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Functionalized periodic mesoporous organosilicas for selective adsorption of proteins

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ABSTRACT

The periodic mesoporous organosilicas (PMO) with an organobridged (—CH_2—) was synthesized and functionalized with amino or carboxylic groups by post-synthesis methods. The functionalized PMO by changing the hydrophilic/hydrophobic property and the net charge could be used to selectively adsorb and purify proteins with different shapes and different isoelectric points (pI). The experimental result showed that Bovine serum albumin (BSA) was adsorbed quicker than hemoglobin (Hb) on the materials, and lysozyme (Lys) could not be adsorbed on these PMO materials at all. The adsorption capacity of amino groups modified PMO (PMO-$(NH_2)_2$) for BSA was 44.67 mg/g and 300.0 mg/gfor Hb on carboxylic groups modified PMO (PMO-$(COOH)_2$). The adsorption behavior of proteins was affected strongly by the interaction among different constituents in the mixture of proteins. In addition, it is found that the adsorption rate of (PMO-$(NH_2)_2$ for adsorption of proteins was much slower than PMO-$(COOH)_2$.

1. Introduction

Mesoporous silica nanoparticles (MSNs) were discovered in early 1990s by the scientists at Mobil Oil Company and by Kuroda and co-workers [1–3]. Periodic mesoporous organosilicas (PMO) containing organic groups in their walls, reported in 1999 by three different groups led by Ozin, Inagaki and Stein, respectively, is one kind of MSNs [4–6]. Unlike the other kinds of MSNs, the surface properties (hydrophilicity/hydrophobicity, interaction with guest molecules, etc.) of PMOs can be facilely adjusted through incorporation of different organic group in the mesoporous wall [7]. This kind of silica-based materials by hybridization of inorganic and organic part is assembled by the bridged silsesquioxane precursors $(R'O)_3Si\text{-}R\text{-}Si(OR')$ (R′: methyl or ethyl. R: organic bridging group). A wide variety of organic moieties R are available for controlling the structure and property of PMO [8] such as short aliphatic groups (methyl, ethyl and vinyl) or rigid aromatic (phenylene, thiophene) groups [9]. PMO own high-specific surface areas, large pore volumes and narrow distributions of pore diameter, which is promising for a wide range of applications such as catalysis and adsorption [10].

Co-condensation and post-synthesis grafting as two different methods were used to functionalize MSNs. The main disadvantages of the co-condensation (one-pot synthesis) are the limited achievable density of functional groups and the totally disordered products in extreme cases. On the contrary, post-synthesis grafting method could keep the mesostructure of the starting silica phase [11]. Grafting various functional groups brings further surface functionalities to enlarge its application scope because the terminal functional groups exposed could offer the active sites more easily for adsorption and so on. Furthermore, grafting suitable groups increase also the stability and biocompatibility of the materials [12]. Ha and co-worker [13] studied the adsorption of lysozyme onto SBA-15 and PMO materials owning the functional groups of bis[3-(trimethoxysilyl)propyl] amine (BTMS-amine), 1,4-bis(triethoxysilyl)benzene (BTES-benzene) and 4-bis(triethoxysilyl) biphenyl (BTES-biphenyl). Wan et al. [14] reported that PMO materials had superior enrichment properties for BSA and myoglobin compared to SBA-15 materials with similar mesostructural parameters. Ethylene-bridged PMO was used for protein refolding by taking advantage of the unique surface and pore characteristics [15]. Recently, horseradish peroxidase (HRP) was immobilized on PMO [16], which not only increased the enzyme activity and stability but also could be reusable for many cycles.

Amino groups are one of the most widely modified functional groups used to the enrichment, separation and immobilization of proteins. The carboxylic group also has a wide range of potential applications because of its negative charge, high polarity [17,18] and superior cation-exchange capacity, which could serve as anchoring sites for biomolecules (such as enzymes, antibodies and other proteins) and polypeptide synthesis [19,20].

The interaction between proteins and MSNs is accomplished by three main ways: physical adsorption, encapsulation and

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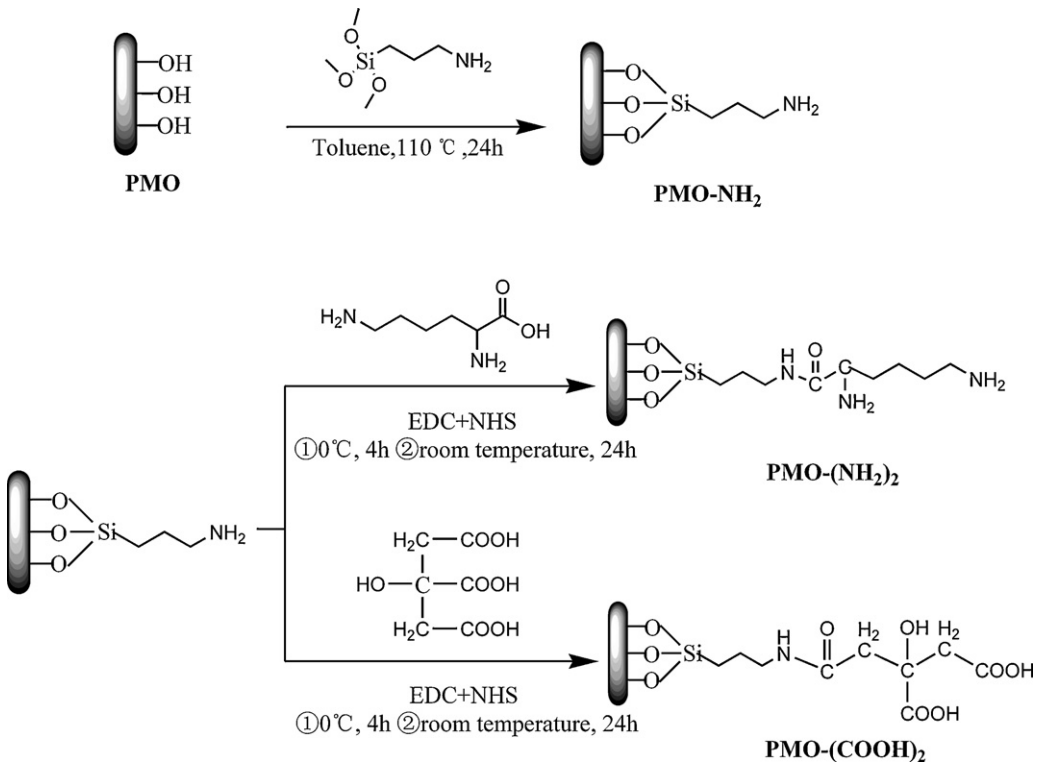

Fig. 1. Preparation procedures of PMO materials.

chemical binding. Many studies have highlighted that Pore size of the material is one of the most important factors affecting selective adsorption proteins [12]. The pH of buffer solutions [21–24] is another significant factor affecting the protein adsorbing behavior on the surface of MSNs. Many studies have shown that the maximum adsorption of protein occurs at or near the isoelectric (pI) point of the protein [25,26]. Other factors affect the interaction including the adsorption temperature [27], the material properties [28–31] and the properties of proteins.

Until now there have been few reports using PMO for protein selective adsorption, and the information on the interaction between PMO and proteins need to be further studied. Encouraged by Wan and co-workers' work [14], amino (PMO-$(NH_2)_2$) and carboxyl group functionalized PMO (PMO-$(COOH)_2$) were synthesized by post-synthesis grafting method. Then the PMO materials were characterized and used as sorbents to investigate the adsorption behavior of proteins with different pI.

2. Experimental

2.1. Chemicals

P123 triblock copolymer [poly(ethylene oxide)-block-poly(propylene oxide) block-poly(ethylene oxide), EO20-PO70-EO20, M_{av} = 5800], bovine serum albumin (BSA), hemoglobin (Hb) and lysozyme (Lys) were obtained from Sigma–Aldrich (Vienna, Austria) and used directly. 3-Aminopropyltriethoxysilane (APTS) was purchased from Alfa Aesar (Karlaruhe, Germany). 1,2-Bis-(trimethoxysilyl)ethane (BTME, 96%) was obtained from Nanjing Capatue Chemical Co., Ltd (Nanjing, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and N-hydroxy succinimide (NHS) were purchased from Aladdin Chemistry Co., Ltd (Beijing, China). The double-distilled water was used throughout the experiments.

2.2. Synthesis of materials

In a typical synthesis of PMO material [32,33], one gram of P123 and 2.98 g (1.0 mol/L) of KCl were dissolved in 40 g of 0.167 mol/L HCl at 40 °C. Then 1.40 g of BTME was added to the solution under stirring. The final reactant molar composition used was P123: KCl: HCl: H_2O: BTME = 0.035:8:1.34:444:1. After being stirred for 10 min, the solution was kept in static condition at 40 °C for 24 h. Then the mixture was transferred into an autoclave and heated at 100 °C for another 24 h. The white precipitates was collected by filtration and washed by deionized water and extracted by refluxing with ethanol for 8 h to remove the templates. The extraction procedure was repeated for three times to get PMO.

2.3. Modification of PMO

As reported in the literature [34], two hundreds of milligrams of PMO materials were first dried and degassed at 110 °C, and then dispersed in 30 mL of dry toluene. An excess of APTS (2 mL) was added under stirring and refluxed for 24 h at 110 °C. The resulting solid was filtered and washed by toluene, dichloromethane and ethanol for three times, respectively. The intermediate products PMO-NH_2 were obtained through drying at 70 °C.

Eighty milligrams of the intermediate products PMO-NH_2 was suspended in 30 mL of PBS, then 65 mg of lysine or 90 mg of citric acid was added. The reaction was activated by adding 100 mg of NHS and 150 mg of EDC·HCl and accomplished by stirring at 0 °C for 4 h and at room temperature for 24 h in sequence. Finally, the powder of PMO-$(NH_2)_2$ or PMO-$(COOH)_2$ was collected by filtration, washed with ethanol for three times, and followed by drying in vacuum. The preparation of PMO and modified PMO were shown in Fig. 1.