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Using silk woven fabric as support for lipase immobilization: The effect of surface hydrophilicity/hydrophobicity on enzymatic activity and stability

Biqiang Chen [a,b], Chunhua Yin [c], Yuanyuan Cheng [a], Weina Li [a], Zhu-an Cao [b], Tianwei Tan [a,*]

[a] Beijing Bioprocess Key Laboratory, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China
[b] Institute of Biochemical Engineering, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China
[c] School of Applied Science, University of Science and Technology Beijing, Beijing 100083, China

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ABSTRACT

Silk fibers in the form of woven fabric were used as a novel and inexpensive carrier for the immobilization of lipase from *Candida* sp.99–125. In this study, the activity and stability of lipases adsorbed on two silk fabrics with different hydrophilic/hydrophobic properties were compared. Hydrophobic silk fibers functionalized with methyl groups were prepared by treatment with amino-functional polydimethylsiloxane (PDMS). The lipase immobilized on PDMS-treated fiber exhibited an over 2-fold increase in both hydrolysis and esterification activity due to the interfacial activation as compared to its immobilization on a hydrophilic support (native fiber). To characterize the properties of different immobilized derivatives, the effects of pH and temperature were investigated in the hydrolysis of olive oil. The esterification behavior in organic media with variable water contents and operational stability of immobilized derivatives were also compared. The lipase immobilized on the hydrophobic fibers could maintain a constantly high activity at a water content range from 1 to 10% (v/v), while the activity of lipase immobilized on native fibers showed a clear dependence on water content in organic media and decreased rapidly at high water content (> 2%). Furthermore, lipase immobilization on the hydrophobic support exhibited a significantly improved operational stability in esterification reaction system. After 27 batches were recycled, a high esterification yield (97%) was maintained. The results in this work indicate that a hydrophobic surface of fabric fiber promotes the interfacial activation of lipase and that woven silk could be a potential material as an immobilization matrix for industrial process.

1. Introduction

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) catalyze the hydrolysis/synthesis of a wide range of soluble or insoluble carboxylic acid esters and amides [1]. Their broad substrate specificity as well as high regio- and stereospecificity enables their various applications: hydrolysis of oils and fats, surfactants and biofuel, and production of intermediates for organic synthesis [2–5].

Enzyme immobilization has been a popular strategy for most large-scale applications due to the ease in biocatalyst recycling, continuous operation, and product purification [6].

* *Corresponding author*. Tel.: +86 10 64416691; fax: +86 10 64715443.
E-mail address: twtan@mail.buct.edu.cn (T. Tan).
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Moreover, immobilization also improves stability of a variety of enzymes against several forms of denaturation [7]. Several methods have been reported for enzyme immobilization, including covalent attachment, entrapment, cross-linking, and adsorption [8–10].

In recent years, lipase immobilization on the surface of a solid support through hydrophobic interactions has been widely reported [11–15]. Activation at the hydrophobic interface is the characteristic property of lipase, which was first reported by Sarada and Desnuelle [16]. In the absence of interfaces, lipase contains a hydrophobic oligopeptide (termed the 'lid') covering its active site and making it inaccessible to substrates. However, in the presence of hydrophobic interfaces, a conformational rearrangement occurs, turning the 'closed form' of the lipase into an 'open form'. As a result of the exposure of hydrophobic surface, the corresponding functionality of lipase is observed [17]. Taking advantage of this catalytic mechanism, the interfacial adsorption of lipases on hydrophobic support has been developed as a popular strategy to prepare immobilized lipase with high catalytic activity [18]. The matrix for interfacial adsorption also may be a hydrophilic support, whose surface has been coated with hydrophobic groups. This functionalization transforms a hydrophilic material into a highly hydrophobic one, providing excellent properties to interact with the lipase adsorbed. For example, coating the silica surface with octyl groups as a support to immobilize lipase from *Candida antarctica* B has been reported [19]. The adsorption of lipases involves mainly the hydrophobic areas surrounding the active site and the internal face of the lid. Through interfacial adsorption, the adsorbed lipase presents an open form, with the active site accessible for the substrates and thus usually exhibits an improved catalytic activity (interfacial activation).

Fibrous materials, such as cellulose fibers and polyester fibers, have been widely used for enzyme immobilization due to their excellent properties, such as high specific surface area, good mechanical strength, chemical stability, and microbial resistance [20–22]. Among them, silk fiber shows some attractive potential [23]. On one hand, silk fiber having a proteinic backbone presents good biocompatibility and physicochemical properties, which are likely to maintain the biological activity of the fixed enzyme molecule. On the other hand, silk fibroin consists of a variety of amino acid residues, so that there are many reaction sites such as amino, carboxyl and imidazole groups. Thus, surface modification of silk film is relatively easy to fulfill to provide various types of functional groups for facilitated enzyme attachment. Furthermore, the non-porous fiber and the opened microstructure of woven fabrics could reduce markedly diffusion resistance of the substrates or products, compared to porous materials. Additionally, woven silk fabrics are inexpensive, non-toxic and ready available. Several enzymes, such as alkaline phosphatase [24], uricase [25], and glucose oxidase [26], have been immobilized on silk material in the form of fibers or membranes. However, limited studies reported the use of silk fiber fabrics for lipase immobilization and very little work dealing with the effect of hydrophilic/hydrophobic surface of the fabrics has been reported [23].

In this study the potential of silk fiber in the form of a woven fabric has been explored for the immobilization of lipase from *Candida* sp. 99–125. A highly hydrophobic fabric film was prepared by coating native fiber with methyl groups through treatment of with polydimethylsiloxane (PDMS), which has been widely utilized for surface modification to enhance the hydrophobicity and several other surface properties of fabrics [27,28]. The aim of this work is to compare the effect of hydrophilic/hydrophobic surfaces of silk fabrics on the stability and activity of immobilized lipase. The hydrolysis of olive oil and the esterification of dodecanoic acid were chosen as two model reactions for determining the catalytic activity and properties of the immobilized lipases. The operational stability of immobilized derivatives in organic media was also investigated.

2. Materials and methods

2.1. Materials

Silk woven fabrics were donated by Beijing Cta New Century Biotechnology Co. Ltd, Beijing China. The raw silk from *Bombys mori* was degummed and then industrially woven. The fibers underwent no further special surface treatment. The amino-modified polydimethylsiloxane (2–8040 polymers, viscosity of 0.8–5.0 Pa s) was purchased from Dow Corning (Shanghai) Mgmt Co. Ltd, Shanghai, China. Lipase (from *Candida* sp.99–125, crude powders) was prepared in our laboratory [29]. Other reagents and solvents used were of analytical grade.

2.2. Surface modification of silk woven fabric

Prior to treatment and immobilization, the woven fabrics were cut into small pieces (3 cm × 3 cm squares for each piece, weighing about 0.15 g) and immersed in deionized water for 12 h, and then dried at 50 °C for 4 h. Dried fabric films (of about 1.0 g total) were treated by dipping in 0.5% (w/v) solution of amino-modified polydimethylsiloxane in hexane at 35 °C for 1 h in a water bath with slight shaking. Finally, the treated fabric films were rinsed with fresh hexane and then dried in an oven at 70 °C for 1 h to remove the residual solvents.

2.3. Characterization of native and PDMS-treated support

The surface morphology of fabric films was observed by SEM using a S250HK3 (Cambridge, UK) instrument. The ATR-IR spectra of the fibers were recorded by an FT-IR spectrometer (Nicolet Nexus 670 spectrometer) equipped with variable angle horizontal ATR accessory. Surface static water contact angles were measured with OCA20 Contact Angle (Data Physics Co., Germany) instrument; all measurements were carried out at ambient humidity and temperature and a minimum of ten readings were taken at different locations on the surface of each film to determine the average values.

2.4. Immobilization procedure of lipase

Lipases from *Candida* sp. 99–125 were immobilized by adsorption onto silk fiber films. The crude lipase powders