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Physical immobilization of 60 kDa Chaperonin linked lipase from *Pseudomonas aeruginosa* BN-1

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Abstract: The 60 kDa chaperone linked lipase from *Pseudomonas aeruginosa* was subjected to physical adsorption on silica 60 and acrylic beads. It was found that higher enzyme loading was achieved on silica gel than acrylic bead. The half life of immobilized enzyme was greater compared to the free enzyme. The adsorption of the enzyme onto a solid phase also resulted in increased thermo and solvent stability. It was observed that soluble enzyme showed maximum stability at 70°C while immobilized enzyme showed stability up to 80°C for 45 minutes. The stability of immobilized enzyme increased up to 48 hours from 24 hours against different organic solvent at 1.0M concentration. It was noted that enzyme immobilized on acrylic beads have greater reusability compared to silica immobilized enzyme

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INTRODUCTION

The use of enzymes to replace conventional inorganic catalysts for various industrial operations has been expanded in recent years. However, their utilization has been limited due to progressive losses in catalytic activity primarily associated with transient enzyme stability. Such enzyme instability can be protected by immobilization onto a solid phase support. Immobilization not only enhances the stability of the enzyme but also provides control over catalytic processes. It not only gives an advantage in product separation from enzyme and substrate but it is also makes the process more economical and efficient since immobilized enzymes can be reused several times[1,2].

Lipases catalyze several reactions including i.e. in aqueous environment they hydrolyzes triglycerides into glycerol and fatty acids. In non-aqueous media these enzymes catalyzes transesterification, esterification, aminolysis, acyl exchange and thio trans-esterification reactions[3]. Due to these properties, lipases have many commercial applications[4]. Lipases have been immobilized on different supports using different methods includinh physical adsorption, covalent binding and entrapment[5]. The present paper deals with immobilization of lipase through adsorption on to silica 60 and acrylate beads. The kinetic parameters of immobilized lipase are compared with free soluble enzyme.

MATERIALS AND METHODS

Enzyme preparation

Lipase from *Pseudomonas aeruginosa* BN-1 was produced, harvested and purified as described earlier[6].

Enzyme assay

Colorimetric assay was based on the hydrolysis of 4-nitrophenyl palmitate.[7] In the reaction mixture Tris-HCl buffer (55mM pH 8.0) was used instead of phosphate buffer.

One lipase unit "U" is defined as µmol of 4–nitrophenol released per minute under assay conditions.

Protein estimation

Total protein was estimated by the method of Lowry[8], using bovine serum albumin (BSA) as a standard.

Immobilization

Purified lipase (10ml) was mixed in duplicates with 1.0gm of silica and acrylate resin separately. The mixtures were kept at 25°C for 90 minutes with occasional shaking. To check the degree of adsorption, sample were drawn at regular time intervals and centrifuged at 5000rpm for 2 minutes. The clear supernatant was assayed for total protein and lipase activity. The amount of lipase bound to the solid support was calculated by the following

Amount of lipase bound to solid support = Total lipase in binding medium - lipase present in binding medium after removal of solid support

formula

Reusability of immobilized enzyme

The immobilized lipase was assayed for 15 cycles of 30 minutes each using the enzyme assay described earlier.

Thermal stability of free and immobilized enzyme

Free and immobilized enzyme was incubated for 1 hour at various temperatures (30-70°C) in 55mM Tris-HCl buffer (pH 8.5). Residual lipase activity was determined under standard assay conditions and activity of immobilized and free enzyme stored at 4°C was taken as 100%.

Stability of free and immobilized enzyme in organic solvents

Free and immobilized lipase was incubated with various organic solvent at 37°C for 48 hours in 55mM Tris-HCl buffer (pH 8.5). Residual lipase activity was determined under standard assay conditions. Enzyme activity without any organic solvent was taken as 100%.

Half life of free and immobilized enzyme

Free and immobilized lipase was incubated at 50°C for 10 hours. Sample were drawn at regular time interval and assayed under standard conditions for residual activity. At zero hour, enzyme activity was considered to be 100%.

RESULTS AND DISCUSSION

Lipases have multi faceted property being hydrolytic in aqueous conditions while promote trans- esterification in non-aqueous conditions.. Because of its wide application in array of enzyme facilitated reactions, it is imperative to maintain optimal biochemical activity of lipase. Immobilization of enzymes is one of techniques for enhancing its stability even under adverse conditions. Lipases contain hydrophobic domains thus this makes it easier to attach the enzyme on to any hydrophobic support through hydrophobic and van der Waals interaction. This property has been exploited for the single step purification of lipase from *Burkholderia multivorans*[9]. It was found that attachment of lipase with acrylate beads and silica is more than 80% efficient which is in agreement with the previous investigations[10]. The immobilized enzyme was subjected to continuous assay and it was observed that more than 50 % of residual biochemical activity remains intact even after 20 cycles (Figure 1). The results obtained were better than finding describe in earlier reports where lipase was immobilized on silica through adsorption[11].

The half life of free enzyme was 90 minutes which is lower than that of immobilized lipase. In fact the half life of acrylate and silica 60 immobilized enzyme was 180 minutes (Figure 2). Similarly, the immobilized lipase retains more than 55% of activity at 70°C. There was no detectable shift in the optimum temperature for biochemical activity but marked increase in thermal stability was observed (Figure 3). Both the thermal stability and increase in the half life of the immobilized enzyme is probably due to the solid external support. It was already reported that immobilized enzymes have better thermal stability than corresponding native enzyme[12].

The matrix extends hydrophobic support to the molecule which ultimately minimizes the disruption of protein structure with less loss of activity. Nuwani *et al.*[10] observed the similar effect with thermophilic *Bacillus* lipase immobilized on silica and polypropylene HP 20. It has been observed that an increase in thermo stability of *Mucor javanicus* lipase immobilized on the silica nanoparticles[13].

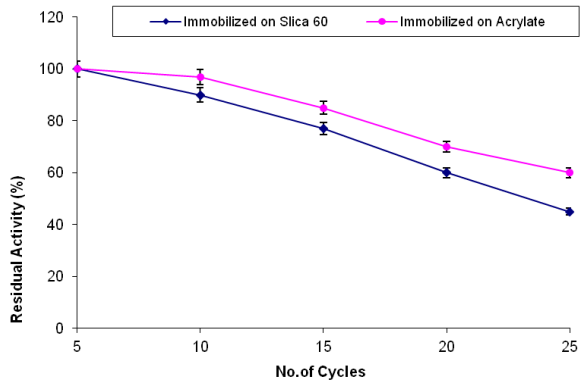

Figure 1: Reusability of immobilized lipase on acrylate and silica.

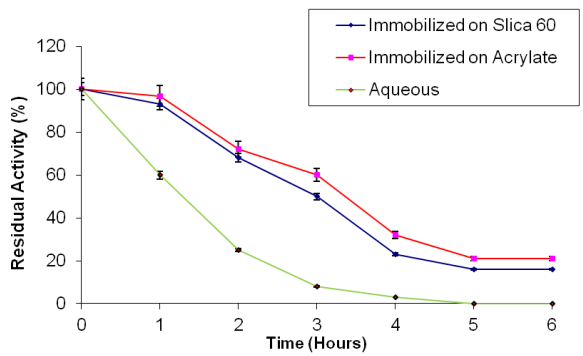

Figure 2: Half lives of immobilized and aqueous enzymes.

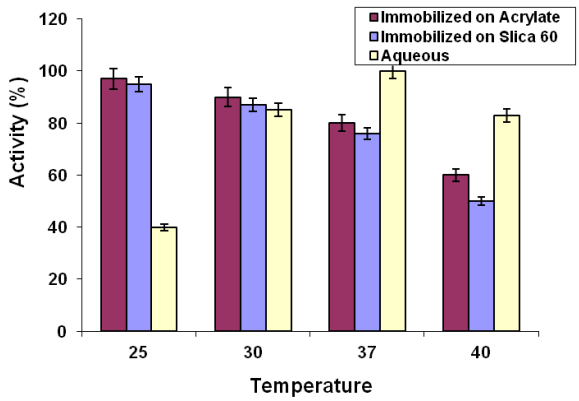

Figure 3: Thermal stability of immobilized enzyme.

In a previous study[13], it was observed that no change occurred in the optimum pH for maximal biochemical activity for the immobilized enzyme (Figure 5), who immobilized *Rizhopus oryaze* lipase

on to silica aerogels. The stability of immobilized lipase also increases to 48 hours in the organic solvents whereas as native enzyme lost the activity very rapidly after 24 hours (Figure 6). There are reports about the increase stability of immobilized enzyme in various organic solvents, where they were employed in the *trans* esterification reactions.

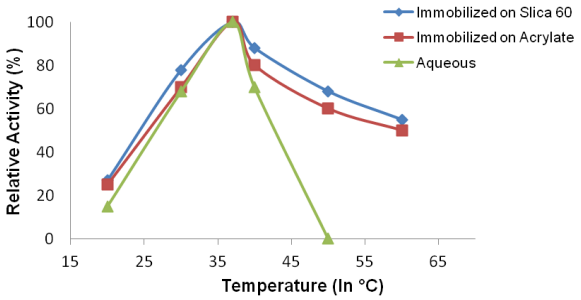

Figure 4: Effect of temperature on enzyme activity.

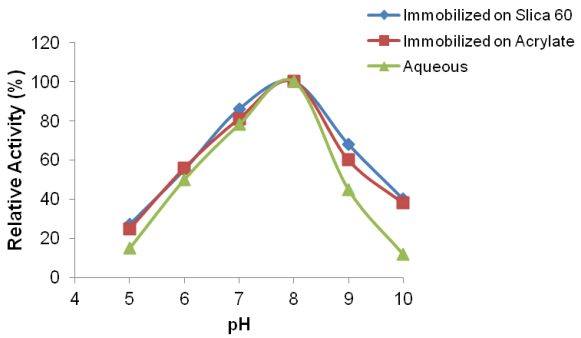

Figure 5: Effect of pH on enzyme activity.

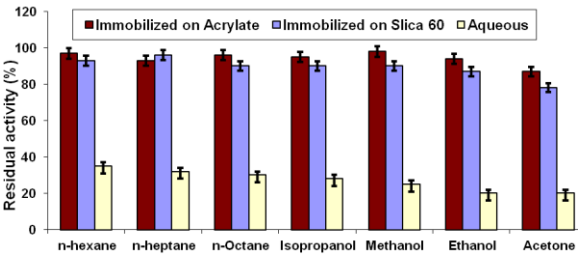

Figure 6: Effect of organic solvents on free and immobilized enzyme after 48 hours.

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