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Review Article

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

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HIGHLIGHTS

- Lipases are the biocatalysts with outstanding prospects in industry and medicine.
- Simultaneous immobilization-purification may enhance lipase activity and stability.
- Lipases have a mechanism of interfacial activation in the presence of hydrophobic interfaces.
- The lipase immobilization on hydrophobic supports is a much-utilized strategy.
- Bio-affinity is a promising approach to increase lipase final yield and activity.

Keywords:
Bio-affinity
Immobilization
Interfacial activation
Lipase
Purification

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-Beikdashti 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;

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Ameri et al., 2017). Most of these applications necessitate homogeneous or at least reasonably pure preparations that the high cost of the purified lipases has limited their usage in industry. Furthermore, the activity will be lost at every stage in the purification process; so reducing purification steps is important to maximize the yield. The use of affinity chromatography can be considered as powerful means of purifying proteins. On the other hand, enzymes are water-soluble molecules that need to be separated from the reaction media to be re-used; this is momentous for improving the economy of the process (Sheldon, 2007; Bready and Jordaan, 2009; Garcia-Galan et al., 2011). In order to overcome this problem, enzyme immobilization technology is a useful way to enhance activity, stability, enzyme loading, easier product recovery, and purification (Motevalizadeh et al., 2015). Also, it is beneficial to use crude enzyme extract instead of pure enzyme for immobilization. Since, the development of efficient simultaneous immobilization-purification methods will permit to obtain high final purification yields of enzymes, and avoiding possible unwanted reactions (Barbosa et al., 2015). The use of functionalized supports is an efficient method to specifically immobilize proteins with certain structural features (Ghasemi et al., 2014; Khoobi et al., 2014; Khoobi et al 2015, Khoobi et al., 2016). Functionalized supports are defined as matrices that present functionalities with various physical or chemical properties on their surface to interact with a certain protein (Barbosa et al., 2013).

The present mini-review will discuss simultaneous immobilization-purification strategies of lipases in a single step such as, control of the immobilization conditions using interfacial activation in the presence of hydrophobic interfaces such as 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.

Lipases

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 enhanced and the demand for lipases could not be supplied from animal sources (Sangeetha et al., 2011). Nowadays, lipolytic enzymes are currently attracting an enormous attention because of their biotechnological applications. Microbial lipases belong to the family of serine hydrolases and their activities depend on a catalytic triad containing of serine, histidine, and aspartate. Commercial microbial lipases are produced from bacteria, fungi, and archea. Bacterial lipases were first observed in 1901 in the strains of *Serratia marescens* and *Pseudomonas aeruginosa.* Bacterial lipolytic enzymes were classified into 8 families (Hasan et al., 2006).

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 some 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 called 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 (Fernandez-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 moieties (Fig. 2). On the other hand, as lipase adsorption is dependent to interfacial affinity, some lipases become attached only on specific hydrophobic supports. This phenomenon permits to separate different types of lipases in a single sample. Lipase from porcine pancreas immobilized on octyl-supports very slowly but fairly rapidly on phenyl-derivatives. This strategy is simple to implement in industrial level.

Specific lipase-lipase interaction

Recently, it has been reported that lipases may form

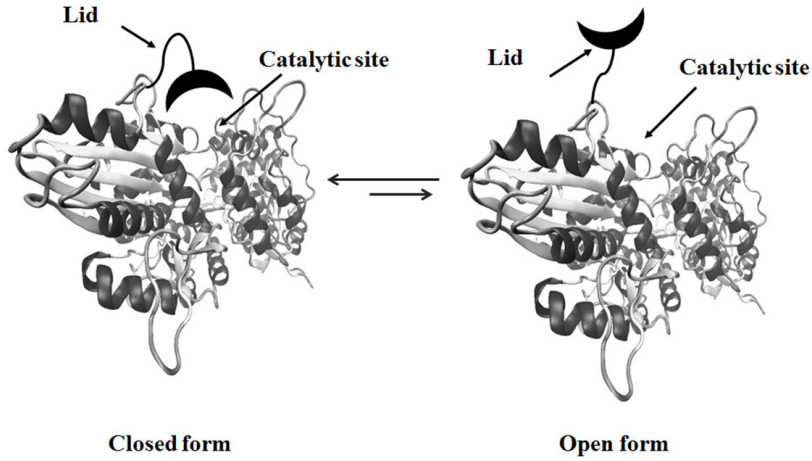

Figure **1.** Structure of open and closed forms of lipase.

bimolecular aggregates by means of interactions between the hydrophobic pockets surrounding the catalytic centers (Fernández-Lorente et al., 2003; Palomo et al., 2003). This capacity can be used as a notable tool to purify lipases. The lipase immobilization on a support as an open form would permit the adsorption of other lipase molecules (Fig. 3)**.** Volpato et al. (2009) investigated some types of lipases from different sources as an adsorbent matrix in order to purify lipases existing in a crude extract of *Staphyloccocus warneri*. Analyzing the crystal structure of lipase in *Pseudomonas fluorescens* (PFL), *Thermomyces lanuginosa* (TLL), *Candida antarctica* (CAL-A), *Rhizomucor miehei* (Palatase 20000L) (RML), and *Geobacillus thermocatenulatus* (BTL2) have shown the existence of lysine residues (Lys) normally in the opposite side of the catalytic center, whereas the area near the active center has almost no Lys residues. Thus, the lipase immobilization on glyoxyl-agarose (Mateo et al., 2006) produce a hydrophobic support by orientation of the enzyme with the active center exposed to the reaction medium. In this methodology, diethyl-*p*-nitrophenyl phosphate (D-pNP) is used for irreversible inactivation of immobilized lipase and changed to open form.

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

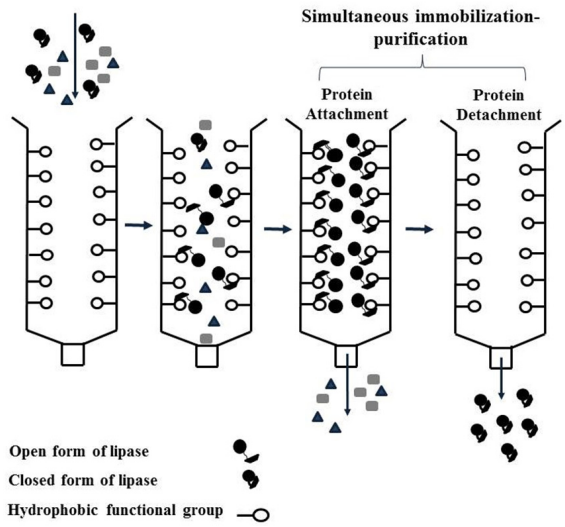

Figure 2. Immobilization-purification on hydrophobic supports by interfacial activation. The supports can be modified with phenyl- or octyl-derivatives, aromatic, cyclic or longer aliphatic hydrophobe moieties. Lipases are changed from close form to open form in the present of hydrophobic groups and can be attached to the supports.

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, archea, 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

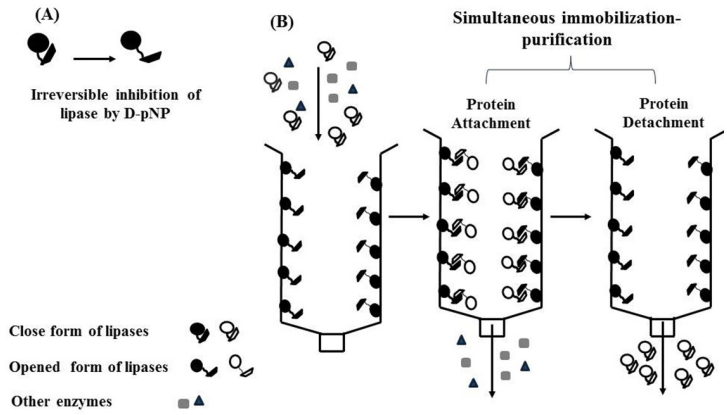

Figure 3. One step immobilization-purification by specific lipase-lipase interactions: (A) irreversible inactivation of lipase and changed to open form with D-pNP; (B) the lipase immobilization on a support as an open form would permit the adsorption of other lipase molecules by interfacial activation on hydrophobic supports.

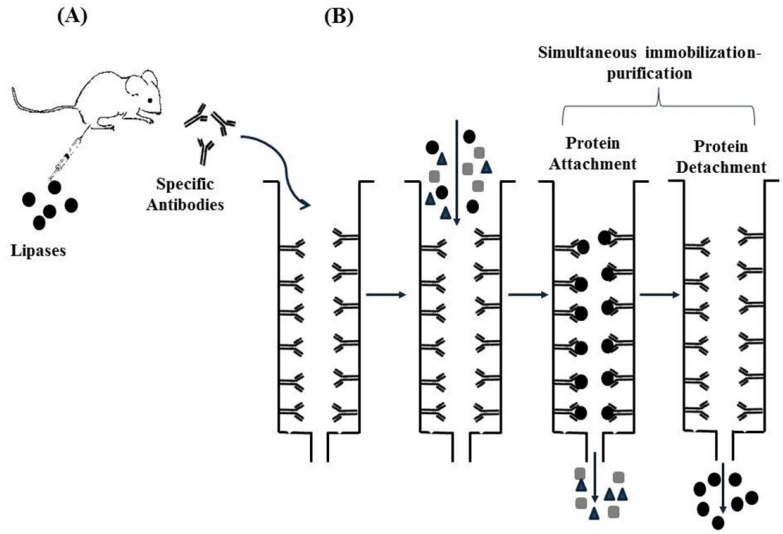

Figure 4. Immobilization-purification by immunoaffinity: (A) specific antibodies production against lipase in experimental animal; (B) the utilization of antibodies on a support, for the immobilization-purification of lipase.

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.

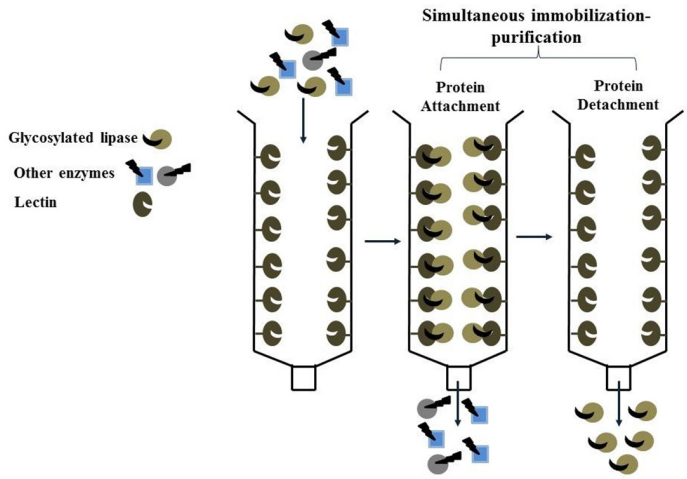

Figure 5. Lectin is highly specific for sugar moieties that can be used for glycolipases immobilization.

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 founded 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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