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2008 Nanotechnology 19 135707
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A Pd/SBA-15 composite: synthesis, characterization and protein biosensing

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Received 16 August 2007, in final form 25 January 2008
Published 26 February 2008
Online at stacks.iop.org/Nano/19/135707

Abstract

In this paper, palladium (Pd) nanoparticles have been successfully encapsulated into the channels of modified SBA-15 in situ via a facile, ethylene glycol (EG)-assisted sonochemical method. The products were confirmed by x-ray diffraction, transmission electron microscopy (TEM) and nitrogen adsorption analysis. The Pd/SBA-15 composite was used for the realization of direct electron transfer of hemoglobin (Hb). Electrochemical results showed that the Pd nanoparticles in the channels of SBA-15 could enhance the direct electron transfer between Hb and the electrode surface. The composite modified electrode displayed excellent electrochemical behavior. The sensor fabricated by the composite showed an excellent response to the reduction of hydrogen peroxide (H$_2$O$_2$), and the linear range for the determination of H$_2$O$_2$ was from 1.8 to 119.3 μM with a detection limit of 0.8 μM.

1. Introduction

Mesoporous materials are the focus of research due to their porous structure and high surface area, and the past decade has seen the development of innovative synthetic methods that employ self-assembled surfactants as structure-directing agents [1–4]. These mesoporous materials have uniform pore size and periodic porous structural properties. They have remarkable applications such as in molecular sieves, adsorbents, gas sensors, protein immobilization, etc [1, 5–9].

Interest has grown greatly in the synthesis of nanosized materials because of their novel electronic, optical and catalytic properties in the past decade [10–12]. Among all the methods available to prepare such materials, one of the most interesting is templated synthesis, where the desired product is encapsulated into the channels and pores of a host [13–16]. In particular, mesoporous materials with tunable pore size ranging from 2 to 30 nm have been the focus of special attention as hosts. Synthesis of nanosized materials within mesopores represents a constricted growth process that provides control of the size of nanosized materials to give them optimal dimensions. Many nanoparticles have been reported to be incorporated inside the channels of mesoporous silica via various methods such as impregnation [17], supercritical fluids [18], γ-radiation treatment [19], sonochemical methods [20], ion-exchange methods [21] and covalent grafting [22].

The sonochemical method has proved to be a useful technique for the rapid growth of materials with unexpected properties [23, 24]. The chemical effects of ultrasound are due to cavitation phenomena involving the formation, growth and implosive collapse of bubbles in the liquid, which generates localized hot spots having a temperature of roughly 5000°C, pressures of about 500 atm and a lifetime of a few microseconds [25]. These extreme conditions can drive chemical reactions such as oxidation, reduction, dissolution and decomposition, which have been employed to prepare nanoparticles. Sonication of the precursor in the presence of support materials provides an alternative means of trapping the produced nanoparticles [26].

Hemoglobin (Hb) is a soft globular heme protein, with four electroactive iron hemes, a molar mass of approximately 64 500 g mol$^{-1}$ and a protein dimension of 5.3 \times 5.4 \times 6.5$ nm. The isoelectric point (pI) of Hb is about 6.8–7.0 [27]. Hb has important biochemical functions such as in electron transport, dioxygen transport, storage and dioxygen-related chemical transformations [28]. Since heme proteins are important in living systems, it is necessary to know their biological activity. Hb can be used as a model molecule for the study of the
direct electron transfer between heme proteins and an electrode because of its commercial availability and relatively well-known structure. However, it may be difficult to do this due to its macromolar mass and large protein dimension. Of all the ways to reach this target, the use of inorganic materials is much attractive because of their chemical and thermal stability, good mechanical strength, biocompatibility, and their potential for improving the stability of the enzymes and proteins under extreme conditions [29]. Mesoporous materials are a typical example of inorganic materials. Their special properties, such as surface characteristics and morphology, as well as the mesopore distributions, made them quite suitable for the immobilization of proteins. Among these mesoporous materials, mesoporous silicates are the most extensively used as biocompatible materials in the area of electrochemical biosensing. Heme proteins, such as Hb, show direct electron transfer in a series of mesoporous silica materials and the excellent results showed that these materials are very suitable for such intentions [30, 31].

The polylol reducing method has been utilized extensively for the synthesis of metals and semiconductor nanoparticles [32–35]. In this paper, the advantage of both the sonication and polylol reducing methods was taken to encapsulate palladium (Pd) nanoparticles into the channel of mesoporous SBA-15. Compared with the methods reported before [36–40], the way used here was easy and fast. The obtained Pd/SBA-15 composite was used for the realization of direct electron transfer between Hb and the electrode surface. Electrochemical results showed that the Pd nanoparticles in the channels of SBA-15 could enhance the direct electron transfer.

2. Experimental details

2.1. Chemicals and reagents

Palladium chloride (PdCl₂) was purchased from Sigma Chemicals Co., Ltd and was dissolved in concentrated hydrochloric acid (HCl) then diluted to form a solution of 0.02 M. Ethylene glycol (EG) was from Shanghai Chemical Reagent Co., Ltd. Bovine heart Hb was purchased from Sigma and used without further purification. Hydrogen peroxide (H₂O₂, 30 wt% solution) was purchased from Shanghai Biochemical Reagent Co., Ltd. All the other reagents are of analytical grade and used as received.

2.2. Synthesis of SBA-15 and Pd/SBA-15 composite

2.2.1. The synthesis of SBA-15. Mesoporous SBA-15 was synthesized as described in the literature [3, 4]. In a typical process, 4.0 g of copolymer poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic P123, molecular weight 5800, EO₂₀PO₇₀EO₂₀) was dissolved in 30 g of water and 120 g of 2 M HCl solution with stirring at 35 °C. Then 8.5 g of tetraethyl orthosilicate (TEOS) was added to the above solution with stirring at 35 °C for another 24 h. The mixture was then transferred to an autoclave and aged for 24 h at 100 °C. The solid product was recovered by filtration, rinsed with deionized water and dried at room temperature. Calcination was carried out in an air atmosphere at 550 °C for 6 h at a heating rate of 1 °C min⁻¹ to remove the template. Before the preparation of the Pd/SBA-15 composite, the obtained SBA-15 was first modified with aminopropyl-triethoxysilane (APTS) by the method described previously [41].

2.2.2. The synthesis of Pd/SBA-15 composite. In the process of preparation, 1 ml of PdCl₂ solution was added to 30 ml of EG containing 0.1 g of modified SBA-15. The mixture was stirred for half an hour in order to immerse the solution into the mesopores. Then the mixture was irradiated by ultrason at a power of 200 W for 0.5 h and a final temperature of about 80 °C was detected for the solution. When the solution was cooled to room temperature, the solid was separated from the solution by centrifugation. The solid was washed with alcohol and water, and then dried at room temperature in a vacuum for one day.

2.2.3. Controlled experiments. To understand the effect of ultrasound, four controlled experiments were performed: (a) heating the mixture of PdCl₂ and modified SBA-15 to 80 °C for 0.5 h in a water bath; (b) sonicating PdCl₂ in ethylene glycol without SBA-15; (c) sonicating PdCl₂ in water instead of ethylene glycol; (d) sonicating PdCl₂ and unmodified SBA-15 in ethylene glycol for 0.5 h. The solids resulting from controlled reactions (a), (b), (c) and (d) were separated, washed and dried by the same procedures as mentioned in the preparation procedure.

2.3. Immobilization of Hb and fabrication of the biosensor

For the immobilization of Hb, 20 mg of Pd/SBA-15 was added to 4 ml of the stock solution of protein (2 mg ml⁻¹, 0.1 M phosphate buffer solution (PBS) and pH 7.0) and stirred at room temperature for 24 h. The resulting Hb/Pd/SBA-15 composite was collected by centrifugation and dried for further tests. For comparison, SBA-15 without Pd was reacted with Hb solution in the same way as that of Pd/SBA-15 to give a composite of Hb/SBA-15.

The glassy carbon electrode (GCE) was first polished with 1.0, 0.3 and 0.05 μm alumina powder successively, followed by rinsing thoroughly with doubly distilled water. The polished electrode was then sonicated in acetone and doubly distilled water and finally allowed to dry at room temperature. For the preparation of the electrode, 10 mg of the Hb/Pd/SBA-15 composite was resuspended in 0.5 ml of water, and 5 μl of this suspension was deposited onto the electrode surface. The electrode was then left to dry at 4 °C for at least 24 h. The biosensor was stored under the same conditions when not in use.

2.4. Characterization and measurements

Powder x-ray diffraction (XRD) patterns were obtained on an ARL X'TRA x-ray diffractometer using Cu Kα radiation. Nitrogen adsorption and desorption isotherms were measured at 77 K on a Micromeritics ASAP 2020 analyzer. The specific surface area was determined using the standard BET method, while the pore size distribution was calculated by the BJH
3. Results and discussion

3.1. Characterizations of the final product

Figure 1(A) shows the low-angle XRD patterns of the as-prepared Pd/SBA-15 composite resulting from the ultrasound-assisted preparation and the original SBA-15 precursor in a 2θ range of 0.7°−5°. It can be seen that the low-angle peaks of Pd-incorporated SBA-15 are almost the same as those of the SBA-15 precursor, indicating that the ordered structure of the SBA-15 substrate is maintained after sonication and insertion of Pd nanoparticles. Figure 1(B) shows the wide-angle XRD of Pd-incorporated SBA-15. The peak located at about 22.64° is a typical peak of the amorphous silica composition of SBA-15. Besides, there is still another weak peak at about 39.3° which belongs to the 100 plane of the cubic phase of metallic Pd (JCPDF 46-1043). This supports the existence of Pd in the channels of SBA-15.

TEM measurements were carried out to study the morphologies of the original SBA-15 and the Pd/SBA-15 composite. Figure 2 shows typical TEM images of the SBA-15 precursor and the as-prepared Pd/SBA-15. Shown in figure 2(A) is the periodic structure of the SBA-15 precursor with a pore diameter of about 6 nm. A typical TEM image of the Pd/SBA-15 composite is shown in figure 2(B). It can be seen that highly dispersed Pd nanoparticles with an average size of about 5 nm were uniformly distributed in the channels of SBA-15. There are also a few large particles (~20 nm) anchored on the external surface of SBA-15. After ultrasound irradiation, the as-prepared Pd/SBA-15 maintains the periodic mesoporous structure compared with the original precursor.

Figure 3 shows the N2 isotherms of Pd/SBA-15 and SBA-15, which are typical type IV isotherms. The data affecting pore structures are summarized in table 1. It can be seen from table 1 that the BET surface area, average pore diameter and pore volume decreased after ultrasound irradiation. This implies that part of the pore space has been occupied during the modification of the pore surface, and some of the pores are then loaded with Pd nanoparticles, which can be clearly seen in figure 2(B).
3.2. Controlled experiments

The role of ultrasound irradiation was studied in the controlled experiments. As described above, the mixture of modified SBA-15 and PdCl₂ in EG was heated to 80 °C and aged for 0.5 h (controlled experiment (a)). The TEM result showed that no obvious loading of Pd nanoparticles inside the pores of SBA-15 were observed, indicating that treatment only by heat cannot reduce Pd²⁺ to Pd in such a short time. When sonicating PdCl₂ in EG without SBA-15 (controlled experiment (b)), the particles obtained were about 20–40 nm in size. These particles were agglomerated into large aggregates (figure 4(A)). The individual particles of their aggregates are much bigger than those observed in figure 2(B). This suggests that the existence of SBA-15 can control the growth of Pd.

No particles could be seen after irradiating PdCl₂ in water for even an hour, indicating that only EG can act as the reducing agent in the reaction. In controlled experiment (d), when sonicating PdCl₂ and unmodified SBA-15 in EG, the solution turned black in only 5 min. The TEM result (figure 4(B)) shows that most of the Pd particles are loaded outside the channels of SBA-15. This may be due to the absence of amino functional groups in the unmodified SBA-15. Amino functional groups had a good chemical interaction with Pd²⁺. They adsorbed the ions into the channels of the modified SBA-15 and protected the ions to be reduced in a relatively long time, so the Pd particles were mostly in the channels and with no aggregation.

No peaks of palladium oxide appear in the XRD spectrum (figure 1(B)). The state of Pd has also been investigated by XPS measurement. Although the load of Pd is very low, there is still a weak peak at an average binding energy of 336 eV. It can be ascribed to the 3d⁸/² transition of metallic Pd [42]. This result is in accordance with XRD data that the sonication yields only metallic Pd.

Based on the results of the controlled experiments, the role of ultrasound can be described as follows: first, sonochemical reactions arise from acoustic cavitation in the EG solution. The energy generated by the extremely high temperature (>5000 K) and pressure (>20 MPa) of ultrasonication is transferred by EG to reduce Pd(II) inside the channels to Pd(0) in very small particles. Second, the small particles grow up into larger ones by the collapse of the bubbles later on. The growth of the small particles is restricted due to the confinement effect of the channels. Besides, the modification of SBA-15 was also necessary to adsorb most of the Pd(II) into the channels. There are still a few Pd(II) anchored on the external surface of SBA-15, and they will also be reduced to Pd(0) by ultrasonication. Those small particles on the external surface can grow up into larger aggregations with successive ultrasonication without the confinement of channels. As can be seen in figure 5, the aggregation of Pd particles becomes larger with the increase in ultrasonication time. This explains the presence of the Pd aggregation on the external surface of SBA-15.

3.3. UV–vis absorption spectroscopic analysis

UV–vis spectroscopy is a useful tool for monitoring the possible change of the Soret absorption band in the heme group region [43]. The band shift may provide information
Figure 5. The aggregation of Pd particles outside of pores at (A) 10 min; (B) 20 min and (C) 30 min.

Figure 6. UV–vis spectra of (a) Pd/SBA-15, (b) Hb/Pd/SBA-15 and (c) free Hb in pH 7.0 PBS.

Figure 7. Cyclic voltammograms of (a) Pd/SBA-15/GCE; (b) Hb/SBA-15/GCE, and (c) Hb/Pd/SBA-15/GCE in 0.1 M PBS 7.0 at 100 mV s$^{-1}$.

3.4. Direct electrochemistry of the Hb/Pd/SBA-15 composite modified electrode

The cyclic voltammograms (CVs) of different electrodes are given in figure 7. No peak appeared at the Pd/SBA-15 modified electrode, indicating that Pd/SBA-15 is inelectroactive, while a couple of well-defined redox peaks are observed at the Hb/Pd/SBA-15 composite modified electrode at $-0.370$ and $-0.337$ V, which can contribute to the Fe(III)/Fe(II) center of

on possible denaturation for the heme protein, particularly that of conformational change. Figure 6 shows the UV–vis spectra of Pd/SBA-15, Hb/Pd/SBA-15 and Hb in 0.1 M PBS7.0, respectively. It can be seen that both free Hb and immobilized Hb showed a maximum absorbance at 406 nm. The absorption peak was attributed to the Soret band of Hb, suggesting no significant denaturation occurred to the protein after immobilization onto Pd/SBA-15.
the immobilized Hb. Although there is a couple of redox peaks at the electrode modified with the Hb/SBA-15 composite, the peak currents were much smaller than those of the Hb/Pd/SBA-15 composite modified electrode. The reduction current ratio of the Hb/Pd/SBA-15 composite modified electrode to the Hb/SBA-15 composite modified electrode is 1.36. This reveals that the existence of Pd in the channels of SBA-15 can facilitate the direct electron transfer between Hb and the electrode surface.

The CVs of the Hb/Pd/SBA-15 modified electrode displays well-defined peak shapes at different scan rates (figure 8(A)). Obviously, both the anodic and cathodic peak currents of the immobilized Hb increase linearly with the increase of scan rate (inset of figure 8(A)), indicating a surface-controlled electrode process.

From integration of the reduction peak of the Hb/Pd/SBA-15 modified GCE at 100 mV s\(^{-1}\), the surface coverage of Hb is calculated to be \(3.47 \times 10^{-11}\) mol cm\(^{-2}\), which is close to the theoretical monolayer coverage of Hb (ca. \(1.89 \times 10^{-11}\) mol cm\(^{-2}\)) on the basis of the crystallographic dimensional structure of Hb and assuming that the biomolecule adopts an orientation with the long axis parallel to the electrode surface [44].

Small peak-to-peak separation always indicates a fast electron transfer rate. The electron transfer rate constant \(k_2\) can be estimated by the Laviron equation [45]:

\[
\log k_2 = a \log(1 - a) + a(1 - a) \log a - \frac{RT}{nFV} \frac{a(1 - a) nF \Delta E_p}{2.3RT}.
\]

Here, \(a\) is the charge-transfer coefficient, \(R\) is the gas constant, \(T\) is the absolute temperature, \(\Delta E_p\) is the peak potential separation and \(V\) is the scan rate. The peak-to-peak separations at 100, 150, 200, 250, and 300 mV s\(^{-1}\) are 68, 70, 78, 82, and 84 mV, respectively, giving an average \(k_2\) value of \(2.17 \pm 0.34\) s\(^{-1}\). This value is much larger than those reported [46, 47].

3.5. Effect of solution pH on the direct electron transfer of Hb

The pH of the solution is very essential to the electrochemical behavior of proteins in most cases. In this research, the Hb/Pd/SBA-15 composite modified electrode showed a strong dependence on solution pH (figure 8(B)). All the changes in the peak potential and current caused by pH (from 4 to 9) were reversible. For example, the CVs of the Hb/Pd/SBA-15 composite modified electrode at pH 7.0 were reproduced after immersion in pH 8.0 buffer and then returned to pH 7.0 buffer. The \(E^0\) of the heme Fe\(^{III}\)/Fe\(^{II}\) redox couple for the Hb/Pd/SBA-15 electrodes showed a linear relationship with pH in the range of 4.0–9.0 with a slope of \(-42.3\) mV pH\(^{-1}\), suggesting that there was nearly one electron participating in the electron transfer process. Thus, the reaction equation for the electrochemical reduction of Hb may be described as follows [48]:

\[
\text{Hb heme Fe(III) + H}^{+} + e^{-} = \text{Hb heme Fe(II)}.
\]

3.6. Electrocatalytic reduction of H\(_2\)O\(_2\) by the Hb/Pd/SBA-15 composite modified electrode

Heme proteins have peroxidase activity and can be used to reduce hydrogen peroxide (H\(_2\)O\(_2\)) through electrochemical catalysis. Based on its excellent electrochemical behavior, the Hb/Pd/SBA-15 composite was immobilized on the surface of GCE and applied to construct a sensor.

The CVs of the Hb/Pd/SBA-15 and Pd/SBA-15 composite modified electrodes in PBS 7.0 before and after the addition of H\(_2\)O\(_2\) are shown in figure 9. It can be seen that, at a bare Pd/SBA-15 modified GCE, no redox response of H\(_2\)O\(_2\) can be observed. However, at the Hb/Pd/SBA-15 modified GCE, an obvious catalytic reduction peak appears at the potential of \(-0.35\) V. The cathodic peak current of Hb increases but the anodic peak current decreases with increasing concentration of H\(_2\)O\(_2\), showing a typical electrocatalytic reduction process. Further experiments showed that the reduction peak currents had a linear response to H\(_2\)O\(_2\) in the range from 1.8 to 119.3 \(\mu\)M with a detection limit of 0.8 \(\mu\)M (inset in figure 9). The linear regression equation was \(y = 0.02x + 0.2\). From the slope of 0.02 \(\mu\)A \(\mu\)M\(^{-1}\), the sensitivity of the proposed Hb sensor was calculated to be 290 mA M\(^{-1}\) cm\(^{-2}\), which is much larger than those of 2.85 mA M\(^{-1}\) cm\(^{-2}\) to H\(_2\)O\(_2\) at \{TiO\(_2\)/Hb\}_15 [49] and 0.56 mA M\(^{-1}\) cm\(^{-2}\) to H\(_2\)O\(_2\).
Figure 9. Cyclic voltammograms of Hb/Pd/SBA-15/GCE ((a), (d) and (e)) and Pd/SBA-15/GCE ((b) and (c)) in 0.1 M PBS 7.0 containing no H$_2$O$_2$ ((a) and (b)), 0.18 mM H$_2$O$_2$ (d) and 0.38 mM H$_2$O$_2$ ((c) and (e)), respectively. Inset: plot of the catalytic current versus H$_2$O$_2$ concentration. The scan rate is 100 mV s$^{-1}$.

at Hb-single-walled carbon nanotubes [50]. The relative standard deviation (RSD) of the peak current in six successive determinations at a H$_2$O$_2$ concentration of 10 $\mu$M was 3.42% for Hb/Pd/SBA-15 modified GCE.

3.7. Stability and reproducibility

Additional experiments were carried out to test the reproducibility and stability. No obvious change was found after the Hb/Pd/SBA-15 modified electrode was immersed in PBS and stored in the refrigerator at 4 $^\circ$C for 20 h. The electrode could keep 95% of its initial response to H$_2$O$_2$ within two weeks.

4. Conclusion

In summary, PdCl$_2$ have been successfully reduced to Pd metal and encapsulated into the pores of SBA-15 by a simple, one-pot polyol-assisted ultrasound method. XRD and TEM showed that the ordered structure of SBA-15 remained after ultrasound irradiation. Controlled experiments demonstrated that the role of ultrasound could be ascribed to the extreme conditions caused by the collapse of bubbles and thus providing the energy for the reduction of Pd(II) to Pd (0) by ethylene glycol. The resulting Pd/SBA-15 can be used for the construction of a sensor and the electrochemical results revealed the Pd particles inside the channels of SBA-15 could enhance the direct electron transfer between Hb and the electrode surface.

Acknowledgments

We greatly appreciate the support of the National Natural Science Foundation of China for the Key program (20635020), Creative Research Group (20521503), and General program (20773065, 20635020, 20575026). We also thank the support of the National Basic Research Program of China (2007CB936302), Jiangsu Natural Science Foundation of China (BK2006114) and the Modern Analysis Center of Nanjing University.

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