Journal of Molecular Catalysis B: Enzymatic 127 (2016) 18–25

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Preparation and characterization of sol–gel hybrid coating films for covalent immobilization of lipase enzyme

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ARTICLE INFO

Article history:
Received 19 November 2015
Received in revised form 15 January 2016
Accepted 14 February 2016
Available online 17 February 2016

Keywords:
Lipase
Immobilization
Sol–gel
Hydrolytic activity
Synthetic activity

ABSTRACT

In this study UV-curable hybrid epoxy-silica polymer films were prepared via sol–gel method. Lipase (EC 3.1.1.3) from *Candida rugosa* was covalently immobilized onto hybrid epoxy-silica polymer films and immobilization capacity of polymer films was found 7.22 mg g^{-1}. The morphology of the polymeric support was characterized by scanning electron microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). Immobilized and free enzymes were used in two different reaction systems: hydrolysis of *p*-nitrophenyl palmitate in aqueous medium and synthesis of *p*-nitrophenyl linoleate (from *p*-nitrophenol and linoleic acid) in *n*-hexane medium. The effect of temperature on hydrolytic and synthetic activities was investigated and observed maximum activities at 50 °C and 45 °C for immobilized enzyme, orderly. K_m values for free enzyme were determined 0.71 and 1.12 mM by hydrolytic and synthetic activity assays, respectively, while these values were observed as 0.91 mM and 1.19 mM for immobilized enzyme.

At the end of 30 repeated cycles, 56% and 59% of initial activities remained for hydrolytic and synthetic assays, respectively. Native enzyme lost its activity completely within 20 days, whereas the immobilized enzyme retained for hydrolytic and synthetic activities was approximately 82% and 72%, respectively, under the same storage time.

1. Introduction

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are atypical enzymes that catalyze both hydrolysis and formation of the ester bond between glycerol and long-chain fatty acids [1]. Lipases also catalyze various bioconversion reactions in a wide variety of organic solvents such as the hydrolysis, esterification, transesterification, aminolysis, and acidolysis [2]. In the last decade, there has been an increasing interest in the use of lipases for their properties such as high specificity, efficient reaction rate, non-toxicity and biodegradability, reproducibility under normal laboratory conditions. Also they are able to recognize very different substrates [3]. Lipases are used as versatile industrial applications, including cleanser, biosensor and biodiesel production, bio-catalytic determination of pharmaceuticals, nourishment and flavor businesses, cosmetics and perfumery production [4,5].

Lipases are produced in almost every living cell, but due to a number of advantages, microbial lipases are the most widely used group for practical applications because of low costs of production and easy modification of properties. Among the lipases from various sources, *Candida rugosa* lipase (CRL) received much attention due to its high activity and broad specificity [5,6].

Immobilization of enzymes is one of the most essential facets of modern biotechnology. This process frequently overcome the structural instability and allow repeated use, native enzymes have been successfully immobilized on different supports using various techniques and enables long-term stability/recyclability of the biocatalyst [5,7,8]. Because of the environmental conditions, free lipases are easily inactivated and hard to be recover for reuse. The separation of products in presence of free lipases is tedious. Hence, further industrial uses of free lipases are restricted because of high costs and complex downstream processing [4]. These drawbacks of the free lipases are overcome through immobilization techniques. As a very promising strategy, immobilization of lipases on solid supports is economically advantageous in order to facilitate the stability and reusability of these enzymes. Immobilized enzymes are preferable to free enzymes because of their easy separation from the reaction mixture makes them suitable for constant use.

A variety of support materials have been used in immobilization of lipases like silica [9], magnetic poly(glycidyl methacrylate-

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http://dx.doi.org/10.1016/j.molcatb.2016.02.007
1381-1177/

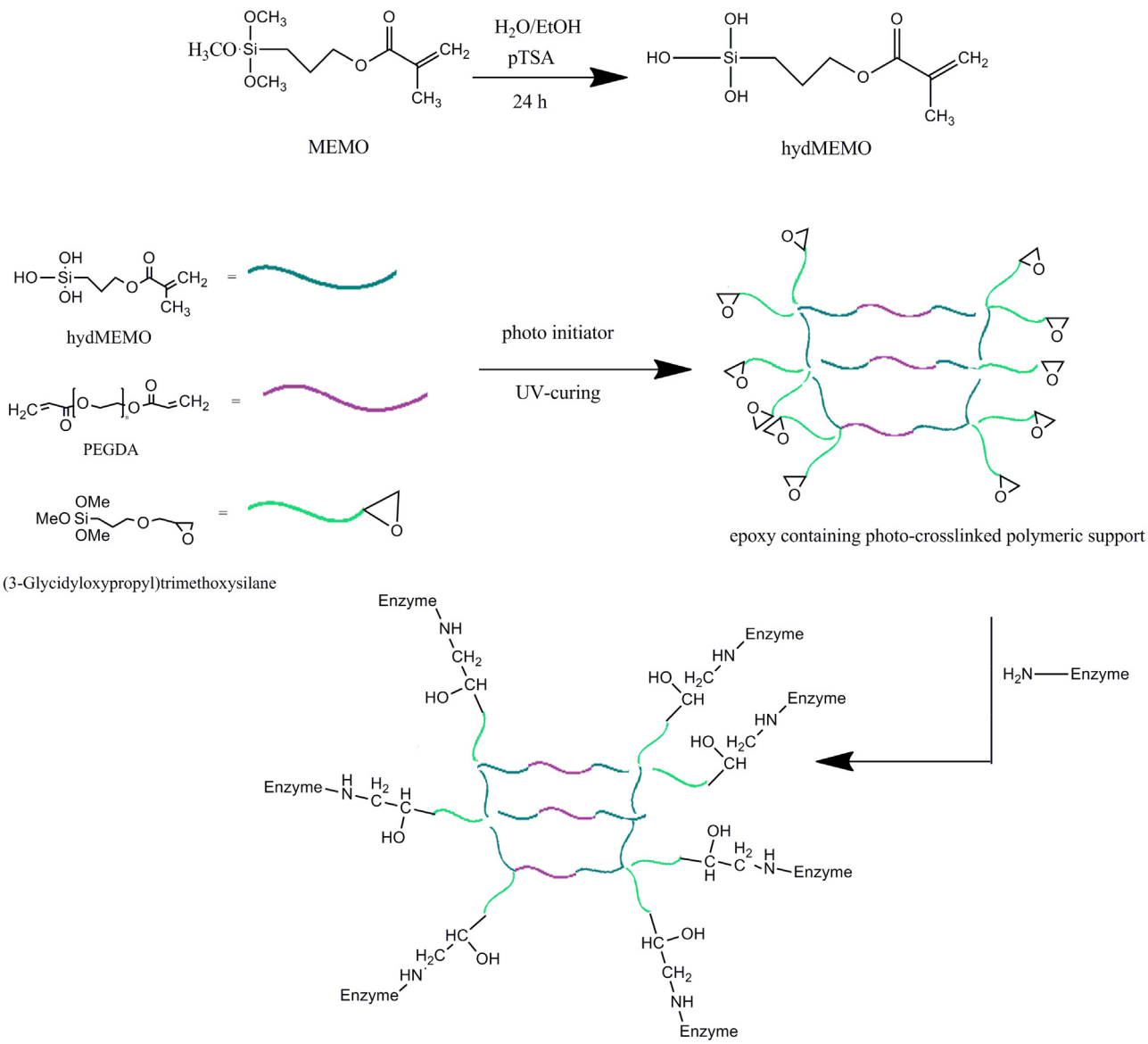

Fig. 1. Preperation of hybrid epoxy-silica polymer film via sol–gel process and enzyme immobilization on functionalized support (MEMO: 3-(methacryloxy)propyltrimethoxysilane *p*-TSA: para-toluenesulfonic acid).

methyl methacrylate) beads [10], polypropylene hollow fiber membrane modified with hydrophobic polypeptide [11], polysulfone ion exchange membranes [12], mesoporous silica [13], poly(hydroxyethyl methacrylate)-based membranes [14], calcium alginate gels [15], celite [16], nylon-6 [17], chitosan and agarose [18].

In this study UV-curable hybrid epoxy-silica polymer films were prepared via sol–gel process. The most generally utilized system for preparing hybrid materials is the sol–gel technique, which permits acquiring cross-linked inorganic silica into the polymer network to improve the properties of the material. Hybrid materials can be prepared by a radiation-curing technique such as using a UV curable binder system [19]. In this study, lipase from *C. rugosa* (CRL) was covalently immobilized onto organic/inorganic hybrid SiO_2—matrix films. Polymeric support material was characterized by using FTIR and SEM. Enzyme activity of free and immobilized lipase was examined in two different reaction systems: 1. hydrolysis of *p*-nitrophenyl palmitate in aqueous medium, 2. synthesis of *p*-nitrophenyl linoleate (from *p*-nitrophenol and linoleic acid) in organic medium. Temperature, kinetic parameters, and storage stabilities for free and immobilized enzymes were evaluated for both hydrolytic and synthetic activities.

2. Materials and methods

2.1. Reagents

Lipase from *C. rugosa* (700 U mg^{-1}), *p*-nitrophenol (*p*NP), linoleic acid (LA), *p*-nitrophenyl palmitate (*p*NPP), 3-(methacryloxy)propyltrimethoxysilane (MEMO), *p*-toluene sulfonic acid, poly(ethylene glycol) diacrylate, (3-glycidyloxypropyl) trimethoxysilane were purchased from Sigma Aldrich Chemical Co. (St., Louis, USA). Bradford reagent and ovalbumin were commercial product of BioRad (BioRad Laboratories, Hercules CA, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of polymeric support material

A hybrid epoxy-silica polymer film was prepared through sol–gel process. The sol–gel precursor was prepared by using