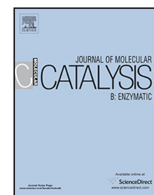
Journal of Molecular Catalysis B: Enzymatic 132 (2016) 67–74

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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



Immobilization of lipase onto functional cyclomatrix polyphosphazene microspheres

Chen Chen, Xue-yan Zhu, Qiao-ling Gao, Fei Fang, Li-wei Wang, Xiao-jun Huang*

MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form 16 June 2016
Accepted 3 July 2016
Available online 5 July 2016

Keywords:
Enzyme immobilization
Lipase
Polyphosphazene microsphere
Thiol-ene

ABSTRACT

Functional polyphosphazene cyclomatrix microspheres (PMS) containing glucose and dodecyl moieties were utilized for physical adsorption of lipase. In this regard, *Candia Rugosa* lipase was immobilized onto the above microspheres through physical adsorption, which exhibited catalytic hydrolysis of glycerol triacetate as the model reaction. As observed under transmission electron microscope (TEM) and scanning electron microscope (SEM), the size of all the microspheres ranged from 400 nm to 1 μm with a mean diameter of 680 nm. Zeta potential analysis demonstrated that the microspheres were negatively charged. The influences of buffer pH, substrate concentration and temperature were also studied along with reusability. The obtained results demonstrated that the immobilized lipase on deprotected glycosylated PMS (D-GPMS) showed the best performance and possessed the highest enzymatic loading activity around 1.0884 mmol L^{-1} min^{-1} g^{-1}, and retained nearly 50% of its initial activity even after 10 cycles of application. Moreover, the lipase-microsphere complex displayed high pH adaptability from 7 to 9 and temperature stability above 40 °C. From the viewpoint of biocompatible PMS support, the GPMS-lipase conjugate have potential applications in field such as food, medicine and environment.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Lipase, a type of hydrolase, is frequently used in various chemical reactions, such as hydrolysis of triglycerides and other carboxylic esters into monoacyl glycerols and free fatty acid [1,2], enabling lipase as particular industrial biocatalysts in biochemical, biomedical, and food industrial processes [3–5]. However, there is only a few direct usage of free lipase in industry due to several limitations including high cost, low stability and difficulties in separation and reuse [6–9]. Therefore, researchers have turned to immobilization methods to immobilize lipases on supporting materials in many enzymatic reactions. It is widely acknowledged that enzyme immobilization is a promising approach when it comes to continuous separation, long-term activity, enhanced stability and reusability [10]. Enormous efforts have been devoted to enhance the loading capacity and to increase the activity of lipase fixed onto solid supports such as membrane, microspheres, nanofibers, nanotubes and other porous material [11–15]. From this perspective, the selection of immobilized interfaces depends on hydrophilicity, inertness toward the enzyme and biocompatibility, especially for long-term usage and activity retaining [16–18]. Other strategies aimed to achieving high loading capacity consistently focus on the chemical and morphological factors, such as affinity towards lipase, availability of reactive functional groups and large surface area [19,20].

A wide range of polymeric supports, e.g. carboxymethylcellulose, chitosan, collagen [21,22], ion exchange resins and biofriendly polymers [23,24] have been widely used as solid supports for enzyme immobilization owing to the ease of interface functionalization and feasibility in nano- or micro- structure control [25,26]. Among the bio-friendly polymeric species, polyphosphazenes are hybrid inorganic-organic polymers, which contain bio-degradable inorganic phosphazene structures and versatile organic parts through classical nucleophilic substitution [27]. Linear polyphosphazenes have been widely applied in different biotechnological applications, such as biosensors, controlled release, drug delivery, scaffolding materials [28–31]. Moreover, highly tunable linear polyphosphazene based materials have proven to be efficient supports for enzyme immobilization. Cuetos et al. [32] generated a synthetic self-sufficient redox biocatalyst by employing $\{NP[O_2C_{12}H_{8-x}(NH_2)_x]\}_n$ as a starting material to covalently coimmobilize Baeyer-Villigermono oxygenase and NADPH recycling enzyme. An anionic poly[bis(methacrylate)phosphazene] hydrogel for lipase entrapment was prepared by Qian et al. [33]

* Corresponding author.
E-mail address: hxjzxh@zju.edu.cn (X.-j. Huang).

http://dx.doi.org/10.1016/j.molcatb.2016.07.003
1381-1177/© 2016 Elsevier B.V. All rights reserved.

to obtain a reversible enzyme binding material. However, there still exists a requirement for polyphosphazene based material with less complicated synthetic route along with higher loading capacity. In this concern, polymeric micro particles are more desirable for enzyme immobilization than the linear ones, since high surface area can enhance enzyme loading per unit mass of support [34]. Thus, a combination of biocompatible polyphosphazene and nano- or micro- structured interfaces may be a potential route to achieving better enzyme loading properties.

Cyclomatrix polyphosphazenes, in the form of microspheres, nanotubes or even nanosheets, can be synthesized under a relative mild condition via a one-step precipitation crosslinking process between hexachlorocyclotriphosphazene (HCCP) and multifunctional amineor phenols [35–37]. Owing to the highly crosslinked phosphazene structure, the cyclomatrix polyphosphazenes generally present remarkable thermal stability, solvent resistance, water dispersibility and biocompatibility [38,39]. Such properties make cyclomatrix polyphosphazene microspheres (PMS) suitable for enzyme immobilization. Although rarely used in lipase immobilization, PMS is also allowed to vary its surface hydrophilicity and biocompatibility through altering the monomers or post-functionalization to get improved performance in lipase catalytic properties. In the current work, HCCP was crosslinked with bisphenol-S (BPS) and 4,4′-diallyl bisphenol-S (DABPS) to achieve poly[(cyclotriphosphazene-*co*-BPS-*co*-DABPS)] microspheres (VPMS). The introduction of the above-mentioned two monomers generated sub-microspheres, which could be viewed through transmission electron microscope (TEM) and scanning electron microscope (SEM). Subsequently, β-D-glucose and 1-dodecanethiol were employed to modify the VPMS via thiol-ene reactions to form glucosyl polyphosphazene microsphere (GPMS) and dodecyl polyphosphazene microsphere (DPMS). The detailed chemical structure of PMSs was reflected by FT-IR and the elemental composition was obtained from X-ray photoelectron spectroscopy (XPS). Zeta potential analysis was also done to investigate the surface charge of the related particles. Surface hydrophilicity of microspheres was characterized by water contact angle (WCA). Other various analytical techniques were used to study the enzyme modified PMS, particularly in terms of optimal temperature and pH value for enzymatic activity, lipase concentration, thermal stability and reusability. In this regard, this work was intended to investigate biocompatible PMS based material for lipase immobilization, which will shed light on further practical applications of these materials in the field of food, medicine and enviroment.

2. Experimental section

2.1. Materials

Hexachlorocyclotriphosphazene (Bo Yuan New Materials & Technique, Ningbo, China) was purified by recrystallization from heptane and subsequent vacuum sublimation at 60 °C. Triethylamine (TEA) was dried over CaH_2 prior use. 2,3,4,6-Tetra-*O*-acetyl-1-thiol-β-D-glucopyranose (SH-$GlcAc_4$, Chemsynlab, Beijing, China) was used without any further purification, bisphenol-S (BPS 98%) was purchased from Aladdin Reagent, China and 4,4′-diallyl bisphenol-S (DABPS 98%) was purchased from Weidelong Chemistry, Jintan, China. trifluoroethanol (TFE, Aladdin Reagent, China, 99.5%), 1-dodecanethiol (Aladdin Reagent, China, 98%) and 2,2-dimethoxy-2-phenylacetophenone (DMPA, Aladdin Reagent, China, 99%) were used as received. Candia Rugosa Lipase (CRL) powder (1150 units per mg solid), Bradford reagent, bovine serum albumin (BSA, molecular mass: 67 000 Da), and glycerol triacetate were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and used as received.

2.2. Synthesis of poly[(cyclotriphosphazene-co-BPS-co-DABPS)] microspheres (VPMS)

To a 100 mL round-bottomed flask, placed in an ultrasonic bath (240 W) and containing a solution of HCCP (0.087 g, 0.25 mmol), BPS (0.125 g, 0.5 mmol) and DABPS (0.169 g, 0.5 mmol) in acetonitrile (30 mL) was added excess TEA (2 mL) at 30 °C. Immediately after addition of TEA, the reaction mixturechanged to a milky white color. After 4 h, the resultant particles were obtained through centrifugation, followed by washing three times with alcohol and deionized water successively. The particles were finally dried at 60 °C under vacuum to yield VPMS active microspheres directly as white powder. The yield of the microspheres was 75.1%.

2.3. Synthesis of PMS containing glucose moiety (GPMS)

VPMS (300 mg) was dispersed in TFE (5 mL) and the mixture was then transferred to a quartz reactor. To the reactor were added 2,3,4,6-Tetra-*O*-acetyl-1-thiol-β-D-glucopyranose (600 mg, 2 mmol) and DMPA (33 mg, 0.128 mmol) sequentially, followed by gentle bubbling of N_2through the mixture for 10 min to eliminate dissolved oxygen. The thiol-ene reaction was initiated by UV irradiation (max = 365 nm, 0.6 mW/cm^2) and conducted for 2 h. After the first click reaction, the acetyl protecting group was removed by the addition of 1 M solution of CH_3ONa in CH_3OH to the polymer solution to obtain deprotected GPMS (D-GPMS). The resultant particles were obtained by centrifugation, followed by washing three times with alcohol and deionized water successively. The particles were finally dried at 60 °C under vacuum to yield deprotected GPMS as light yellow powder.

2.4. Synthesis of PMS containing dodecyl moiety (DPMS)

VPMS (300 mg) was dispersed in TFE (5 mL) and the mixture was then transferred to a quartz reactor. Excess 1-dodecanethiol (2 mL) and DMPA (33 mg 0.128 mmol) were next added sequentially to the reactor, followed by gentle bubbling of N_2 through the mixture for 10 min to eliminate dissolved oxygen. The thiol-ene reaction was initiated by UV irradiation (max = 365 nm, 0.6 mW/cm^2) and conducted for 2 h. The resultant particles were obtained via centrifugation, followed by washing three times with alcohol and deionized water successively. The particles were finally dried at 60 °C under vacuum to yield DPMS as white powder.

2.5. Preparation of enzyme immobilized PMSs

Lipase solutions were prepared by adding an appropriate amount of lipase powder in PBS(0.05 M, pH 7.0). The insoluble impurities of the enzyme solution were removed by centrifugation at 4000 rpm for 15 min. VPMS, DPMS and D-GPMS (50.0 mg) were added into the lipase solution (20 mL, 2.5 mg/mL) before carefully grinding. The resultant mixture was gently stirred for 3 h in a flask placed in a water bath at 25 °C. The PMSs-lipase complex was obtained through centrifugation at 10,000 rpm for 15 min. The amount of proteins immobilized on the support was determined by measuring the protein concentration of the lipase solution in the supernatant using the Bradford method and BSA as a standard. It should be noted that after each lipase adsorption, the PMSs-lipase complex was washed three times by PBS solution. The loading capacity of PMSs was calculated by the following relationship:

$$A_e = \frac{(C_0 - C) \times V - C_W \times V_W}{m}$$

Download English Version:

https://daneshyari.com/en/article/69310

Download Persian Version:

https://daneshyari.com/article/69310

Daneshyari.com