Enzyme and Microbial Technology 105 (2017) 24–29

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Preparation of glutaraldehyde-treated lipase-inorganic hybrid nanoflowers and their catalytic performance as immobilized enzymes

Hye Rin Lee[a], Minsoo Chung[b], Moon Il Kim[b,*], Sung Ho Ha[a,*]

[a] Department of Advanced Materials & Chemical Engineering, Hannam University, Daejeon 34054, Republic of Korea
[b] Department of BioNano Technology, Gachon University, Gyeonggi, 13120, Republic of Korea

ARTICLE INFO

Keywords:
Protein-inorganic hybrid nanoflowers
Candida rugosa lipase
Reusability
Glutaraldehyde treatment

ABSTRACT

The use of protein-inorganic hybrid nanoflowers for the immobilization of enzymes has received a significant degree of attention owing to their capability to retain high enzymatic activity and stability. However, the relative lack of reusability due to the weakness of the flower-like structure has limited their practical applications. Herein, we have developed a simple but efficient method to synthesize highly robust enzyme-inorganic hybrid nanoflowers, which relies on further crosslinking of the enzyme molecules entrapped in the hybrid nanoflowers by treatment with glutaraldehyde (GA). By employing lipase from *Candida rugosa* as a model enzyme with copper phosphate during 3 days incubation followed by the additional GA treatment for only 1 h, we could successfully synthesize GA-treated lipase nanoflowers having similar flower-like morphology and hydrolytic activity (*ca.* 95% compared with the free lipase) as conventionally synthesized lipase nanoflowers without GA treatment. Importantly, the conventional lipase nanoflowers seemed not to be reusable because they lost most of their activity (~90%) after recycling 4 times, whereas GA-treated lipase nanoflowers exhibited higher retention of their initial activity (over 70%) after 4 reuses, which was also accompanied by an efficient maintenance of their flower-like morphology. Based on our results, we expect that this simple GA-mediated strategy to synthesize enzyme-inorganic hybrid nanoflowers can be readily extended to other enzymes for various biotechnological applications.

1. Introduction

Immobilization of enzymes to improve their catalytic performance, and especially their reusability and stability, has been extensively studied to facilitate the development of practical applications and improve their economic efficiency [1]. The improved stability of immobilized enzymes was thought to be arisen from the efficient maintenance of enzyme's native three-dimensional structure by the proper restriction of enzyme mobility in a fixed space, representatively by preparing chemical or physical linkages between enzyme molecules and appropriate support materials [2,3]. Improved reusability was also achieved by making enzymes "insoluble" by the proper conjugation with insoluble support materials which facilitates its separation and reuse. Such immobilized enzymes were proven to reduce costs in many industrial applications by enabling continuous process as well as facilitating proper control of the process [4]. However, catalytic activity of most of the enzymes after the immobilization was generally lower than the initial one due to the possible blocking of enzymes' active sites during the immobilization procedures and increased mass transfer limitation of substrate toward the enzymes. Thus, there have been many studies to develop a highly efficient immobilization strategy of enzymes, which exhibits greatly enhanced stability and reusability as well as minimal loss of initial activity. For examples, diverse materials, such as silica, carbon, hydrogels, and polymeric resins, and methods, such as physical adsorption, chemical covalent attachment, and entrapment, have been so far investigated for enzyme immobilization [5–7]. Some of them are currently adopted for usage in several enzyme-mediated industrial processes. In this research field, the nanobiocatalytic approach, in which enzymes are associated with nanostructured materials, is particularly interesting [8–12], and has created new opportunities for both conventional and newly emerging areas of enzymatic applications including biosensors, bioanalytical devices, and biocatalysis.

In this regard, enzyme-inorganic hybrid nanomaterials, such as enzyme-polymer conjugates or nanogels, produced by a variety of immobilization techniques, such as conjugation, cross-linking, and self-assembly, have received a significant attention because of their effectiveness to retain and stabilize enzymatic activity [13]. Recently, an innovative strategy to synthesize hybrid nanostructures having flower-

* Corresponding authors.
E-mail addresses: moonil@gachon.ac.kr (M.I. Kim), shha@hannam.kr (S.H. Ha).

http://dx.doi.org/10.1016/j.enzmictec.2017.06.006
Received 26 February 2017; Received in revised form 26 May 2017; Accepted 10 June 2017
Available online 13 June 2017
0141-0229/ © 2017 Elsevier Inc. All rights reserved.

like shapes (nanoflowers) was reported, based on the combination of enzyme molecules with copper or calcium phosphate [14,15]. The hybrid nanoflowers were more active and stable than conventionally immobilized enzymes owing to their large surface area and effective confinement of enzyme molecules in their interior. Nanoflowers composed of laccase and copper phosphate were extremely stable and highly active even compared to free laccase, and thus were successfully applied to detect phenol in aqueous solution [16]. Lipase which is one of the widely utilized enzyme in many biosynthetic industries, was also efficiently entrapped within nanoflowers [17–19]. Lipase hybrid nanoflowers showed high activities and yielded even greater than that of native lipase with excellent long-term stability. Furthermore, several groups have utilized hybrid nanoflowers for different applications, such as detections of hydrogen peroxide [20] and glucose [21] and protein digestion [22]. Although enzyme-inorganic hybrid nanoflowers exhibit greatly enhanced enzymatic activity and stability, they are generally not recyclable and reusable, which critically limits their widespread use in practical applications. The weak reusability of nanoflowers has been mainly attributed to their elaborate flower-like structures consisting of many hierarchically shaped petals that are easily broken during the centrifugation steps of the recycling process. Therefore, there is a significant incentive for the design of innovative methods to synthesize highly robust and recyclable protein-inorganic nanoflowers by reinforcing the interactions between their petals.

Towards this goal, we developed, for the first time in this study, a simple but efficient method to synthesize recyclable protein-inorganic nanoflowers using simple glutaraldehyde (GA) treatment to further crosslink the enzyme molecules inside the nanoflowers. Although GA treatment has been employed for many years to prepare several robust immobilized enzymes [23], there have been no reports so far of its usage in the synthesis of protein-inorganic nanoflowers. Using this technique, we successfully synthesized nanoflowers containing a lipase and copper phosphate, whose hydrolytic activity and morphology were similar to those of conventional lipase nanoflowers without GA treatment. The lipase nanoflowers generated after the additional GA treatment were very robust and exhibited significantly enhanced reusability, which would be highly beneficial for practical applications.

2. Materials and methods

2.1. Materials

Lipase from *Candida rugosa*, copper(II) sulfate pentahydrate, 4-nitrophenyl butyrate (4-NB), GA, and phosphate buffered saline (PBS, 1×, pH 7.4) were purchased from Sigma-Aldrich (Milwaukee, WI). All chemicals were of analytical grade and used without further purification. Aqueous solutions were prepared using ultrapure DNase/RNase free distilled water (Invitrogen).

2.2. Synthesis and characterization of lipase nanoflowers

Conventional nanoflowers were prepared by adding 50 μL aqueous $CuSO_4$ (120 mM) to 3 mL PBS (pH 7.4) containing lipase at different concentrations, and incubating the mixture at room temperature (RT) for 3 days. GA-treated lipase nanoflowers were synthesized following the same procedure, but with an additional treatment with GA (0.1% w/w) for 1 h at RT.

The resulting lipase nanoflowers were analyzed by scanning electron microscopy (SEM) (CX-200TA, COXEM, Korea), after the nanoflower suspension was filtered and dried on a membrane (pore size: 0.1 μm). For the transmission electron microscopy (TEM) analyses, the nanoflowers was applied by drop casting the particle suspensions on a carbon-coated copper TEM grid (Electron Microscopy Sciences, USA) followed by drying at room temperature. All samples were observed using a Jeol EM-2010 microscope (Jeol Co.). For X-ray diffraction (XRD) analysis (D/MAX- 2500, Rigaku Corporation, Tokyo, Japan), the precipitate of lipase nanoflowers was washed with deionized water, and dried at 80 °C for 1 day. Fourier transform infrared (FT-IR) spectra of the nanoflowers were obtained using a FT-IR spectrophotometer (FT/IR-4600, JASCO, Easton, MD). The yield of lipase encapsulation in the nanoflowers was determined by measuring the amount of protein in the supernatant using the Lowry protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard [24].

Table 1
Encapsulation yield (%) of lipase in the nanoflowers.

	Lipase (mg/mL)			
Copper(II) sulfate (mM)	0.02	0.05	0.1	0.5
0.4	47.8	29.7	50.2	26.5
0.8	67.0	44.6	53.4	51.4
2	76.5	59.5	67.5	58.7
4	28.7	40.9	59.6	33.8

2.3. Measurement of lipase activity

The enzymatic activity of GA-treated lipase nanoflowers (0.1 mg/mL), conventional lipase nanoflowers without GA treatment (0.1 mg/mL), and free lipase (0.1 mg/mL) were evaluated by determining the absorbance intensity using the lipase substrate 4-NB (500 μM in 10 mM sodium phosphate buffer, pH 6.5) at 25 °C for 120 min. Periodically, 100 μL aliquots were taken from the reaction mixture and their absorbance at 400 nm was measured using a UV–vis spectrophotometer (BioMATE3S, Thermo, USA) equipped with a desktop computer for data acquisition. The conversion from 4-NB into 4-nitrophenol was calculated from the concentration of consumed 4-NB. For parallel assays, free lipase (0.1 mg/mL), conventional lipase nanoflowers without GA treatment (0.1 mg/mL), GA-treated lipase nanoflowers (0.1 mg/mL), and negative control sample without any enzyme were incubated with 4-NB (500 μM in 10 mM sodium phosphate buffer, pH 6.5) at 25 °C for 30 min. Subsequently, absorbance intensity was measured using a Tecan Infinite M200 pro microplate reader (Mnnedorf, Switzerland) with a transparent 96 well-plate.

2.4. Determination of nanoflower reusability

The reusability of the GA-treated and conventional lipase nanoflowers was assessed after repeated cycles of: the typical enzymatic reaction (0.1 mg/mL lipase nanoflowers and 500 μM 4-NB in 10 mL sodium phosphate buffer, pH 6.5 at 25 °C for 30 min), recovery of the lipase nanoflowers by centrifugation at 11,000 rpm for 3 min, and excessive washing (5 times) with sodium phosphate buffer (10 mM, pH 6.5). The excessively washed sample was reused for the measurement of the residual enzymatic activity. The relative activity (%) was calculated as the ratio of the residual activity to the initial activity.

3. Results and discussion

To develop robust protein-inorganic hybrid nanoflowers, the reaction mixture containing copper(II) phosphate and the enzyme was first incubated for 3 days at RT to generate the hybrid nanoflowers, and then allowed to react with GA for one additional hour to further crosslink the enzyme molecules entrapped inside the nanoflowers. We hypothesized that this would act as strengthening 'glue' that tightly binds the petals, and hence GA-treated nanoflowers would be highly robust, exhibiting significantly enhanced reusability during conventional recycling procedures by centrifugation. Thus, this method could be potentially used in various enzyme-related biotechnology applications that require the synthesis of protein-inorganic nanoflowers.

Before testing the GA-mediated synthesis method, we first prepared nanoflowers from a PBS solution containing *Candida rugosa* lipase as a

Download English Version:

https://daneshyari.com/en/article/4752731

Download Persian Version:

https://daneshyari.com/article/4752731

Daneshyari.com