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A novel electrochemical chiral interface based on sandwich-structured molecularly imprinted SiO_2/AuNPs/SiO_2 for enantioselective recognition of cysteine isomers

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ABSTRACT

SiO_2 nanospheres were synthesized by the sol–gel method using tetraethoxysilane (TEOS) as the precursor, which was then modified with 3-aminopropyltriethoxysilane (APTES) for the grafting of $-NH_2$ to the SiO_2 surface. Au nanoparticles (AuNPs) were anchored to the $-NH_2$ grafted SiO_2, and then L-cysteine (L-Cys) templates were introduced to the SiO_2/AuNPs through forming Au—S bond. Another layer of SiO_2 was then coated on the L-Cys doped SiO_2/AuNPs as the coating layer, and then the templates were removed by calcination to form a sandwich-structured molecularly imprinted SiO_2/AuNPs/SiO_2. Finally, the molecularly imprinted chiral interface was applied for electrochemical enantioselective recognition of Cys isomers, and discernible differences in the current signals could be observed on the differential pulse voltammograms ($I_{D\text{-cys}}/I_{L\text{-cys}}$, 2.36).

1. Introduction

Amino acids are inarguably among the most important and predominant chiral compounds in nature [1]. Among the chiral amino acids, L-cysteine (L-Cys) plays a key role in the biological activity of proteins whereas D-Cys may produce non-negligible cytotoxicity [2], and thus it is of great importance to effectively discriminate Cys isomers. Among the reported methods for recognition of chiral amino acids, electrochemistry has shown great potential [3–5], because it can convert chiral recognition events into discernible changes in electrochemical signals. Although Raman spectrometry [6] and colorimetry [7] have been applied for recognition of L/D-Cys, little attention has been paid to the enantioselective recognition of Cys isomers via electrochemical methods.

The past decades have witnessed the applications of molecular imprinting technique (MIT) in chiral recognition [8], and the enantioselective recognition of chiral amino acids and other chiral compounds by combining MIT and electrochemistry has been reported by several research groups [9–14]. However, the commonly used matrixes in MIT are polymers, which are often suffered from the collapse of imprinted cavity during the removal of template molecules under harsh conditions. Therefore, the design of structurally rigid materials for MIT-based chiral recognition still remains as an urgent and challenging task.

Herein, a novel electrochemical chiral interface based on sandwich-structured molecularly imprinted SiO_2/AuNPs/SiO_2 is constructed, which is then used for the recognition of Cys isomers. Since AuNPs have a high affinity for Cys via forming Au—S bond, the templates of L-Cys can be stably combined with the AuNPs at the chiral interface; meanwhile, the high mechanical strength of SiO_2 in the hybrids is favorable for the preservation of the complementary cavities after the L-Cys templates are removed. Therefore, electrochemical chiral recognition of Cys isomers can be achieved at the molecularly imprinted SiO_2/AuNPs/SiO_2 chiral interface.

2. Experimental

2.1. Reagents and apparatus

L/D-Cys, tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES) were purchased from Aladdin Chemistry Co., Ltd. (China). Other chemicals not mentioned were received from Sinopharm Chemical Reagent Co. (SCRC, China). All solutions were prepared with ultrapure water (Milli-Q, Millipore).

FT-IR spectra were measured by a FTIR-8400S spectrometer (Shimadzu, Japan). XRD analysis was carried out on a Rigaku D/max 2500PC diffractometer. SEM and TEM images were recorded on a Supra55 field-emission scanning electron microscope (Zeiss, Germany) and a JEM 2100 transmission electron microscope (JEOL, Japan),

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respectively. The UV–vis spectra were measured by a U-3900 UV–vis spectrophotometer (Hitachi, Japan). All electrochemical measurements were performed on a CHI 660D electrochemical workstation in a conventional three-electrode cell.

2.2. Synthesis of L-Cys doped SiO_2/AuNPs

SiO_2 nanospheres were synthesized by the sol–gel method using TEOS as the precursor [15], and then 400 mg of the SiO_2 nanospheres were dispersed into 20 mL of isopropanol. Next, 0.1 mL of APTES was added to the dispersion, and the mixture was heated to 85 °C and stirred for 2 h. The products were centrifuged, washed and lyophilized at − 40 °C, and $-NH_2$ grafted SiO_2 was obtained.

The $-NH_2$ grafted SiO_2 was dispersed in 40 mL ethanol, and then the dispersion (pH 5.8) was added dropwise to 10 mL AuNPs solution prepared according to the method previously reported [16]. Due to the electrostatic attractions between negatively charged AuNPs and the protonated $-NH_2$ [17], AuNPs were anchored to the $-NH_2$ grafted SiO_2. Next, 0.5 g of polyvinylpyrrolidone (PVP) stabilizer was added to the solution. After being stirred overnight and centrifuged, the obtained SiO_2/AuNPs was dispersed in 10 mL water, and the dispersion was added to 1 mL L-Cys aqueous solution (4 mM). L-Cys templates were doped into the SiO_2/AuNPs through forming Au$-$S bond. After reaction for 12 h, the L-Cys doped SiO_2/AuNPs were collected by centrifugation and lyophilized at − 40 °C.

2.3. Preparation of sandwich-structured molecularly imprinted SiO_2/AuNPs/SiO_2

50 mg of the L-Cys doped SiO_2/AuNPs was dispersed in the mixture of water (57 mL), ethanol (26 mL) and ammonia (1.5 mL), and then 1.5 mL TEOS dissolved in ethanol (3.6 mL) was added dropwise to the mixture. After continuous stirring for 18 h, the formed sandwich-structured SiO_2/AuNPs/SiO_2 was collected, thoroughly washed and dried. The SiO_2/AuNPs/SiO_2 was then calcined at 500 °C for 2 h to remove the L-Cys templates, and therefore molecularly imprinted SiO_2/AuNPs/SiO_2 was obtained. The whole process illustrating the preparation of molecularly imprinted SiO_2/AuNPs/SiO_2 is shown in Fig. 1A. For the control experiment, non-imprinted SiO_2/AuNPs/SiO_2 was also prepared by the same procedures without the addition of L-Cys templates.

2.4. Electrochemical recognition of Cys isomers

100 mg of the imprinted SiO_2/AuNPs/SiO_2 or non-imprinted SiO_2/AuNPs/SiO_2 was dispersed in 0.3 mL water, and then 40 μL of the dispersion was dropped onto a piece of indium tin oxide (ITO, 4 × 2 cm) and left to be air dried.

The molecularly imprinted SiO_2/AuNPs/SiO_2 coated ITO (MISiO_2/ITO) was immerged into 25 mL of 0.1 M PBS containing 1 mM L- or D-Cys (pH = 7.0), and then a positive potential of 0.25 V was exerted to the imprinted electrode for 1000 s for the rebinding of L/D-Cys (isoelectric point of Cys, 5.05 [18]). Next, the residual L/D-Cys in the solution was determined by differential pulse voltammetry (DPV) using glassy carbon electrode (GCE) as the working electrode at a scan rate of 8 $mV\ s^{-1}$, and the peak currents ratio was calculated to assess the recognition ability of the MISiO_2/ITO.

3. Results and discussion

3.1. Characterization of imprinted SiO_2/AuNPs/SiO_2

Fig. 1B shows the FT-IR spectra of L-Cys, L-Cys doped SiO_2/AuNPs/SiO_2 and imprinted SiO_2/AuNPs/SiO_2. For L-Cys (curve a), the peaks at 2552, 2081 and 692 cm^{-1} are attributed to S$-$H stretching, C$-$O stretching and C$-$S bending, respectively [19], and the characteristic peaks of L-Cys still exist on the L-Cys doped SiO_2/AuNPs/SiO_2 (curve b), suggesting that L-Cys templates are doped into the matrix with the aid of AuNPs. The L-Cys templates can be removed through calcination, since the characteristic peaks of L-Cys disappear completely on the spectra of imprinted SiO_2/AuNPs/SiO_2 (curve c).

The XRD patterns of SiO_2, SiO_2/AuNPs and imprinted SiO_2/AuNPs/SiO_2 are shown in Fig. 1C. The typical peak centered at 23° is characteristic of SiO_2 (curve a), and the peaks at around 38.2°, 44.5°, 64.7° and 77.6° can be assigned to the (111), (200), (220) and (311) crystalline planes of Au (JCPDS 1-1172, curve b) [20]. The dominant crystal growth plane is (111), implying the crystalline structure of SiO_2/AuNPs is face centered cubic [20]. Noted that all the diffraction peaks of Au and SiO_2 can be found on the imprinted SiO_2/AuNPs/SiO_2 (curve c), indicating that the crystalline structure of SiO_2/AuNPs is not altered after calcination.

The UV–vis spectra of AuNPs, SiO_2/AuNPs and imprinted SiO_2/AuNPs/SiO_2 are shown in Fig. 1D. The AuNPs show a sharp surface plasmon resonance (SPR) absorption at 520 nm, which is due to the collective oscillation of free electrons in colloid AuNPs [21]. The characteristic band of AuNPs can still be observed on the spectra of SiO_2/AuNPs and imprinted SiO_2/AuNPs/SiO_2 with a slight red-shift (525 nm), suggesting successful incorporation of AuNPs to the $-NH_2$ grafted SiO_2 and stable existence of AuNPs even after calcination at 500 °C. This slight red-shift could be ascribed to the increase in the local refractive index of the SiO_2 shell surrounding the AuNPs [22].

The FESEM images of SiO_2, SiO_2/AuNPs, L-Cys doped SiO_2/AuNPs/SiO_2 and imprinted SiO_2/AuNPs/SiO_2 are recorded. The spherical SiO_2 nanoparticles prepared by the classical Stöber method [15] show an average diameter of 320 nm (Fig. 2A). After the AuNPs are grafted to the silica nanospheres, a slight aggregation of the formed SiO_2/AuNPs is observed (Fig. 2B), which could be attributed to the large surface van der Waals force among the particles of AuNPs [23]. After another layer of SiO_2 is coated on the SiO_2/AuNPs via TEOS hydrolysis, the aggregation is attenuated and the size of the sandwich-structured hybrids (SiO_2/AuNPs/SiO_2) is increased to 460 nm (Fig. 2C), indicating the thickness of the SiO_2 outlayer is around 70 nm. After calcination, the obtained imprinted SiO_2/AuNPs/SiO_2 retains the similar structure to that of doped SiO_2/AuNPs/SiO_2 (Fig. 2D), suggesting the as-prepared SiO_2/AuNPs/SiO_2 is structurally rigid and is beneficial for the preservation of complementary cavities of the templates during the calcination process.

The successful grafting of AuNPs and formation of the sandwich-structured hybrids are further verified by TEM characterization. The AuNPs are homogeneously distributed on the surface of the silica inner layer (Fig. 2E), and the lattice fringes of 0.23 nm (Fig. 2F) correspond to lattice spacing of the (111) plane of the AuNPs [24], implying the successful grafting of AuNPs. Noted that PVP plays an important role in the stable existence of AuNPs, since it is a good stabilizing agent for transition metal particles [25]. The TEM image of the SiO_2/AuNPs/SiO_2 (Fig. 2G) clearly reveals that the AuNPs are located between two layers of silica, further confirming the formation of the sandwich-structured hybrids. In addition, the thickness of the silica outlayer is evaluated to be around 70 nm, agreeing well with the FESEM results.

3.2. Electrochemical chiral recognition of Cys isomers with imprinted SiO_2/AuNPs/SiO_2

Electrochemical chiral recognition of Cys isomers with the imprinted SiO_2/AuNPs/SiO_2 is studied. The MISiO_2/ITO is first immerged in 1 mM L- or D-Cys dissolved in 0.1 M PBS (pH = 7.0), and then a positive potential of 0.25 V is exerted to the imprinted electrode for the rebinding of L- or D-Cys. Next, the residual L- or D-Cys in the solution is