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Enzyme-containing silica inverse opals prepared by using water-soluble colloidal crystal templates: Characterization and application

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ABSTRACT

Enzyme-containing silica inverse opals are prepared for the first time by using water-soluble polyacrylamide (PAM) colloidal crystals as templates. Glucose oxidase-containing silica inverse opals (GOD@SIOs) are firstly fabricated and characterized by SEM, TEM, CLSM, and N_2 adsorption-desorption. Compared with free GOD, the GOD@SIOs are more stable against extreme pH, heat, and chemical denaturants. GOD@SIOs can be used in fast and sensitive visual detection of glucose. In order to verify that this method is suitable for various enzymes, organophosphorus hydrolase (OPH)-containing silica inverse opals are also prepared and successfully applied as sensors for visual and spectrometric detection of organophosphorus based compounds. These enzyme-containing silica inverse opals offer a novel approach to in situ immobilize biomolecules.

1. Introduction

Inverse opals or three-dimensionally ordered macroporous (3DOM) materials, featuring open and interconnected macropore structure, tunable pore sizes, and nanosized wall components, hold great promise in catalysis, photonic devices, sensors, separation processes, and other potential applications [1–5]. The general processes for fabrication of inverse opals involve the preparation of colloidal crystals templates (CCTs), the fluid chemical precursor infiltration of the void space among spheres, the conversion from the precursor into a solid skeleton, and the removal of the CCTs by thermal treatment, solvent extraction, or chemical etching [6]. The most commonly used CCTs for synthesizing inverse opals materials are composed of monodisperse silica or polymer spheres. However, the removal of the templates usually requires harsh conditions, i.e. treatment with hydrofluoric acid (toxic and caustic) or hot alkali solution for silica and calcination, pyrolysis, or organic solvent extraction for polymer spheres [6], which significantly devalues their practical applications in many fields. For example, when the inverse opals materials contain bioactive substance (i.e. proteins, enzymes, DNA), these conventional template removal processes are lethal to the bioactivity of the biomolecules. Thus, it is of great importance to find a new material which could be easily removed through an environmental-friendly way for CCTs fabrication. Polyacrylamide (PAM), a water-soluble polymer which is biodegradable and does not persist or accumulate in the environment, has been recognized as useful in terms of technological applications and scientific investigations [7,8]. Using the PAM CCTs to obtain inverse opals materials is generally benign to human health and environment. However, to the best of our knowledge, there is no report of preparing inverse opals materials using PAM colloidal crystals as template.

Enzymes, which display high chemo-, regio-, and enantioselectivity, can catalyze a variety of chemical reactions under mild and environmentally benign conditions. In spite of these excellent properties, improvement of the intrinsic properties and extra stabilities of the enzymes should be achieved before their use

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Nomenclatures

PAM	Polyacrylamide
3DOM	Three-dimensionally ordered macroporous
CCTs	Colloidal crystals templates
GOD	Glucose oxidase
OPH	Organophosphorus hydrolase
HRP	Horseradish peroxidase
GOD@SIOs	GOD-containing silica inverse opals
OPH@SIOs	OPH-containing silica inverse opals
GOD@SiO_2	GOD entrapped in conventional sol-gel silica
LOD	The limit of detection
PBS	Phosphate buffer solution
SEM	Scanning electron microscopy
TEM	Transmission electron micrographs
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CLSM	Confocal laser scanning microscopy
EDS	Energy dispersive spectroscopy

as industrial biocatalysts. Immobilization of enzymes is a very important method for the improvement of their stability, activity, selectivity, and resistance to inhibitors [9–13]. Entrapping enzymes in silica matrixes has emerged as a promising platform for improving the stability and realizing the recovery and reuse of the enzymes [14,15]. In order to overcome the difficulty of the diffusional restriction, porous sol-gel silica with micro-, *meso*-, or macroporous structures has gained particular attention over the past decades for entrapping enzymes [16–18]. In our previous reports, we have immobilized enzymes on 3DOM silica materials [19,20]. 3DOM materials should be prepared firstly, and then the enzymes can be immobilized on 3DOM supports through physical adsorption, cross linking or covalent binding. The process of physical adsorption is easy to perform but possesses the concomitant problem of enzyme leaching from the support. Covalent linkage of enzymes to the 3DOM support can obviate this disadvantage but may result in significantly reduced activity. Also, poly(norepinephrine) coating of the 3DOM support might block off the interconnected pores and result in enhanced mass transfer limitations [19]. Cross-linked enzyme aggregates (CLEAs) in 3DOM supports can improve the thermal and mechanical stability but result in the poor activity recovery of enzyme [20]. However, it should be noted that in situ encapsulation of enzyme in 3DOM sol-gel silica or inverse opals during the preparation process has never been reported. In situ encapsulation enzymes in 3DOM support could maintain the activities without any other agents making them inactive during immobilization process. In addition, functionalization or modification of the 3DOM support is avoided. Thus, in the present study, enzyme-containing silica precursor was infiltrated into the interstitial voids between spheres of PAM CCTs. After gelling and aging processes, the template was removed via phosphate buffer solution (PBS) treatment, and then enzyme-containing inverse opals were obtained (the preparation process was depicted in Scheme 1). Compared with traditional sol-gel silica monolith or particle for enzyme immobilization, the inverse opals possess open and interconnected macropore structure and nanosized walls which can considerably favor the diffusion of the substrates, and thus facilitate their accessibility to the enzymes active sites. What's more, the fabrication process is mild and compatible to biomolecules. Thus, it is reasonable to believe that this method can be a general platform for preparation of bioactive inverse opals.

Glucose oxidase (GOD, β-D-glucose:oxygen-1-oxidoreductase, E.C. 1.1.3.4) has a dimeric structure and can catalyze the oxidation of β-D-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide (H_2O_2). For decades, GOD is widely used in enzymatic glucose sensors due to its high affinity and selectivity for glucose [21–23]. Organophosphorus hydrolase (OPH, E.C. 3.1.8.1) is a homodimeric enzyme that can hydrolyze abundance of organophosphorus compounds by producing less toxic products such as *p*-nitrophenol and diethyl phosphate [24–26]. In this study, GOD and OPH were chosen as model enzymes for preparing enzyme-containing silica inverse opals and then used for detection of glucose and methyl parathion, respectively.

2. Materials and methods

2.1. Materials

Glucose oxidase (GOD, E.C. 1.1.3.4, ≥100 U/mg) was purchased from Shanghai Sanjie Biological Technology Co., Ltd. (Shanghai, China). Horseradish peroxidase (HRP, E.C. 1.11.1.7, ≥150 U/mg) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Organophosphorus hydrolase (OPH, E.C. 3.1.8.1) was purchased from Schengenbiya Bioengineering Technology Co., Ltd. (Beijing, China). All other reagents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of polyacrylamide microspheres and colloidal crystals templates

Monodisperse polyacrylamide microspheres were prepared through a dispersion polymerization approach. For a typical process, acrylamide (AM, 10.16 g) and polyvinylpyrrolidone (PVP K30, 6.10 g) were dissolved into a mixture of distilled water (30 ml) and anhydrous ethanol (70 ml). The obtained solution was transferred into a three-necked flask equipped with a mechanical stirrer and argon inlet. After sealing in an argon atmosphere, the mixture reacted at 75 °C under stirring until the system was homogeneous. Then, azodiisobutyronitrile (AIBN, 0.06 g) as an initiator was added into the mixture with a stirring speed of 60–80 r/min at 75 °C. After 7 h of reaction, the suspension was centrifugalized at 845*g* for 2 h to separate the PAM microspheres, and then washed with fresh anhydrous ethanol for three times. The resulted PAM microspheres were dispersed into anhydrous ethanol and centrifugalized at 845*g*. Finally, the PAM colloid crystal template was obtained by drying in desiccator at room temperature for two weeks.

2.2.2. Fabrication of enzyme-containing silica inverse opals

The fabrication of enzyme-containing silica inverse opal was based on the sol-gel technique. The sol was obtained by mixing 4.5 ml of tetraethyl orthosilicate (TEOS), 0.25 ml of HCl (0.1 M), and 1.4 ml of deionized water. Then, the mixture was suffered from sonication to obtain a homogeneous sol. 3 ml of the obtained sol was mixed with 0.21 ml of enzyme solution to produce enzyme-containing sol. Several pieces of PAM CCTs were immersed into the enzyme-containg sol under vacuum. CCTs were taken out and the sol on the surface was wiped. Then the CCTs were transfered into a filter flask and kept in vacuum for 12 h at room temperature to remove the ethanol generated during the reactions. Dried material was washed several times with PBS to remove the PAM template. Finally, enzyme-containing silica inverse opals were obtained. Adsorbed enzyme was prepared by incubating 3DOM materials into enzyme solution for 2 h. Then adsorbed enzyme was washed by PBS for 3 times.

2.2.3. Enzymatic activity assay

The enzymatic activity assay was performed as follows: glucose solution (1.5 ml, 13%) and PBS (1.5 ml, 0.1 M, pH 7.0) containing HRP