Sol-gel encapsulation of *Arthrobacter* sp. lipase : resolution of drug intermediates

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INTRODUCTION

Heterogenous catalysis using immobilized biocatalysts for biotransformations is considered to be more advantageous than homogeneous catalysis as they can be easily separated from reaction mixture and regenerated for multiple uses providing pure form of product, therefore are useful for industrial applications (Chen et al 2008, Mateo et al 2007, Palomo et al 2005). Immobilization of enzymes is considered to be an important tool to enhance their thermal and operational stability. Encapsulation/entrapment is the only immobilization method that does not require any interaction between the catalyst and the support, but pore size of the support needs to be smaller than the immobilized catalyst and suffer from mass transfer effects. Immobilized lipases on porous supports can act only on the fraction of substrate that is soluble in the reaction medium and is able to penetrate the porous structures of immobilized biocatalyst. Several factors including choice of support and selection of an immobilization strategy may affect the activity, recovery and reusability of enzymes in immobilization process (Chaubey et al 2006, Fernandez-Lorente et al 2001, Hsu et al 2000). Thus, exploiting good supports and immobilization strategy has been an attractive work for enzyme engineering. Therefore, use of different immobilization strategies may provide immobilized lipases with different activity/selectivity characteristics for various applications.

Enzymatic resolution of drugs/ drug intermediates at IIIM Jammu, India is well established, and lipase/esterase from a number of microbial strains have been used for various resolution applications (Singh et al 2008, Gupta et al 2008, Kaiser et al 2006, Koul et al 2005, 1998). Recently it has been demonstrated that immobilization of lipase can be used as an important tool for enantioselectivity enhancement during resolution of chiral auxiliaries and drug intermediates (Chaubey et al 2009, 2006). Present work demonstrates comparative characteristics of some sol-gel encapsulated *Arthrobacter* sp. lipase vis-à-vis free enzyme for resolution of fluoxetine drug intermediate (antidepressant) and ß-amino alcohols (antidiabetic agents and β-blockers).

MATERIALS AND METHODS

Preparation of cell biomass/enzyme: *Arthrobacter* sp. cell biomass was grown in 1% peptone, 0.5% beef extract and 0.5% sodium chloride for 18h. After collection of biomass by centrifugation at 10,000x*g,* enzyme was isolated by ultrasonication method.

Enzyme activity/ protein estimation: Enzyme activity in free and immobilized enzymes was measured by titremetry using pH Stat Metrohm 718 using tributyrin as substrate. Protein estimation was performed by Bradford method using Bovine serum albumin (BSA) as standard protein.

Sol-gel encapsulation of *Arthrobacter* sp. lipase: Sol-gel encapsulation was carried out by addition of enzyme after hydrolysis of silicates e.g. tetraethylorthosilicate (TEOS) precursor with soluble additives such as polyvinyl alcohol (PVA), polyethylene glycol (PEG). Composite immobilizates were prepared with aminopropyltriethoxy silane (APTES) as additional precursor

with and without magnetic particles. After polymerization for 24h, the gel was washed well with phosphate buffer followed by drying in air at room temperature. The dried encapsulated lipase was used for further stability and resolution studies.

Resolution of racemic drug intermediates: Resolution of racemic drug intermediates; fluoxetine and ß-amino alcohols was carried out with *Arthrobacter sp.* Lipase (ABL) cell biomass, crude enzyme extract and different immobilizates. All the reactions were performed at 20g/L substrate concentrations with 5 units/ml enzyme under stirred conditions at room temperature.

RESULTS AND DISCUSSION

Bioencapsulation of ABL enzyme was carried out using TEOS precursor and composites with APTES precursors. Table I shows activity and protein binding of different immobilizates prepared during present study. The encapsulated enzyme in sol-gel supports prepared from tetraethylorthosilicate resulted in 90 units/gm activity. Modified sol-gel matrices prepared in presence of PVA as additive resulted in higher activity 120 units/gm, whereas entrapment with PEG and fructose resulted in 80 units/gm activity. Nonmagnetic composite sol-gel supports prepared from aminopropyltriethoxysilane and tetraethylorthosilicate obtaining 30 units/gm activity of entrapped enzyme. Magnetic composite sol-gel supports prepared using 3-aminopropyltriethoxysilane and tetraethylorthosilicate precursors in the presence of magnetic particles obtaining 40 units/gm activity.

Encapsulated enzyme	Activity (U/g)	Protein (mg/g)
Sol-gel	90	25
Sol-gel/PVA	120	25
Sol-gel/PEG	80	25
Sol-gel composite	30	21
Magnetic sol-gel composite	40	24

Table 1 : Immobilizates prepared using different precursors

Encapsulated enzymes were found to have better stability towards wide pH and temperature range (pH 5-9) as compared to cell biomass/free enzyme as shown in Fig.1. Encapsulated enzymes when tested for their enzyme activity after keeping in buffers of different pH for 48h at room temperature, they retained more than 80% activity. Free enzyme however lost about 50% activity within 24h. Encapsulated enzymes were also thermally stable as compared to free enzyme and retained about 40-50% activity after 1h incubation at 80°C, whereas free enzyme gets deactivated beyond 50°C. On reuse there was no reduction in enzyme activity observed after 15 cycles. Improved properties of immobilizates prepared by encapsulation method may be attributed to the availability of protected microenvironment within the micro-porous structure of the sol-gel/ sol-gel composite support. The activity and reaction rates catalyzed by encapsulated enzymes though suffer from diffusion and mass transfer effects, yet present better stabilities for prospective use under harsh reaction conditions.

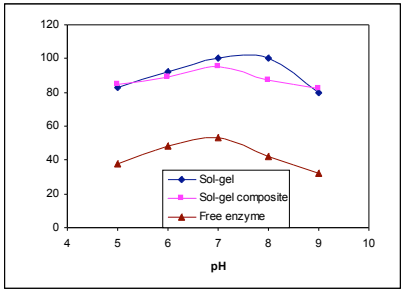


Figure1(a) : pH stability of encapsulated ABL

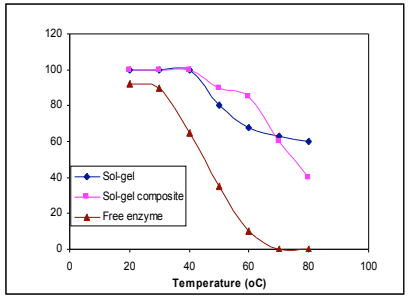
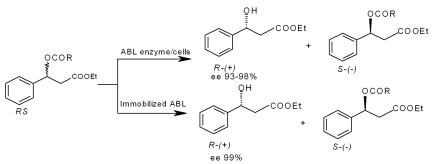


Figure1(b): Thermal stability of encapsulated ABL

Resolution alkylacylates of fluoxetime drug intermediate and -amino alcohols (10-20mg/ml) were carried out with encapsulated immobilizates vis-à-vis cell biomass. The reactions were carried out for 24h at room temperature.



(a) Fluoxetine drug intermediate
(b) -amino alcohols

Figure 2: Resolution of racemic drug intermediates

Results obtained by HPLC presented an improved enantiopurity (ee) of the hydrolysed product in the reaction mixture (Fig.2). Since the encapsulated enzyme is available inside the porous structure of the sol-gel network, resolution reaction takes place by diffusion of substrate through porous network of the support. Thus, substrate-enzyme complex is formed inside the pores in such a

manner that only one isomer is capable to bind the enzyme active site. The configuration of enzyme structure due to restricted movement of the active site may also contribute to the selectivity of the enzyme, which is otherwise not possible with free enzyme. Therefore, the substrate-enzyme binding in case of encapsulated enzyme is different from that of free enzyme imparting different enantioselectivity with the encapsulated enzyme. The improved selectivity of binding with selective isomer form the racemic mixture in the immobilized enzyme may be attributed to the availability of enzyme active site with better configuration for binding with only one isomer of the racemic substrate.

CONCLUSIONS

Encapsulation of ABL within sol-gel supports and sol-gel composite supports provided stable immobilizates for obtaining improved enzyme stability and enantioselectivity (99%ee) for resolution of racemic fluoxetine drug intermediate and -amino alcohols vis-à-vis free enzyme. There were no loss in activity or change in enantioselectivity on reuse of encapsulated enzyme.

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