easily under alkaline conditions than the alkaline proteases used for laundry purpose. Hence, lipases have found a new significant role in detergent industries. The currently known lipases are not generally stable in detergents and hence required to be protected from the proteases, which otherwise inhibit lipase activity. Malik et al 2000 immobilized commercially available lipase onto zirconia

coated alkylamine glass-beads through the process of glutaraldehyde coupling and employed it in washing of cloths in the presence of various detergents. The aim of the work was to study the immobilization and kinetic characteristics of porcine pancreas lipase immobilized on bristles of polypropylene brush.

EXPERIMENTAL SECTION

A. Material

Lipase from porcine pancreas (40-70U/mg) was purchased from Sisco Research Laboratory. Acetone, methanol, ethanol and phenolphthalein were from E. Merck. Tris-base, calcium chloride, sodium benzoate, sodium hydroxide and hydrochloric acid were from Qualigen. All other chemicals used were of AR grade.

B. Immobilization of porcine pancreas lipase onto bristles of plastic brush

1) The introduction of -NH_2 group on bristles of plastic brush:

It was carried out as described by Indian patent of Dr. C.S. Pundir, Ms Manu Bhambi & Mr. N.S. Chauhan (2004).

2) Activation of bristles of brush:

The bristles were activated by glutaraldehyde by putting the brush into 2.5 % glutaraldehyde in 0.05 M sodium phosphate buffer pH 7 in a 250 ml beaker. After keeping it at room temperature for 2 h, the excess of glutaraldehyde was discarded and the brush was repeatedly washed in 0.05 M sodium phosphate buffer pH 7 until the pH of washing discard was 7.0.

3) Immobilization of lipase onto activated bristles:

The glutaraldehyde activated brush was kept into enzyme (lipase) solution at 4°C for 24 h. After that, the brush was taken off and the protein concentration was estimated in the unbound enzyme by Lowry method. The activity of enzyme immobilized onto bristles was tested as follow.

C. Assay of enzyme

The activity of lipase was assayed according to Gotthiff Naher with modifications. 5.0ml olive oil emulsion was added to 5.0 ml 0.1M tris buffer (pH 8.0) and incubated at 35°C for 10 min in a 100ml conical flask. Bristles of plastic brush bound lipase were dipped and incubated at 35°C for 20 min. The reaction mixture was kept at room temperature for 20 min and then 10ml of acetone and methanol in a ratio of 1:1 was added to stop the reaction. Titration was carried out against the 0.025N NaOH after adding 1% phenolphthalein as an indicator. In case of control, 1ml of lipase solution was kept in boiling water bath for 5 min so that it became inactive due to thermal denaturation of protein and further processed by the similar method as for the testsample.

D. Kinetic Properties of immobilized lipase

The following kinetic properties of immobilized lipase were studied and compared with those of free enzyme.

1) Effect of pH:

In order to determine the optimum pH of immobilized enzyme, tris-HCl reaction buffer in the pH range of 5.0 to 8.0 was used. The activity of immobilized lipase was determined by assaying with different buffers of different pH values.

2) Effect of incubation temperature:

To determine the effect of incubation temperature the maximum activity of immobilized enzyme, the reaction mixture was incubated at 25, 30, 35, 37, 40 and 45°C for 20 min. The activity of immobilized lipase was determined as described above.

3) Effect of time of incubation:

To determine the incubation time for maximum activity of immobilized enzyme, the reaction mixture containing 5ml olive oil emulsion and 5.0 ml 0.1M tris-HCl buffer, pH 8.9 was incubated for 10, 20, 30, 40 and 50 min.

4) Effect of substrate concentration:

To determine the effect of triglyceride (substrate) conc. on the immobilized lipase, the following olive oil (substrate) conc. was used: 1mM, 3mM, 6mM, 10mM, 20mM, 30mM, 40mM, 50mM, 100mM, and 200mM.

RESULTS AND DISCUSSION

For immobilization of enzyme first conditions were standardized and standardized conditions for immobilization of

enzyme pH 7.5, time of incubation of enzyme with bristles of plastic brush- 36 hr., optimum temperature- 4-8°C, conc. of enzyme- 3mg/ml were used (complete data not shown here).

A. Immobilization of enzyme and assay

The Commercially available lipase from porcine pancreas has been immobilized covalently onto bristles of a plastic brush and 75% retention of initial activity of free enzyme under optimum immobilization conditions (Table-1).

Table1 Immobilization of Porcine Pancreas Lipase onto Plastic Bristles

lipase added to plastic brush (mg)	3.0 mg/ml
lipase coupled to plastic brush (mg)	1.2 mg/ml
Total activity added (µg/ml/min)	0.615
Total activity bound (µg/ml/min)	0.435
% Retention	40 %

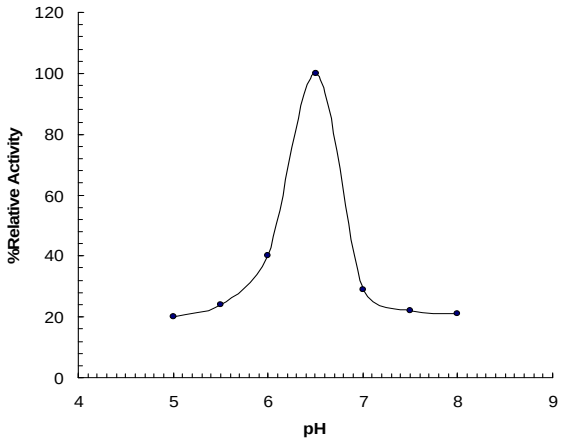

Fig.1 Effect of pH on immobilized lipase

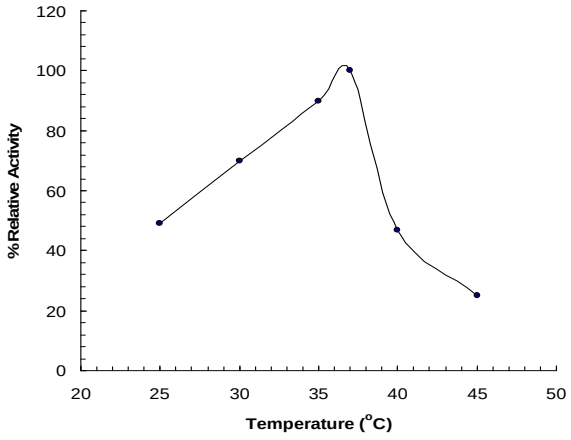

Fig. 2 Effect of temperature on immobilized enzyme

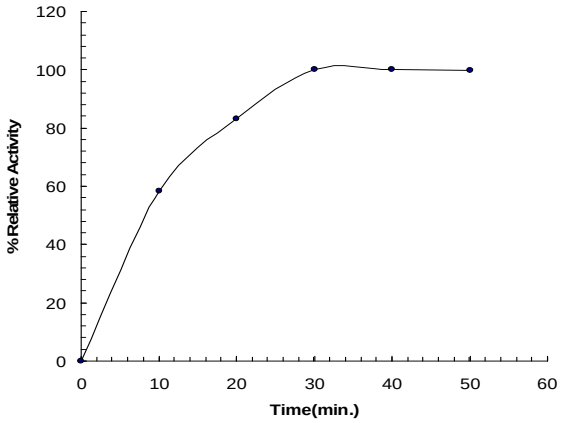

Fig. 3 Effect of time of incubation on immobilized lipase

B. Kinetic Properties of immobilized lipase

The maximum activity of bristles of plastic brush bound lipase was attained at pH 6.5, which is lower than that of free enzyme pH 7.5 (Fig.1). Optimum pH of an enzyme is displaced upon immobilization, particularly when the support material is charged. The immobilized lipase showed maximum activity at 37°C, which is higher of free enzyme (35°C) (Fig. 2). The time of incubation was also increased from 20 to 30 min after immobilization (Fig. 3), which might be due to diffusion of the substrate from bulk to the active center of the immobilized enzyme. The substrate triglyceride (olive oil) concentration required for the maximum activity or saturation of immobilized amylase was five times lower than that of free enzyme. Km for starch, as calculated from Lineweaver-Burk plot, was increased as compared to free enzyme, indicating the decreased affinity of the enzyme towards the substrate (olive oil) after immobilization. Vmax was changed from 1.515 to 1.45µmol/min after immobilization. The changes in kinetic properties of enzyme after immobilization are controlled by four factors: change in enzyme conformation and its microenvironment, steric effects, and bulk and diffusional effect. A comparison of kinetic properties of lipase coupled to bristles of plastic brush with those of free enzyme is given in Table 2.

Table 2 Kinetic parameters of free and immobilized porcine pancreas lipase onto plastic bristles

Parameters	**Free Lipase**	**Lipase bound to plastic brush**
Optimum pH	7.5	6.5
Optimum temperature	35°C	37°C
Time of incubation	20 min	30 min
Saturation conc. of olive oil	50mM	40mM
Km for olive oil	4.242×10^{-3} M	20.48×10^{-3} M
Vmax	1.515 µmol/min	1.45 µmol/min

C. Storage and stability

The immobilized lipase on a plastic brush was stored in a beaker (250 ml) containing sodium phosphate buffer (0.1 M, pH 7.0) at 4°C. The enzyme showed no noticeable loss of activity during its regular use on cotton clothes (50 times) for about one month.

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