Fabrication of Nanoelectrode Ensembles of Porous Gold Nanoshells and Direct Electrochemistry of Horseradish Peroxidase Immobilized on the Electrode

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Novel nanoelectrode ensembles of porous gold nanoshells were prepared on a glassy carbon electrode surface through $NH_2(CH_2)_2SH$ self-assembly approach. The direct electrochemistry of horseradish peroxidase immobilized on the NAuE with high surface area was investigated using electrochemical methods.

In recent years there has been growing interest in materials with specific nanomorphologies because of the expectation of novel properties. Nanostructures with hollow interiors have received broad attention in recent years because of their novel properties and potential applications.[1–3] For example, they can serve as extremely small containers for encapsulation—a process that has been extensively explored in applications related to catalysis, drug delivery, and protection of environment-sensitive materials such as enzymes.[4–6] Although the majority of current work on hollow nanostructures is focused on their optical, electrical, and magnetic properties and the corresponding devices, a new application field in biosensor, has begun to emerge.[7,8]

Heterogeneous electron-transfer (eT) reactions of redox proteins, especially horseradish peroxidase (HRP), at a variety of electrodes have been widely investigated to better understand the complex mechanisms of biological eT, and also for their potential applications in biotechnology.[9,10]

In this letter, we report a novel fabrication of nanoelectrode ensembles of porous gold nanoshells (NAuE) on a glassy carbon electrode surface through $NH_2(CH_2)_2SH$ (AET) self-assembly approach and the electrochemical behaviors of HRP immobilized on the NAuE. To our knowledge, there have been no reports on the NAuE.

Gold hollow nanospheres were synthesized by reacting aqueous $HAuCl_4$ solutions with solid templates such as silver nanoparticles. The Ag colloids were prepared by the reduction of silver nitrate in ethylene glycol in the presence of polyvinylpyrrolidone (PVP).[11] The final silver templates were collected and redispersed in distilled water followed by centrifugation. Because the standard reduction potential of $AuCl_4^-/Au$ pair (0.99 V, vs SHE) is higher than that of Ag^+/Ag pair (0.80 V, vs SHE), silver nanoparticles suspended in solution can be oxidized by $HAuCl_4$ through replacement reaction.[12] The elemental gold produced nucleates and grows around the template surface. An incomplete shell with pore in the wall is formed in the initial stages.

In order to obtain porous gold nanoshells, the residual silver core was removed by adding a concentrated ammonia solution. NH_3 molecules form coordinate compounds with Ag surface atoms, thus reducing the redox potential of the Ag/Ag^+ pair and resulting in the oxidation of silver metal by oxygen from the environment. The formed silver ion complexes diffuse out from the interior of the gold nanoshells into the solution through the pores in the incomplete shell. Upon complete extraction of the silver core, porous gold nanoshells were collected and redispersed in distilled water followed by centrifugation.

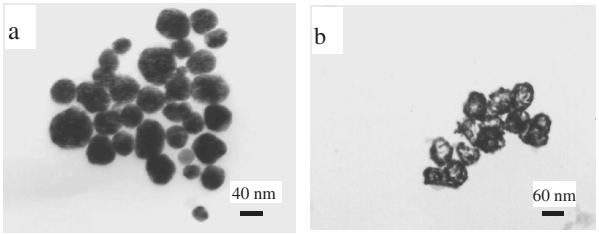
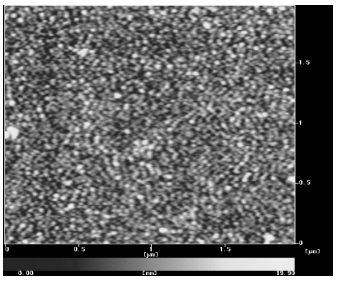


Figure 1. (a) TEM images of silver nanoparticles, (b) TEM images of porous gold nanoshells.

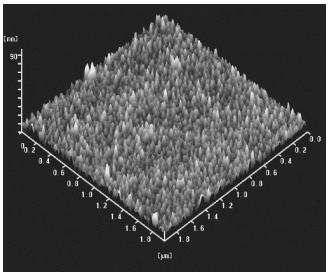
The morphologies of the samples were observed with a JEM-100S transmission electron microscope (TEM). Figure 1a shows the TEM image of the silver nanoparticles that were used as templates. These nanoparticles are sphere particles with an average size of 50 nm. Figure 1b gives a TEM image of porous gold nanoshells with an average size of 60 nm. The strong contrast between the dark edge and light center exhibits its hollow nature. The incomplete shells show that there have many pores in the wall.

The NAuE was fabricated on glassy carbon electrode (GCE) through AET self-assembly approach. In a typical preparation, the GCE was polished with diamond paper, then with alumina (0.05 μm)/water slurry, and rinsed thoroughly with copious deionized water. After that, it was oxidized for 1 min at 1.5 V (vs SCE) in the solution containing 10% HNO_3 and 2.5% K_2CrO_7, which formed –COOH on the surface of the GCE. A 5 μL 1% AET solution was then transferred onto the resulting GCE surface with a microsyringe. Under a certain range of temperature (35–50 °C), AET can bond with the surface of the GCE through covalent bond formed by the dehydration reaction between the $–NH_2$ of AET and the –COOH on the surface of the GCE.[13] Then the resulting GCE was immersed in the solution dispersing porous gold nanoshells for 1 h in order to assemble the nanoshells by the –SH of AET reacting with Au particles. The HRP/NAuE was prepared by incubating the NAuE in 3 mg/mL HRP phosphate buffer solution (PBS) (pH 7.8) for 12 h to attach HRP molecules to the electrode surface. After that, the prepared electrodes were rinsed thoroughly with deioned water, and were ready for characterizations and electrochemical applications. Electrochemical experiments were performed using a CHI 660 electrochemical workstation (CH Instruments, USA).

The surface morphologies of the NAuE were imaged using tapping-mode AFM with a SPI 3800N Probe Station atom force



(a) 2-D image



(b) 3-D image

Figure 2. The tapping-mode AFM images of the NAuE surface.

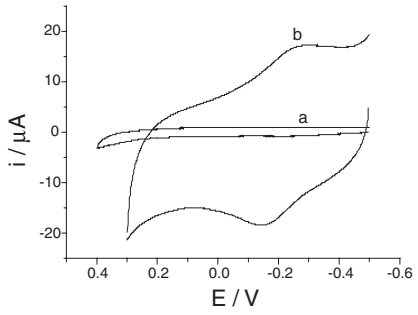


Figure 3. Cyclic voltammograms of the NAuE (a) and the HRP/NAuE (b) in pH 7.0 PBS at 100 mV/s.

microscope. Figure 2 shows well-arranged and close packed 2-D arrays of porous gold nanoshells with comparatively uniform size and shape.

The electrochemical behaviors of the NAuE and the HRP/NAuE in pH 7.0 PBS are shown in Figure 3. No peak is observed at the NAuE (Figure 3a). The HRP/NAuE diaplays a cathodic peak at −0.264 V and an anodic peak at −0.145 V (Figure 3b), which are attributed to the redox process of HRP immobilized on porous gold nanoshells. The high surface area of porous gold nanoshells is helpful to the immobilization of HRP. The adsorption of HRP on porous gold nanoshells surface plays an important role in facilitating the electron exchange between the HRP and the substrate. Figure 4 diaplays a pair of redox peaks for the direct eT behavior of immobilized HRP at various scan rates. The anodic and cathodic peak currents are proportional to the scan rate (inset in Figure 4), which indicates the electrode reaction is typical of surface-controlled quasi-reversible process.

The effect of solution pH (from 4.0 to 9.0) on the electron transfer of HRP/NAuE was studied. An optimal pH range occurs

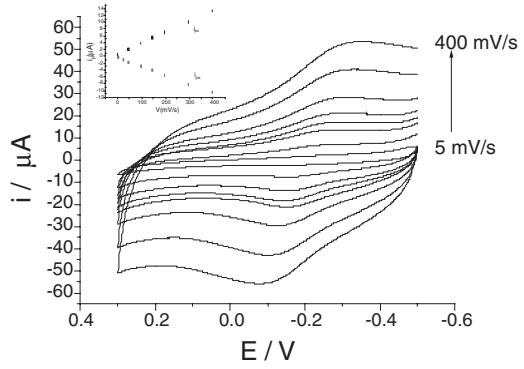


Figure 4. Cyclic voltammograms of the HRP/NAuE in pH 7.0 PBS at 5, 30, 50, 100, 150, 200, 300, and 400 mV/s. (Inset) Plot of peak current vs scan rate.

between 6.0 and 7.0 with a maximum peak current at pH 7.0. The same result was also observed for soluble HRP,[14] indicating that the porous gold nanoshells arrays do not alter the optimal pH value for electron transfer of immobilized HRP.

In summary, novel nanoelectrode ensembles of porous gold nanoshells have been fabricated through AET self-assembly approach. The reversible eT of HRP immobilized on the NAuE is achieved, which shows promise in biosensing field.

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