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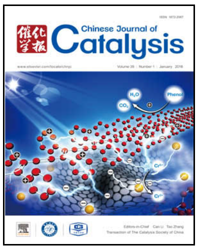

Article

Immobilization of penicillin G acylase on paramagnetic polymer microspheres with epoxy groups

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ABSTRACT

Paramagnetic polymer microspheres were synthesized by the inverse suspension polymerization method through polymerization of glycidyl methacrylate, ally glycidyl ether and methacrylamide on the surface of silica-coated Fe_3O_4 nanoparticles using *N*,*N*′-methylene-bis(acrylamide) as a cross-linking agent. Penicillin G acylase (PGA) was covalently immobilized on the surface of the paramagnetic microspheres by reacting the amino groups of the PGA molecules with the epoxy groups of the paramagnetic polymer microspheres. The effect of the SiO_2 coating and the amount of paramagnetic Fe_3O_4 nanoparticles on the initial activity and the operational stability of the immobilized PGA was investigated. The results indicated that SiO_2 played an important role in the polymerization process and paramagnetic polymer microspheres with a SiO_2-coated Fe_3O_4 nanoparticles mass content of 7.5% are an optimal support material for PGA immobilization. Immobilized PGA on the paramagnetic polymer microspheres shows a high initial activity of 430 U/g (wet) and retains 99% of its initial activity after recycling 10 times. Furthermore, immobilized PGA exhibits high thermal stability, pH stability and excellent reusability, which can be rapidly recycled by the aid of magnet.

1. Introduction

Penicillin G acylase (PGA) is an important industrial biocatalyst that is widely used for the enzymatic production of 7-aminodeacetoxycephalosporanic acid and 6-amimopenicillanic acid, which are the major pharmaceutical intermediates for producing semisynthetic β-lactam antibiotics [1,2]. However, the stability and reusability of PGA are major drawbacks that have restricted its wide industrial application [3,4]. Therefore, as an industrial biocatalyst, it is extremely urgent to immobilize PGA on solid supports to obtain recoverable and stable heterogeneous biocatalysts. Many kinds of supports have been reported for the immobilization of PGA, including acrylic resins [5], aldehyde-agarose [6], gelatin-chitosan [7], magnetic polymer beads [8], functionalized silica [9] and mesoporous silica [10–12].

The optimal support material for the immobilization of PGA should meet the following requirements: (1) A high density of

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reactive groups on the support surface for multipoint grafting of enzymes [13] and (2) a large surface area to immobilize more enzymes on the support surface [14]. In general, the surface area can be increased by decreasing the particle size of the support. However, support materials with a small particle size are difficult to separate from the reaction mixture by conventional methods. For example, GAMM support with epoxy groups can meet the first requirement mentioned above and has been used as a support for covalent immobilization of PGA and glucose isomerase [15,16]. However, with a decrease in the particle size, the reuse of GAMM support in the substrate by filtration is time-consuming and inconvenient. Magnetic separation is an attractive alternative to centrifugation or filtration. Therefore, among all supports, magnetic materials have attracted increasing attention for the immobilization of enzymes. Magnetic supports are mainly magnetic silica composites and polymer microspheres, which are prepared by encapsulating magnetic particles in a silica shell or in organic polymers [17,18]. Yang et al. [19] prepared paramagnetic aldehyde-functionalized mesostructured cellular foams (PAMCFs) by grafting 3-aminopropyltriethoxysilane-modified Fe_3O_4 (NH_2-Fe_3O_4) nanoparticles on the outer surface of aldehyde-functionalized mesostructured cellular foams (AMCFs). PGA/PAMCFs-15 showed a high initial activity of 9563 U/g and retained 89.1% of its initial activity after it was recycled 10 times. Furthermore, PGA/PAMCFs can be easily recycled by a magnetic field instead of tedious separation by high-speed centrifugation that is usually used for mesoporous materials.

Herein, we prepared paramagnetic polymer microspheres as supports for the immobilization of PGA to combine the excellent properties of polymer microspheres with the unique paramagnetic properties of magnetic nanoparticles [20]. The magnetic nanoparticles were synthesized by a coprecipitation method, and then the paramagnetic polymer microspheres were synthesized by inverse suspension polymerization with glycidyl methacrylate (GMA), ally glycidyl ether (AGE) and acrylamide in the presence of silica-coated Fe_3O_4 nanoparticles. The structure, composition and morphology of the paramagnetic polymer microspheres were characterized by Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD), vibrating sample magnetometry and nitrogen sorption. The initial activity, operational stability, thermal stability and pH stability of the immobilized PGA were investigated.

2. Experimental

2.1. Chemicals

Penicillin G acylase (520 U/mL) was purchased from Zhejiang Haider Co. Ltd., Jinhua, China. Penicillin G potassium salt was purchased from CSPS Heibei Zhongrun Pharmaceutical Co. Ltd., Shijiazhuang, China. Tetraethyl orthosilicate (TEOS) and ammonium hydroxide (25 wt%) were bought from Shanghai Lingfeng Chemical Reagent Co. Ltd., Shanghai, China. Other reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

2.2. Synthesis of paramagnetic polymer microspheres

2.2.1. Preparation of magnetic Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were prepared by a conventional co-precipitation method [21]: 0.0137 mol of $FeCl_3·6H_2O$ and 0.0075 mol of $FeSO_4·7H_2O$ were dissolved in 100 mL of deionized water in a three-neck flask under a nitrogen atmosphere and then the mixture was heated to 65 °C under mechanical stirring. Then, aqueous ammonia (25 wt%) was added dropwise into the solution until the pH value reached 11.0. After the mixture was stirred at 65 °C for 2 h, the Fe_3O_4 nanoparticles (designated as Fe_3O_4) were separated from the mixture by the aid of magnet, washed several times with deionized water until the pH decreased to 7.0 and then dried at 60 °C under vacuum for 12 h.

2.2.2. Preparation of SiO_2-coated magnetic Fe_3O_4 nanoparticles

The SiO_2-coated magnetic Fe_3O_4 nanoparticles were prepared by the Stober method [22]. 1.5 g of Fe_3O_4 nanoparticles were added into the mixture composed of 120 mL of ethanol, 30 mL of deionized water and 3.75 mL of aqueous ammonia (25 wt%). The suspension was vibrated ultrasonically and then 2.1 g of TEOS was slowly added into the suspension under continuous stirring. After stirring at room temperature for 12 h, the SiO_2-coated magnetic Fe_3O_4 nanoparticles (designated as Fe_3O_4/SiO_2) were separated by the aid of a magnet, washed with deionized water until the pH reached 7.0 and then dried at 60 °C under vacuum for 12 h.

2.2.3. Preparation of paramagnetic polymer microspheres

The paramagnetic polymer microspheres were prepared by the inverse suspension polymerization method [23]. Typically, 1.07 g of a mixture of Span-60 and Tween-20 (8:3, mass ratio) was added into 120 mL of the mixed solvent of *n*-heptane and tetrachloroethylene (3:1, volume ratio) in a glass flask. Then, 28 g of the mixture composed of Fe_3O_4/SiO_2 nanoparticles, GMA, AGE, *N,N'*-methylene-bis(acrylamide), methacrylamide and carboxamide (1:2:2:7:1:20, mass ratio) was added into the glass flask within 2 min with 0.55 g of azobisisobutyronitrile as an initiator under a nitrogen atmosphere. After the resulting mixture was reacted at 55 °C for 4 h, the produced paramagnetic polymer microspheres were separated with the aid of a magnet and washed with ethanol several times. Finally, the paramagnetic polymer microspheres were extracted with 50 mL of ethanol for 48 h and 50 mL of *n*-heptane for 48 h successively and dried at 60 °C under vacuum for 12 h.

2.3. Immobilization of PGA

0.1 g of paramagnetic polymer microspheres was added to 5 mL of PGA solution that was diluted with phosphate buffer (1 mol/L, pH = 7.5, $V_{buffer}:V_{PGA}$ = 5.25:1). The mixture was incubated in a water bath at 28 °C for 12 h at a rotating speed of 150 r/min. Then, immobilized PGA was separated with the aid of a magnet and washed with phosphate buffer (0.1 mol/L, pH = 7.5) to remove the noncovalently coupled enzymes. The wet immobilized PGA was stored at 4 °C for the subsequent testing