Journal of Molecular Catalysis B: Enzymatic xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Review

Increasing the hydrolytic activity of lipase in oil/water two-phase system using surfactant–enzyme nanocomposite

Xun Cao, Sheng Xu, Yan Ni, Yuan-yuan Lu, Huan-huan Li, Ke-quan Chen*, Ping-kai Ouyang

College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211816, PR China

ARTICLE INFO

Article history:
Received 8 October 2016
Received in revised form 8 February 2017
Accepted 8 February 2017
Available online xxx

Keywords:
Lipase
Sodium deoxycholate
Nanocomposite
Oil/water two-phase

ABSTRACT

In this study, a lipase/Na $_nCo_m(DC)_{n+2m}$ composite was successfully synthesized via the co-precipitation of *Candida rugosa* lipase (CRL), sodium deoxycholate (NaDC), and cobalt ions. Composite characterization confirmed that CRL was successfully embedded in Na $_nCo_m(DC)_{n+2m}$, with particle diameters from 50 nm to 120 nm. Furthermore, the nanocomposite not only exhibited lipase hydrolytic activity but also showed surfactant function. As a result, the nanocomposite showed a 2.8-fold increase in hydrolytic activity as compared to the free CRL. In addition, the nanocomposite exhibited excellent adaptability over a wider temperature and pH range. After the 180-min hydrolysis of sunflower oil, the fatty acid yield was 0.88 mmol by CRL/Na $_nCo_m(DC)_{n+2m}$, which was 3 times higher than that with free CRL. Therefore, the CRL/Na $_nCo_m(DC)_{n+2m}$ nanocomposite may have wide biocatalytic application at the interface of oil/water two-phase systems.

Contents

1. Introduction

Lipases constitute a special group of enzymes that can catalyze the hydrolysis of triacylglycerols and other hydrophobic esters [1]. With a variety of biotechnological applications, lipases have extraordinarily broad substrate specificity and can adapt to a wide

* Corresponding author at: College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Puzhu Road 30#, Nanjing City, PR China.
E-mail address: kqchen@njtech.edu.cn (K.-q. Chen).

http://dx.doi.org/10.1016/j.molcatb.2017.02.006
1381-1177/

range of esters with different structures [2]. In recent years, the development of new applications of lipases to products and processes has been rapidly and globally increasing, particularly in the areas of detergents, oils and fats, and fine chemical engineering [3,4]. Lipases are characterized by their high activity in terms of hydrolyzing triacylglycerols at substrate–water interfaces but very low activity towards soluble oils or non-natural substrates due to the closed conformation of lid, which covers the active site of the lipase [5,6]. However, the activity of lipases decreases following the hydrolysis of the substrate, which results in an irreversible interfacial inactivation of the lipase at high interfacial tension [7]. By accumulating at the interface of immiscible fluids, surfactants can reduce interfacial tensions as well as increase the solubility and mobility of hydrophobic or insoluble organic compounds [8]. Furthermore, it is likely that the surfactants can shift the close-open equilibrium between the forms of lipases towards the open conformation, exposing the active center to the reaction medium [9]. And surfactants may also increase the stability of the enzymes and prevent the denaturation of enzyme during the catalysis [10]. However, some surfactants can cause protein denaturation due to the surfactant unfolding of secondary and tertiary protein structure [11].

The effect of different types of surfactants on lipase activity has been widely investigated [12]. Nonionic and cationic systems could provide high hydrolysis rates in two-phase emulsions, whereas for anionic systems, almost no reaction activity has been observed [7]. Some papers have reported that when PEG_{6000} (nonionic surfactant) and CATB (cationic surfactant) are applied, the activity of the lipase increased 2.3-fold and 2.5-fold, respectively [13,14]. However, anionic systems have also played an important role in reducing the interfacial tension by populating the interface [15]. Different from many ordinary anionic surfactant molecules, the special structure and properties of sodium deoxycholate (NaDC) make it a broad-spectrum biosurfactant that can act as an emulsifier and solubilizer for apolar materials, particularly bilirubin, fat-soluble vitamins, cholesterol, and lecithin in intestines [16,17]. Although NaDC can reduce the interfacial tension of oil–water systems and increase the solubility of triglycerides, they have been shown to be nonconductive to hydrolysis by lipase near the critical micelle concentration. This phenomenon has been attributed to electrostatic repulsion between the negatively charged NaDC and the lipase [7]. There are no obvious effective methods of relieving the inhibition of electrostatic repulsion and accelerating the hydrolysis rate by a large margin.

Previous studies have shown that the addition of divalent metal ions can react with NaDC via metal coordination–chelation interactions, forming a series of nanocomposites with different sizes and structures [18]. In this study, we used cobalt ions to neutralize the negatively charge of NaDC and prepare a *Candida rugosa* lipase (CRL)/Na $_n$Co $_m$(DC)$_{n+2m}$ nanocomposite via a co-precipitation procedure. Furthermore, the effects of temperature, pH, and thermal treatment on the hydrolytic activity of the nanocomposite were conducted, and the efficiency for hydrolyzing sunflower oil using the nanocomposite in oil/water two-phase system was also investigated.

2. Materials and methods

2.1. Materials

CRL was purchased from Sigma–Aldrich (product of Japan). NaDC was obtained from Sinopharm Chemical Reagent Company, Shanghai, China. Cobalt chloride was purchased from Guangdong Chemical Reagent Engineering-Technological Research and Development Center, China. Sunflower oil was purchased from the local market. Other chemicals were of analytical grade.

2.2. Synthesis of CRL/Na$_n$Co$_m$(DC)$_{n+2m}$ composite

In a typical experiment, a solution (10 mL) containing both NaDC (10 mmol/L) and CRL (0.18 g/L protein concentration) was added to a water solution of cobalt chloride (20 mmol/L, 10 mL). The mixture was stirred (200 rpm) for 0.5 h at room temperature, followed by two cycles of centrifugation at 8000 rpm for 15 min and washing with distilled water. Then the precipitate was freeze-dried.

2.3. Characterization of the CRL/Na$_n$Co$_m$(DC)$_{n+2m}$ composite

N_2 adsorption–desorption assays of particles were conducted on a Micromeritics 3Flex surface characterization analyzer. All the samples were degassed at 70 °C for 10 h before measurement. Specific surface area was determined by the Brunauer–Emmett–Teller (BET) model, whereas the pore volume and pore size were obtained from the corrected form of the Barrett–Joyner–Halenda method.

A Thermo Scientific BioMate 3S spectrophotometer (made in China, designed in USA) was used to measure the UV–vis spectra of the Na $_n$Co $_m$(DC)$_{n+2m}$ and CRL/Na $_n$Co $_m$(DC)$_{n+2m}$ within a range of 220–500 nm. Analyses of the chemical functional groups of the NaDC and Na $_n$Co $_m$(DC)$_{n+2m}$, as well as of the CRL/Na $_n$Co $_m$(DC)$_{n+2m}$, were conducted by means of Fourier transform infrared (FTIR) spectroscopy (Nicolet iS5, USA). The FTIR spectra were collected in transmission mode between 500 and 4000 cm^{-1} at a resolution of 0.5 cm^{-1}.

The morphologies of the samples were characterized by scanning electron microscopy (SEM, Hitachi S-3400 II, Japan) with an accelerating voltage of 20 kV with fit magnification. Transmission electron microscopy (TEM, JEM-200CX, Japan) was used to further show the morphology and dimensions of the CRL/Na $_n$Co $_m$(DC)$_{n+2m}$ composite on a different scale. Sample preparation was accomplished by dispersing the composite in ethanol with ultrasound and then placing a well-dispersed droplet on a copper grid. The samples were then dried and analyzed.

2.4. Measurement of protein content

The protein amount of the CRL solutions or CRL/Na $_n$Co $_m$(DC)$_{n+2m}$ preparations was determined according to the Bradford method assay at 595 nm based on the bovine serum albumin standard line (10–100 μg/mL) [19].

2.5. Enzyme assays for soluble CRL and CRL/Na$_n$Co$_m$(DC)$_{n+2m}$

Because triacetin is a type of simple ester, it was used to measure the hydrolytic activity of the soluble CRL or CRL/Na $_n$Co $_m$(DC)$_{n+2m}$. 200 μL of free CRL solution or an equivalent quantity (in terms of protein) of the CRL/Na $_n$Co $_m$(DC)$_{n+2m}$ was dispersed into a pH-stabilized mixture of triacetin (2.0 g), deionized water (50 mL), and pH 7.0 phosphate buffer solution (10 mL). After stirring for 30 min at 40 °C, the reaction was then stopped by adding 20 mL of an acetone–ethanol mixture (1:1 v/v ration), which inactivated the soluble CRL or CRL/Na $_n$Co $_m$(DC)$_{n+2m}$. Finally, the release of the acid moiety of triacetin was measured by titration with a 0.02 M sodium hydroxide solution. The volume of sodium hydroxide solution consumed was measured and the hydrolytic activity of the soluble CRL or CRL/Na $_n$Co $_m$(DC)$_{n+2m}$ was calculated in the standard way [20]. The relative hydrolytic activity of soluble CRL with NaDC, Na $_n$Co $_m$(DC)$_{n+2m}$, and the cobalt ion mixture were also measured. The rel-

Please cite this article in press as: X. Cao, et al., Increasing the hydrolytic activity of lipase in oil/water two-phase system using surfactant–enzyme nanocomposite, J. Mol. Catal. B: Enzym. (2017), http://dx.doi.org/10.1016/j.molcatb.2017.02.006