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Immobilization of penicillin G acylase on paramagnetic aldehyde-functionalized mesostructured cellular foams

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ABSTRACT

Paramagnetic aldehyde-functionalized mesostructured cellular foams (PAMCFs), synthesized by grafting 3-aminopropyltriethoxysilane modified Fe_3O_4 (NH_2-Fe_3O_4) nanoparticles with larger particle size than the window pore size of MCFs on the outer surface of aldehyde-functionalized mesostructured cellular foams (AMCFs), were investigated as efficient supports for immobilization of penicillin G acylase (PGA). The results show that NH_2-Fe_3O_4 nanoparticles were successfully grafted on the outer surface of AMCFs and PGA molecules were mainly immobilized covalently on the inner surface of PAMCFs, which was because amino groups of NH_2-Fe_3O_4 nanoparticles or PGA molecules reacted with aldehyde groups of AMCFs or PAMCFs to form imine bonds. PGA/PAMCFs-15 showed a rather high initial activity of 9563 U g^{-1} and retained 89.1% of its initial activity after recycled for 10 times. PGA/PAMCFs are easily recycled by magnetic field in order to replace tedious separation of high-speed centrifugation for mesoporous materials.

1. Introduction

Enzyme biocatalysts have been widely studied in the pharmaceutical and chemical industries in recent decades [1]. Penicillin G acylase (PGA, penicillin amidohydrolase, E.C.3.5.1.11, 70 Å × 50 Å × 55 Å), as an important enzyme since its discovery in 1960, is used in production of 6-aminopenicillanic acid (6-APA), 7-aminodeacetoxycephlosporanic acid (7-ADCA) or 7-aminocephalosporanic acid (7-ACA), while 6-APA is a major pharmaceutical intermediate in the semi-synthesis of β-lactam antibiotics [2]. However, the use of free enzyme has numerous drawbacks in its industrial applications, such as difficulty to recycle and separate from products, and lower stability. An important route to tackling these shortcomings is to immobilize enzyme on various supports. Therefore, enzyme immobilization has been extensively investigated. Reported works were made to immobilize PGA on solid supports, including organic and inorganic supports, such as acrylic resins [3], amberlite XAD-7 [4], aldehyde-agarose [5], gelatin [6], concanavalin A [7] and layered double hydroxides (LDH) [8]. As for inorganic supports, a variety of porous materials have been tested for their efficiency of enzyme immobilization, since they afforded high enzyme loading but often suffered a much greater diffusional limitation for enzymes and substrates with larger sizes [9–11].

Enzymes immobilized on nanoparticles are always recycled by high-speed centrifugation due to their small particle sizes. However, magnetic separation is an attractive alternative to centrifugation or filtration. Paramagnetic supports attract more and more interests owing to their easy separability from products by the external magnetic field. Magnetic supports are mainly magnetic silica composites and magnetic polymer microspheres, which are prepared by encapsulating magnetic particles in silica shell or in organic polymers [12,13]. Enzymes immobilized on magnetic mesoporous silica have been widely investigated for many potential applications such as bioseparation [14,15] and catalysis [16,17]. These immobilized enzymes combine the properties of paramagnetic nanoparticles with the outstanding textural properties of mesoporous materials. The magnetic mesoporous silica for enzyme immobilization should have good biocompatibility [18], increase the mass transfer [19], and adsorb a large amount of enzyme. Strategies based on impregnation of mesoporous materials with the magnetic precursor have been developed [18,20]. However, their magnetization was low, leading to the difficulty of magnetic separation, which is a severe shortcoming for application in the magnetic fields. Under the condition with the high saturation magnetization, the pores of mesoporous materials were

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blocked severely [21]. Other works incorporated the magnetic nanoparticles by reverse microemulsions or, as an alternative, a phase-transfer process from an organic medium to an aqueous solution prior to the encapsulation within the mesoporous silica nanospheres [22,23]. Though silica shell could protect the magnetic particles from dipolar interactions, the significant decrease in magnetization stemmed from the silica shell in the core/shell structure. The pore size of the magnetic mesoporous materials synthesized by this method was small, owing to the M41S structure of the shell, which was not suitable to immobilize large enzyme molecules, such as PGA. Therefore, it is critical to synthesize paramagnetic mesoporous materials with high magnetization and large pore size for immobilization of PGA.

Mesostructured cellular foams (MCFs), with a continuous and open 3-D pore system, large pore volume and pore size, was efficient supports to achieve high capacity for immobilization of PGA [11,24–26]. Paramagnetic iron oxide (Fe_3O_4) nanoparticles were chosen as magnetic precursor, thanks to their multifunctional properties, such as biocompatibility, low toxicity, small particle size, and superparamagnetism to an external magnetic field [27]. The major techniques for immobilization of enzyme include ionic and physical adsorption, aggregation and entrapment, and covalent bonding, in which covalent bonding can retain the enzyme activity in the operational progress and hence the immobilized enzyme has good operational stability [28].

In this work, paramagnetic aldehyde-functionalized MCFs (PAMCFs) were synthesized by grafting 3-aminopropyltriethoxysilane modified Fe_3O_4 (NH_2-Fe_3O_4) nanoparticles on the outer surface of aldehyde-functionalized MCFs (AMCFs) due to larger particle size of NH_2-Fe_3O_4 nanoparticles than the window pore size of MCFs, which decreased effect of the preparation procedure on the textural properties of PAMCFs as little as possible. PAMCFs, combining the paramagnetic properties of NH_2-Fe_3O_4 nanoparticles with the outstanding textural properties of MCFs, were efficient supports for immobilization of PGA. PGA was immobilized covalently on the inner surface of PAMCFs. Therefore the operational stability of the immobilized PGA increased greatly with little activity loss and PGA/PAMCFs could be easily recycled by the aid of an external magnetic field.

2. Materials and methods

2.1. Chemicals

P123 ($EO_{20}PO_{70}EO_{20}$, $M_w = 5800$) was purchased from Aldrich. Vinyltrimethoxysilane was purchased from WD Silicone Co. Ltd., China. Penicillin G acylase (PGA, 500 IU/mL) was purchased from Zhejiang Haider Co. Ltd., China. Penicillin G potassium salt was purchased from CSPC Zhongrun Pharmaceutical Co. Ltd., China. Other chemicals were purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd., China. All chemicals were used without further purification.

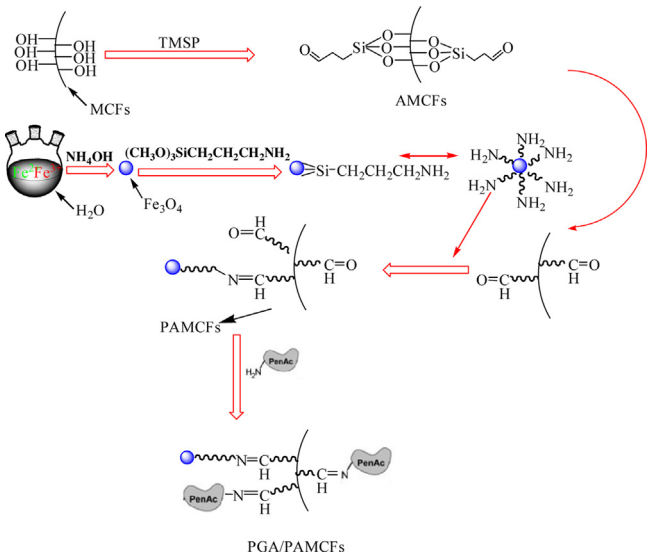
2.2. Synthesis of PAMCFs

2.2.1. Synthesis of MCFs

MCFs were synthesized as described by Patrick Schmidt-Winkel et al. [25]. Typically, P123 (2.0 g) was dissolved in 75 mL of aqueous HCl (1.6 mol L^{-1}) at 37 °C. Then NH_4F (23 mg) was added and 1,3,5-trimethylbenzene (TMB, 3.0 g) followed. After stirring for 60 min, tetraethoxylsilane (TEOS, 4.4 g) was added dropwise to the above solution. After continuous stirring for 20 h, the white cloudy mixture was kept at 100 °C for 24 h. The filtered precipitate was dried at 100 °C for 12 h and then calcined at 500 °C for 8 h in air to obtain MCFs.

2.2.2. Synthesis of trimethoxysilylpropanal (TMSP)

TMSP was synthesized by the method reported by Takeuchi et al. [29]. A solution of 7.51 g (50.6 mmol) of vinyltrimethoxysilane (VTMS), 24.6 mg (0.027 mmol) of $RhH(CO)(PPh_3)_3$ catalyst in 50 mL of benzene was placed in a 100-mL autoclave. The vessel was pressurized to 80 kg cm^{-2} ($CO/H_2 = 1/1$) and maintained at 80 °C for 3 h. The products were isolated by distillation under vacuum at 50 °C. The isomer ratio of normal to iso of TMSP was 54:46 (determined by ^1H NMR).



Scheme 1. The procedure for immobilization of penicillin G acylase on paramagnetic aldehyde-functionalized MCFs.

2.2.3. Synthesis of aldehyde-functionalized MCFs (AMCFs)

Functionalization of MCFs was carried out in toluene solution as follows: 1.00 g of MCFs and 1.07 g of TMSP were added into 50 mL of dry toluene, and heated to 110 °C for refluxing for 12 h. Then the solid was filtered and washed with ethanol excessively. Finally the solid sample was dried in vacuum at 90 °C for 12 h to obtain the white powder of AMCFs.

2.2.4. Synthesis of paramagnetic Fe_3O_4 nanoparticles modified with 3-aminopropyltriethoxysilane (NH_2-Fe_3O_4) [30]

$FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ were dissolved in distilled water and heated to 80 °C. The pH value of the solution was adjusted to about 11 by ammonia solution. The molar ratio of Fe^{3+}:Fe^{2+} was 1.8:1. The resulting mixture was aged at 80 °C for 2 h. The synthesized mixture was allowed to cool down to room temperature and washed with distilled water and ethanol and finally dried in vacuum at 60 °C for 12 h to obtain black Fe_3O_4 powder. 0.298 g of Fe_3O_4 nanoparticles were dispersed in 600 mL of ethanol with 8 mL of H_2O. Then 113 mg of 3-aminopropyltriethoxysilane (APTES) was added and stirred for 7 h. Finally the mixture was separated and dried in vacuum at 60 °C to obtain the dark powder of NH_2-Fe_3O_4.

2.2.5. Synthesis of paramagnetic aldehyde-functionalized MCFs (PAMCFs)

The APTES modified Fe_3O_4 nanoparticles were dispersed in distilled water and then were added into AMCFs suspension solution under stirring. After reacting at 30 °C for 10 h, the solvent was removed by the aid of magnet. Finally the sample was dried in vacuum at 60 °C for 12 h to obtain the dark powder of PAMCFs-*x*, where *x* represented the weight percentage of NH_2-Fe_3O_4 nanoparticles. The preparation procedure is shown in Scheme 1.

2.3. Characterization of materials

Small-angle X-ray scattering (SAXS) measurement was recorded on a PANalytical Empyrean X-ray scattering system. The powder X-ray diffraction (XRD) measurement was carried out on a Bruker AXS D8 Focus X-ray diffractometer operated at 40 kV, 40 mA (Cu Kα radiation, λ = 0.15406 nm), and the diffraction patterns were taken in the range of $10° < 2\theta < 80°$ at the scanning rate of 6° min^{-1}. Nitrogen adsorption–desorption isotherms were measured on a Micromeritics ASAP 2020M surface area and porosity analyzer at 77 K. Pore size distribution was calculated by the Barrett–Joyner–Halenda (BJH) method. The Brauner–Emmet–Teller (BET) surface area was calculated using experimental points at a relative pressure of $P/P_0 = 0.05–0.20$. The total pore volume was calculated by N_2 amount adsorbed at the highest P/P_0 ($P/P_0 \approx 0.99$). Transmission electron microscopy (TEM) images were obtained from a TECNAI 20S-TWIN microscope. All samples were ultrasonically dispersed in the ethanol solvent and then dried over a copper grid coated with carbon film. Fourier transformed infrared spectroscopy (FT-IR) were conducted using a Nicolet Nexus 670 spectrometer in the range of 400–4000 cm^{-1}. The samples were homogenized with dry KBr and then made into thin wafers. Thermogravimetric (TG) was carried out on a Perkin-Elmer Pyris Diamond TG-DTA analyzer, in which the sample was heated programmedly from 40 to 600 °C at the rate of 10 °C min^{-1} in the air atmosphere of 50 mL min^{-1}. The analysis of C, H and N elements was determined by Elementear Vario EL III elementary analyzer. The analysis of Fe element was performed by inductively coupled-plasma atomic emission spectroscopy (ICP-AES) using a TJA IRIS ADVANTAG 1000 instrument. ^1H NMR spectra were recorded