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Aldehydepropyl-functionalized mesostructured cellular foams: Efficient supports for immobilization of penicillin G acylase

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ABSTRACT

The aldehydepropyl-functionalized mesostructured cellular foams (CHO-MCFs) were prepared by post-synthetical functionalization of MCFs with trimethoxysilylpropanal (TMSP), and used as efficient supports for immobilization of penicillin G acylase (PGA). The physicochemical properties of CHO-MCFs were characterized by SAXS, nitrogen sorption, TEM, elementary analysis, solid state ^{29}Si MAS NMR, FT-IR spectroscopy and thermogravimetry. The results show that the aldehydepropyl groups have been grafted successfully on the surface of MCFs, and after functionalization, the BET surface area and pore volume of CHO-MCFs decrease, but the ultra-large and continuous 3D mesoporous structure of CHO-MCFs are retained to be beneficial for immobilization of PGA with large size and diffusion of substrates and products. PGA is immobilized covalently on CHO-MCFs via the reaction to produce Schiff's base between the free amino groups of lysine residues of PGA and the aldehyde groups on the surface of CHO-MCFs, which greatly increases the operational stability of the immobilized PGA with little activity loss due to the short-chain groups of aldehydepropyl grafted on the surface of CHO-MCFs. PGA/CHO-MCFs-10 shows the immobilization yield of 57.6%, the specific activity of 22.2 U/mg and the initial enzymatic activity of 8895 U/g, and retains 93.0% of its initial enzymatic activity after recycled for 10 times.

1. Introduction

Penicillin G acylase [1] (PGA, EC 3.5.1.11, 7.0 nm × 5.0 nm × 5.5 nm) is the key enzyme used in the pharmaceutical industry for production of β-lactam antibiotics, because it can catalyze the hydrolysis of penicillin G potassium salt to produce 6-aminopenicillanic acid (6-APA), which is an intermediate for synthesis of several semisynthetic β-lactam antibiotics. Unfortunately, free PGA's low thermal and solvent stability, impossibility of reusability and difficult purification of products inhibit the wide industrial applications. However, these disadvantages can be overcome by immobilization of free enzymes on various solid materials, which provides many distinct advantages including enhanced stability, easy separation from reaction mixtures and then recycle, possible modulation of catalytic performances, effective prevention of enzyme contamination in the products, and easier prevention of microbial growth [2]. Immobilization of enzyme was first reported in 1916, in which invertase immobilized on charcoal was found to retain the same activity as the native enzyme [3].

Mesoporous silicas are efficient supports for enzyme immobilization because of their high surface area and pore volume, tunable and uniform pore size, high chemical and mechanical stability [4–7]. In addition, they are environmentally acceptable, non-toxic and highly resistant against microbial attacks and organic solvents [8]. Since mesoporous silicas were first explored for enzyme immobilization in 1996 [9], various mesoporous silicas, such as MCM-41 [10,11], MCM-48 [11], SBA-15 [12–15], KIT-6 [16,17], and mesostructured cellular foams (MCFs) [14,18–20], have been studied as supports for immobilization of PGA. Among them, MCFs are composed of uniformly sized, large spherical cells which are interconnected by uniform windows to create a continuous 3D pore system [21], leading to the large pore size and pore volume. This special mesostructure is favorable to immobilize more enzyme molecules with large size to achieve high activity. Moreover, the catalytic hydrolysis of penicillin G potassium salt by PGA is a fast reaction, and the diffusions of the substrates and products are the rate-determining steps [22–25], so MCFs with large pore size and 3D mesostructure are favorable to reduce the diffusion resistance to increase the activity.

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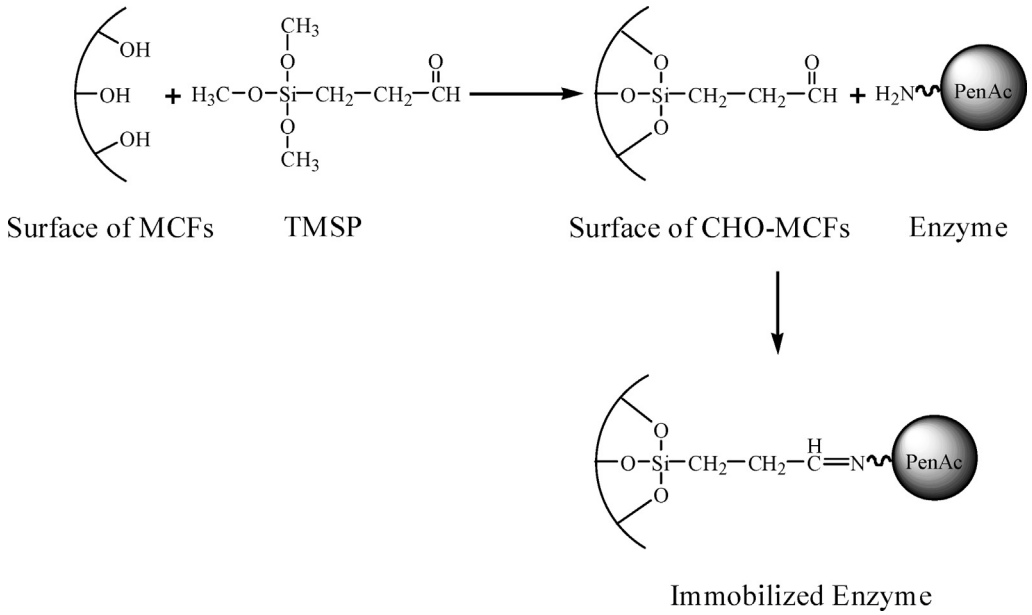

Scheme 1. The procedure for surface functionalization of MCFs and covalent immobilization of PGA.

The interaction between enzyme and mesoporous silicas is an important factor as it affects the amount of enzyme immobilized on mesoporous silicas, as well as the activity and operational stability of the immobilized enzyme in industrial applications. It is noted that the interaction between enzyme and pure mesoporous silicas is physical adsorption, which is too weak to keep enzyme immobilized under the reaction conditions of high concentrations of substrate and product and high ionic strength. The surface functionalization of mesoporous silicas with certain organic groups can enhance the interaction between enzyme and mesoporous silicas via covalent bonding and thus improve the performances of the immobilized PGA. Covalent immobilization can improve the activity, stability and selectivity of the immobilized enzymes [26]. Some researchers used a cross-linking agent, such as glutaraldehyde, to connect amino-modified silicas and amino groups of enzyme via covalent bonding [27–31]. Cubic *Ia3d* mesoporous silica functionalized with glycidoxypropyl groups was synthesized and used as the support for covalent immobilization of PGA. This immobilized PGA behaved higher activity and better operational stability than that via the physical adsorption [16]. PGA immobilized covalently on epoxy-functionalized MCFs exhibited high operational stability compared with that immobilized on pure MCFs [18]. On the other hand, the properties of organic functional groups grafted on the surface of mesoporous silicas affect the interaction between enzyme and functionalized mesoporous silicas. Surface functionalized SBA-15 with thiol (–SH), phenyl ($-C_6H_5$), vinyl (–C=C), amine ($-NH_2$), and carboxyl (–COOH) groups were used to immobilize PGA, in which the enzymatic activity of the immobilized PGA varied from 52.2 to 167.5 U/g of solid [12].

The mesoporous silicas should be modified by γ-aminopropyltriethoxysilane before cross-linking with glutaraldehyde. Glutaraldehyde's method consists of two steps, namely, grafting by γ-aminopropyltriethoxysilane and then cross-linking with glutaraldehyde. To the best of our knowledge, mesoporous materials directly functionalized with aldehydepropyl groups have not yet been reported. In this paper, trimethoxysilylpropanal (TMSP) was used to functionalize MCFs, providing a lot of free aldehyde groups on the surface of aldehydepropyl-functionalized MCFs (CHO-MCFs), which covalently immobilized PGA via Schiff's base formation through the reaction between the free amino groups of lysine residues of PGA and the aldehyde groups on the surface of CHO-MCFs (Scheme 1). Compared with the long-chain organic groups obtained from glutaraldehyde's method with two steps, the short-chain groups of aldehydepropyl grafted on the surface of mesoporous materials can effectively decrease effect of the preparation procedure on the textural properties of the parent mesoporous materials as little as possible, which greatly increases the operational stability of the immobilized PGA with little activity loss.

2. Experimental

2.1. Chemicals

Non-ionic triblock copolymer $EO_{20}PO_{70}EO_{20}$ (MW = 5800) was purchased from Aldrich. Penicillin G acylase (PGA, 29.0 mg/mL) was purchased from Zhejiang Hiader Co. Ltd., China. Penicillin G potassium salt was purchased from Shandong Lukang Pharmaceutical Co. Ltd., China. NaOH aqueous solution and phosphate buffers were prepared according to the reference [11]. Benzene and toluene were refluxed with sodium to dehydration, and all other chemicals were analytical grade and used without further purification.

2.2. Synthesis of Trimethoxysilylpropanal

Trimethoxysilylpropanal (TMSP) was synthesized by the method similar to that reported by Takeuchi and Sato [32]. A mixture of 30 mL of benzene, 4.45 g of vinyltrimethoxysilane (30 mmol) and 28 mg of $RhH(CO)(PPh_3)_3$ (0.03 mmol) was placed in a 100 mL autoclave, then the vessel was pressurized to 8.0 MPa (CO/H_2 = 1/1) and maintained at 80 °C for 3 h under stirring. The products were isolated by distillation under vacuum at 50 °C.

2.3. Synthesis of MCFs

MCFs were synthesized as described in the reference [21]. In a typical preparation method, 2.0 g of P123 was dissolved in 75 mL of 1.6 mol/L HCl aqueous solution. Then 23 mg of NH_4F and 3.0 g of 1,3,5-trimethylbenzene (TMB) were added, and the mixture was