Specific Strategies for One-Step and Simultaneous Immobilization-Purification of Lipases

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ABSTRACT
Lipases are the biocatalysts with outstanding prospects in industry and medicine. They have proven to be useful in various hydrolytic and synthetic reactions. However, there are some limitations for impure lipases that may restrict their widely uses in industrial applications. Purification is sometimes vital for the characterization of the function, structure, and interactions of lipases. The lipase immobilization is also an efficient strategy for increasing the enzyme activity and stability, and getting a simpler recovery. Lipases are naturally produced together with many other proteins that they may occupy some surface of immobilization solid support and decrease the final activity. The coupling of immobilization and purification of lipase will overcome the mentioned problems and obtain the maximum purification yields. The present mini-review will discuss the use of the techniques that permit to join immobilization and purification of lipases in a single step, including control of the immobilization conditions by interfacial activation on hydrophobic supports, the development of specific supports with affinity for lipases, and the use of bio-affinity supports including immuno- and lectin affinity.

Introduction
Lipases are triacylglycerol ester hydrolases (E.C. 3.1.1.3) that indicate a strong ability in many hydrolytic and synthetic reactions in nature. Microbial lipases with unique features from various sources such as bacteria, fungi, and microalgae can be used in biochemical reactions, including esterification, inter-esterification, trans-esterification, aminolysis, acidolysis, and alcoholysis (Hasan-Beikdash et al., 2012; Sangeetha et al., 2011; Mogharabi and Faramarzi 2016; Imanparast et al., 2017). Nowadays, using the microbial enzymes especially lipases is a promising approach to produce of biofuels, detergents, perfumes, cosmetics, leather, enantiopure pharmaceuticals, organic synthetic compounds, foods, and feeds (Ghasemi et al., 2013; Salihu and Alam, 2015;
Lipases are produced from bacteria, fungi, and archea. Microbial lipases belong to the family of serine hydrolases with its corresponding antigen. Lipase was first explored in pancreatic juice in 1856 by Claude Bernard. Animal pancreatic extracts were traditionally used as the source of lipase for industrial applications. However, microbial sources of lipase were discovered when the commercial potential of lipases was recognized. Microbial lipases have a mechanism of interfacial activation in the presence of hydrophobic interfaces (Verger, 1997). In aqueous media, they usually have a secondary structure called the ‘lid’ covering their active sites with a very hydrophobic internal face and a hydrophilic external face (Fig. 1). This conformation is known as the ‘closed form’ of lipases that it is undesirable in aqueous homogenous medium. In the presence of a hydrophobic surface, conformational change shifts towards the ‘open structure’ of lipase. This conformation permits lipases to adsorb on the hydrophobic surfaces via large hydrophobic face of lid. Moreover, these enzymes can be adsorbed on various hydrophobic interfaces such as drops of oil (Derewenda et al., 1992; Basri et al., 1995), gas bubbles (Palomo et al., 2003), hydrophobic support surfaces (Taipa et al., 1995; Palomo et al., 2003), hydrophobic proteins (Fernández-Lorente et al., 2003), lipopolysaccharides (Palomo et al., 2004), etc.

One step immobilization-purification by control of the immobilization

Control of the immobilization process is an efficient strategy for lipase purification. In some cases, it is possible to use specific features of the catalytic mechanism of lipase to separate it from other proteins. Lipases have a mechanism of interfacial activation in the presence of hydrophobic interfaces (Verger, 1997). In aqueous media, they usually have a secondary structure called the ‘lid’ covering their active sites with a very hydrophobic internal face and a hydrophilic external face (Fig. 1). This conformation is known as the ‘closed form’ of lipases that it is undesirable in aqueous homogenous medium. In the presence of a hydrophobic surface, conformational change shifts towards the ‘open structure’ of lipase. This conformation permits lipases to adsorb on the hydrophobic surfaces via large hydrophobic face of lid. Moreover, these enzymes can be adsorbed on various hydrophobic interfaces such as drops of oil (Derewenda et al., 1992; Basri et al., 1995), gas bubbles (Palomo et al., 2003), hydrophobic support surfaces (Taipa et al., 1995; Palomo et al., 2003), hydrophobic proteins (Fernández-Lorente et al., 2003), lipopolysaccharides (Palomo et al., 2004), etc.

Interfacial activation of lipase on hydrophobic supports

The lipase immobilization on hydrophobic supports is a much-utilized strategy. This process permits to immobilize and purify, in a single step, to stabilize the open form of the enzyme and full lipase immobilization was performed on these supports in few minutes (Fernández-Lafuente et al., 1998; Fernández-Lorente et al., 2007; Fernandez-Lorente et al., 2008). In this strategy, the supports were modified with phenyl- and octyl-derivatives, aromatic, cyclic, or longer aliphatic hydrophobic, and other hydrophobic moieties, the lipase immobilization-purification by specific lipase-lipase interactions, and the use of antibody or lectin by highly specific interaction with its correspondence antigen.

Specific lipase-lipase interaction

Recently, it has been reported that lipases may form
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Simultaneous immobilization-purification by bio-affinity supports

Selective attachment to a suitable ligand is maybe the common feature of proteins. Lipases can bind with remarkable specificity and strength to their substrates, inhibitors, and co-factors. In this strategy, the lipase immobilization is performed directly from the crude homogenate or lipase preparation via bio-affinity interactions. However, such affinities cannot be employed for immobilization because the binding may block the catalytic site of enzyme followed by interfering with enzyme activity. For this goal, ligands should be attached to epitopes located at a distance from the active site. The affinities of some biomolecules such as lectin-sugar, antigen-antibody, and biotin-avidin are well known. Bio-affinity immobilization is done by two principal strategies. Firstly, the support is pre-coupled to an affinity ligand and the target protein is added. Secondly, the enzyme is conjugated to affinity ligand that has affinity toward a support. The lipase conjugation can be obtained either by chemical cross-linking preparation or as a protein adsorption (Bilkova et al., 1997; Farooqi et al., 1997; Roy and Gupta, 2006).

Immuno-affinity supports

Immuno-affinity or immuno-adsorption is a specialized form of bio-affinity strategy, and subsequently a general strategy to couple immobilization with purification. This matrix can use monoclonal or polyclonal antibodies, and allows an extremely selective protein adsorption. The principle of the process is based on the highly specific interaction of an antigen with its correspondence antibody and only the target protein becomes immobilized. Specific antibodies can be produced against any protein in proper experimental animals and utilized for the immobilization of the enzyme on a suitable support (Fig. 4) (Saleemuddin, 1999). Both monoclonal and polyclonal antibodies have been employed in the immobilization of enzymes such as β-glucosidase (Melchers and Messer, 1970), gulonolactone oxidase (Sato and Walton, 1983), transglutaminase (Ikura at al., 1984), and tyrosinase (Khan et al., 2005). However, immuno-affinity is not cost...
effective for industrial applications due to its complexity and expensively.

**Lectin affinity**

Lectins are carbohydrate-binding proteins that have been isolated from plants, microorganisms, and animals. They are highly specific for sugar moieties that can be used for glyco-enzymes immobilization (Mislovicova et al., 2000).

Glycosylation is the most common post-translational modification in eukaryotes, archa, and even some bacteria. The largest and best characterized among the lectins is Concanavalin A (Con A). Con A has been widely used in the bio-affinity purification-immobilization of various glyco-enzymes on a variety of supports. Con A maintains its affinity for carbohydrates between pH 5 or lower and above pH 9 makes it appropriate for the immobilization of lipases acting in a wide pH range (Fig. 5). Akhtar et
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Mancheno et al. (2005) immobilized and purified peroxidases from bitter gourd by Con A-adsorbed Sephadex. Mancheno et al. (2005) described that the β-trefoil lectin domain of the pore-forming toxin from the mushroom *Laetiporus sulphureus* (LSL150) that illustrates typical properties of fusion tags which have permitted to set up a novel protocol for the production and purification of recombinant proteins tagged with such a domain. Recently, lipase from *Geobacillus thermocatenolatus* (BTL2) was recombinantly produced tagged to the lectin domain of toxin LSL. The fusion protein was immobilized onto agarose supports via affinity interaction between lectin domains and the galactose units present in the structure of the agarose beads (López-Gallego et al., 2012).

**Conclusion**

The coupling of immobilization and purification of enzymes has many advantages such as enhancing of activity, stability, easier product recovery, and also it is beneficial to obtain high final purification yields of enzymes in a cost effective manner especially in industrial applications.

**Figure 4.** Immobilization-purification by immunoaffinity: (A) specific antibodies production against lipase in experimental animal; (B) the utilization of antibodies on a support, for the immobilization-purification of lipase.

**Figure 5.** Lectin is highly specific for sugar moieties that can be used for glycolipases immobilization.
Better understanding of the immobilization mechanisms on the various supports may create new strategies to attain this goal. The use of interfacial activation capacity of lipase by functionalized supports can be a powerful tool for the one step immobilization-purification. The uses of these supports are still limited in industry and only the lipase immobilization on hydrophobic supports seems very popular at both laboratory and industrial scales. This may be found in the wide uses of lipases in the industrial level.

**Competing Interests**

The authors declare that there is no conflict of interest regarding the publication of this article.

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