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Enzyme@silica hybrid nanoflowers shielding in polydopamine layer for the improvement of enzyme stability

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ABSTRACT

In this study, a novel kind of flower-like monodisperse silica nanoparticles (silica nanoflowers) were successfully synthesized based on the microemulsion phase composed of water-surfactant-oil ternary systems. The obtained silica nanoflowers were used to immobilize penicillin G acylase (PGA). The maximum adsorption capacity of immobilized PGA (PGA@nanoflowers) was 207.3 $mg/_{support}$ and the specific activity was 539.1 $U/_{support}$. In addition, based on the mussel-inspired chemistry, we demonstrated a simple and highly effective strategy to stabilize the enzymes that immobilized on silica nanoflowers by the poly(dopamine) (PDA) coating through in situ polymerization. The effects of pH and temperature on the activity of free PGA, PGA@nanoflowers and immobilized PGA with PDA coating (PDA-coated PGA@nanoflowers) were investigated. The operational stability, storage stability and kinetic properties of the different preparation of PGA were also tested. Compared to free PGA and PGA@nanoflowers, the stabilities of PDA-coated PGA@nanoflowers were all improved significantly. This work demonstrated that the PDA coating presented a new way for surface modification of immobilized enzyme to improve the properties of enzyme. The approach of enzyme@silica hybrid nanoflowers shielding in PDA layer would have great potential in various enzyme immobilization.

1. Introduction

Biocatalysts play a crucial role in various scientific fields thanks to their unique superiorities, such as high substrate specificity, prominent catalytic ability, and mild reaction conditions [1]. As one of the major biocatalysts in the pharmaceutical industry [2], penicillin G acylase (PGA, E.C. 3.5.1.11) can catalyze penicillin G to 6-aminopenicillanic acid (6-APA) and 7-aminodeacetoxycephalosporanic acid, which are the pivotal intermediates for producing semi-synthetic antibiotics [3]. However, the major bottlenecks for the applications of enzymes are their low thermal and poor operational stability as well as the difficulties in reusing enzymes [4,5]. Therefore, significant effort has been devoted to exploiting immobilization strategies to stabilize enzymes and endow the enzymes with high stability, reusability and tailored enzyme activity [6–9].

For immobilizing enzyme, it is necessary to choose proper host materials to improve the properties of enzymes. Mesoporous silica nanoparticles (MSNs), thanks to the high specific surface area, tunable pore size, their high pore volume and biocompatibility, are extensively studied as carriers for enzyme immobilization [8,10–16]. Besides, enzyme immobilized on MSNs exhibits excellent thermal and pH stability, mechanical stability, high resistance to de naturants and organic solvents [14,17–19]. Among the various morphologies of MSNs, flower-like wrinkled mesoporous silica nanoparticles have attracted increasing attention in enzyme immobilizations because of their unique radial and wrinkled conical shape of the large mesopore [20,21]. Compared with the traditional MSNs, the silica nanoflowers not only have the advantages of MSNs, but also show the high surface to volume ratio and tunable interwrinkle distance owing to their three-dimensional dendritic superstructures. The special structure endows a high loading amount of enzyme and is beneficial for the mass transfer of the guest molecules within the porous network [22–24].

The most common method for the immobilization of enzyme on mesoporous silicas is physical adsorption via Van der Waals forces, hydrophobic interactions, hydrogen bonding and ionic interactions [22]. However, the phenomenon of enzyme leakage from the support often occurs due to the relatively weak interactions between the enzyme and support surface [25]. Many studies have been done to prevent enzyme leaching from supports [8,26]. A simple and effective approach is to coat a protected layer on the surface of pre-immobilized enzyme. To date, the materials of coating layer, including the organosilicon [8], polyacrylamide

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[27], protein biopolymer such as silk [28,29], have been applied to prevent enzyme leakage. Inspired by mussel adhesive proteins, polydopamine (PDA) technology has attracted extensive interest [30,31]. Dopamine molecules can easily self-polymerize under alkaline conditions, leading to a facile deposition of PDA coating on the surface of materials [32]. More importantly, the PDA coating is robust and endurable to extreme pH and temperature [33]. This provides a new way for protecting enzymes and improving their stability. However, to the best of our knowledge, few literatures have been reported that the self-polymerization of dopamine as coating layer for improving the stability of enzyme.

Hence, in this work, the PDA coating layer was deposited on the silica nanoflowers through self-polymerization to construct a safety shield to protect the enzyme. Specifically, PGA was chosen as model enzyme. Firstly, silica nanoflowers were prepared by the homogeneous microemulsion phase. Then, the PGA was directly immobilized on the surface of the silica nanoflowers by physical adsorption (denoted as PGA@nanoflowers). In order to prevent enzyme leakage and improve the enzyme stability, the PGA@nanoflowers was coated by PDA (denoted as PDA-coated PGA@nanoflowers). Interestingly, the thickness of PDA coating could be tuned by adjusting the time of polymerization. The properties of free PGA, PGA@nanoflowers and PDA-coated PGA@nanoflowers were investigated in detail. The results demonstrated that the PDA-coated PGA@nanoflowers exhibited enhanced thermal and pH stabilities. The introduction of PDA obviously improves the operation stability of PGA@nanoflowers as well as minimizes enzyme leakage. In addition, Candida antarctica lipase B (CALB) and catalase (CAT) were used to verify the universality of the immobilized enzyme method.

2. Experimental

2.1. Materials

Cetyltrimethyl ammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), and dopamine hydrochloride were purchased from Sigma-Aldrich. Penicillin G acylase (1000 U mL^{-1}) and penicillin G potassium salt (PGAK) were supplied by Huamaike Boitechnology Co., Ltd, China. Catalase (36800 U g^{-1} protein) was purchased from Beijing Dingguo Bioengineering Co. Ltd., China. Lipase B from C. antarctica was purchased from Novozymes. 4-nitrophenyl palmitate (*p*-NPP) and 6-Aminopenicillanic acid (6-APA) were purchased from Alfa Aesar Chemical Co. Ltd, China. Sodium acetate, ethanol, acetone, cyclohexane, *n*-butanol and urea were purchased from Tianjin reagent Corporation. Deionized water was used throughout the whole experiments. All of the chemical reagents used in the experiments were analytical grade and used without further purification.

2.2. Characterization

Scanning electron microscope (SEM) and transmission electron microscope (TEM) images were recorded on a field-emission microscope (Nova Nano SEM450) and a JEM-2100 microscope respectively. Dynamic light scattering (DLS) data were obtained through a Malvern instruments (Zetasizer Nano ZS90). The N_2 adsorption-desorption isotherms were performed on a Micromeritics physisorption analyzer (ASAP 2460) at 77 K [34]. Powdery samples (~0.2 g) were degassed at 90 °C for 6 h. The surface area and pore size distribution of the samples were estimated respectively using the Brunauer-Emmett-Teller (BET) method and the Barrett-Joyner-Halenda (BJH) model. UV–vis measurements were conducted by an optical instrument (Thermo Scientific Evolution 300). Fourier-transform infrared (FT-IR) spectroscopy analysis was recorded on a Bruker Vector 22 spectrophotometer using KBr pellets method. The thermal analysis was carried out in a thermogravimetric analyzer (TGA-DSC 1; Mettler Toledo) under N_2 atmosphere with heating rate of 10 °C/min. Confocal laser scanning microscopy (CLSM) images were obtained using an optical microscope (Leica TCS SP5). Before test, PGA molecules immobilized on the silica nanoflowers were labeled via fluorescein isothiocyanate (FITC).

2.3. Preparation of silica nanoflowers and immobilized PGA

2.3.1. Preparation of the silica nanoflowers

The silica nanoflowers were prepared by a strategy based on the microemulsion phase composed of water-surfactant-oil ternary systems [20]. For the typical preparation, CTAB (1.0 g), *n*-butanol (1.0 g), cyclohexane (12.0 g) and aqueous urea solution (0.4 M, 30.0 g) were mixed and stirred vigorously to form a homogeneous microemulsion solution at 25 °C. Then, TEOS (2.0 g) was dropwise dispersed into the reaction system. The obtained mixture was stirred vigorously at 25 °C for 30 min and then incubated at 70 °C for 20 h. When the reaction was completed, the products were centrifugally separated, and washed extensively with ethanol and acetone. Finally, the silica nanoflowers were dried at 60 °C overnight and then calcined at 550 °C for 5 h to remove the surfactant.

2.3.2. Preparation of PGA@nanoflowers

PGA solutions with different concentrations were obtained by diluting the initial PGA enzyme solution with PBS (50.0 mM, pH 7.0) to perform immobilization. Typically, ten milligrams of silica nanoflowers were added into 1 mL PGA solution as mentioned above. Then, the reaction mixture was incubated at 25 °C for a certain time under shaking condition. After that, the immobilized PGA was centrifugally separated and then washed with fresh PBS for several times. Before and after immobilization, enzyme contents of the solutions were measured by Bradford's microassay method. The loading amount of enzyme immobilized on the silica nanoflowers was calculated via the difference adsorption values between the original PGA amount and the residual amount of PGA in the washing solutions and supernatant.

2.3.3. Preparation of PDA-coated PGA@nanoflowers

Typically, ten milligrams of PGA@nanoflowers were dispersed in a 10.0 mL Tris-HCl buffer solution (50.0 mM, pH 8.5). After stirring for 5 min at room temperature, a certain amount of dopamine (2.0 mg/mL) was added to the above solution. The coating process was maintained for a specific time (2 h, 3 h, 4 h, 5 h, 8 h and 12 h). Correspondingly, a thin PDA coating layer was gradually formed on the surface of the PGA@nanoflowers. Subsequently, the solid products were separated by centrifugation and washed with fresh PBS for several times. Finally, the PDA-coated PGA@nanoflowers was obtained.

2.4. Concentration assay of free PGA

The concentration of PGA was measured using Bradford microassay method [35]. The protein quantification was evaluated based on a colorimetric assay of the Bradford reagent and PGA. Typically, 0.1 mL PGA solution was mixed with a 5.0 mL Bradford reagent and reacted for 5 min. Then its absorbance was measured by UV–vis spectrometer at 595 nm. Finally, the concentration of PGA in the solutions was calculated by spectrophotometric calibration line.