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Nonenzymatic Electrochemical Glucose Sensor Based on mesoporous Au/Pt nanodendrites

Yang Song, a Chenzhou Zhu, 2 He Li, ab Dan Du 2 and Yuehe Lin a*

Herein, an ultrasensitive and reliable nonenzymatic electrochemical glucose sensor has been developed, which is based on mesoporous Au/Pt nanodendrites prepared by a facile route with aided by ultrasonication under mild conditions. The fabricated glucose sensor is capable of detecting glucose with wide linear region of 0.01-100 µM and an ultralow detection limit of 1 nM. The higher sensitivity and catalytic properties can be attributed to the mesoporous structures facilitating the mass diffusion rate and favoring the Pt surfaces accessible to reaction media, and the resulting high electroactive surface area of Au/Pt nanodendrites modified electrodes.

1. Introduction

As glucose sensor has played an important role in biotechnology, food analysis, and clinical diabetes diagnosis for over fifty years, tremendous efforts have been made to develop electrochemical glucose sensors. The pivotal features of glucose sensor for commercial uses are good stability, fast diagnosis and high sensitivity. Enzymatic electrochemical biosensor based on immobilized glucose oxidase is one of the most successful achievements exhibiting good selectivity and high sensitivity; however, enzymatic glucose biosensor cannot work stably or sensitively due to sophisticated enzyme immobilization, and enzymatic activity sensitive to pH, temperature, and humidity. Thus, considerable attention has been paid to develop non-enzyme glucose sensors. It is known that, selectivity, sensitivity and anti-fouling of intermediate, are crucial to develop high-performance non-enzyme electrochemical glucose biosensor, which are often relied on the properties of electrode materials. Therefore, the key issue for developing reliable non-enzyme glucose sensors is to explore proper materials that could effectively facilitate electrochemical oxidation and reduction of glucose with strong anti-interference ability. Generally, carbon and metal materials modified electrodes exhibited poor performance for enzymeless glucose determination because of the slow reaction kinetics of electrochemical oxidation and electrode fouling by adsorption of intermediates which demand to apply a repetitive pulsed potentials to remove from the electrode surface. Alternatively, noble metals are found to be one promising candidate. Recently, various noble metal based nanoparticles (NPs), such as Pt/PAni hydrogel, mesoporous Pt NPs, nanoporous Pt NPs, porous Pd and Au, have been synthesized and employed to develop high performance glucose sensors. As compared, bi-noblemetallic NPs showed brighter future for developing non-enzyme glucose sensor attributed to the enhanced electrocatalytic activity. Especially, Pt binary NPs, including Pt/Pd, Pt/Ag, Pt/Ni and Pt/Cu, have been explored to fabricate non-enzyme glucose sensors. The combination of these two metal components brought the advantage of high surface area and unique synergistic contribution to enhancing the conductivity and accelerating electron transfer, thus improving the electrochemical performance in glucose sensing.

Herein, we prepared a new type of bimetallic Au/Pt nanodendrites by one-step mild reduction process and then used it for non-enzyme glucose sensing. The fabricated non-enzyme glucose sensor exhibited ultrahigh sensitivity as well as good stability and strong capability of inference resisting, indicating the high potential application for clinical glucose detection.

2. Experimental

2.1 Material and chemistry

Pluronic F127, K2PtCl4 (Pt, 44.99%), HAuCl4 (Au, 49.98%), hydrochloric acid (HCl, 39%), sodium hydroxide (NaOH) and ascorbic acid (AA) were purchased from Sigma-Aldrich. Ultrapure water from Millipore Milli-Q water purification system was used for experiments. The phosphate buffered saline (PBS) containing Na2HPO4 and NaH2PO4 was prepared using de-ionized water (18.2 MΩcm), and the pH was adjusted with NaOH and H3PO4. All the reagents are analytical standard and used without further purification.

2.2 Instruments and characterization

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a School of Mechanical and Material Engineering, Washington State University, Pullman, Washington 99163, USA. E-mail: yuehe.lin@wsu.edu
b School of Biological Science and Technology, University of Jinan, Jinan 250022, China. E-mail: lihecd@gmail.com
Transmission electron microscopy (TEM) images were recorded with Philips CM200UT microscope. Scanning electron microscopy (SEM) images were conducted using Robinson Model RBH570R microscope. The energy dispersive X-ray spectrometer (EDX) was measured with Robinson Model RBH570R microscope. X-ray photoelectron spectroscopy (XPS) measurements were recorded on a Kratos AXIS-165 multi-technique electron spectrometer system with a base pressure of $1 \times 10^{-9}$ torr. All the electrochemistry experiments were carried out on CHI 660C electrochemistry workstations with a commercial Ag/AgCl (3M KCl) electrode as reference electrode, a platinum wire as counter electrode and a modified glassy carbon electrode as working electrode. All the experiments were performed under nitrogen atmosphere at room temperature.

2.3 Synthesis of mesoporous Au/Pt nanodendrites

Au/Pt nanodendrites were synthesized by a modified procedure as Yamauchi’s work. Briefly, Pluronic F127 (10 mg) was ultrasonically dissolved into aqueous solution containing 0.9 ml of $K_2PtCl_6$ (20 mM), 0.1 ml of $HAuCl_4$ (20 mM) solution and 22 μL of hydrochloric acid (6 M). After adding 1.0 ml of AA (100 mM) as a reducing agent, the mixture was continuously sonicated in a water bath with a constant temperature (30 °C) for 5 h. The final product was collected by centrifugation at 10000 rpm for 20 min and residual Pluronic F127 was removed by rinsing with acetone and water in five consecutive washing/centrifugation cycles. Finally, the collected product was dried at room temperature for further characterization.

2.4 Au/Pt nanodendrites modified electrode preparation

Glassy carbon electrodes (GCE) were polished with alumina powder (1.0 μm, 0.3 μm and 0.05 μm) following by rinsing with de-ionized water through every steps. The GCE were successively sonicated in doubly de-ionized water, and then allowed to dry at room temperature. Mesoporous Au/Pt nanodendrites were suspended in de-ionized water to form a homogeneous solution of 2 mg/ml. 5 μL of solution was dropped on the surface of GCE, and dried under ambient condition for 1 h. Au/Pt nanodendrites modified GCE were obtained, which would be kept at 4 °C in refrigerator before use.

3. Results and discussion

3.1 Characterization of Au/Pt nanodendrites

Figure 1 exhibits the TEM and SEM images of mesoporous Au/Pt nanodendrites prepared by one-step routine with aiding...
by ultrasonication at room temperature. The nanodendrites size was distributed in the range of 40-50nm. It is clear to see the mesoporous dendritic shape. The corresponding EDX analysis further confirmed the presence of Au and Pt in the Au/Pt nanodendrites with an atomic Pt: Au ratio of 85.7: 9.3, which was close to the stoichiometric ratio of the two metal precursors added (9:1) (Figure 2a). It can be drawn that the precursors input can be effectively reduced by a mild reducing agent. The formation of Au/Pt nanodendrites was further confirmed by XPS measurements. Figure 2 also showed the survey spectra of the Pt 4f (b) and Au 4f (c), which lied at 71.2eV and 83.3eV, respectively. The formation of mesoporous Au/Pt nanodendrites can be attributed to the following factors: 1) triple segment copolymer F127 can effectively help to form the dendritic metal deposition\cite{30}; 2) It is known that reduction rate has important role in the formation of nanoparticles\cite{31}. Ascorbic acid, as a mild reducing agent, leads to homogeneous nucleation of Pt and Au seeds, which means ascorbic acid reduction can bring enough reaction time and nucleation sites for overgrowth of metallic seeds.

3.2 Electrochemical performance of Au/Pt nanodendrites modified electrode

Figure 4. (a) CV curves of APME in 0.1 M NaOH with and without 0.1 mM glucose. (b) CV curves of APME in 0.1 M NaOH with different concentration of glucose. (c) Linear relationship between current and glucose concentration on APME in 0.1M NaOH in the range of 10 nM-0.1 mM.

Figure 3. (a) CV curves of APME with different scan rates (10, 25, 50, 100, 200, 500 mV/s) in 0.1 M NaOH. (b) Linear relationship of the anodic and cathode peak current with the square root of the scan rate.
To investigate the electrochemical performance of Au/Pt nanodendrites modified electrode (APME), electrochemical parameters on the modified electrode are firstly investigated. The effect of different scan rates on the CVs response of APME is revealed in Figure 3a. With the increase of scan rate from 10 mV/s to 500 mV/s, the redox peak current increases gradually. Figure 3b stands for the calibration curve of peak currents to the square root of different scan rates. It indicates that the electrochemical activity of APME is a diffusion controlled process. Figure 4a shows CVs of APME in 0.1 M NaOH solution. The humps in the potentials range from -0.7 V to -0.5 V on the CVs curves are associated with the hydrogen desorption in the anodic scan. The strong hydrogen desorption peaks is due to the core-shell structure provided large specific surface area, which facilitated mass transferring to the active sites and improved electron transport ability, both of which can result in great electrochemical activity of Au/Pt nanodendrites. The conclusion is supported by the aforementioned TEM and SEM images. Figure 4a also presents CVs recorded in 0.1 M NaOH solution with or without 0.1 mM glucose at a potential scan rate of 10 mV/s. The well-defined oxidation peak was at -0.26 V of Au/Pt nanodendrites modified electrode was observed, indicating that Au/Pt nanodendrites possess good electrocatalytic oxidation activity for glucose. The excellent electrocatalytic performance toward oxidation of glucose can be attributed to the significantly increased electroactivity surface area of nanodendrites. APME showed an increasing current response to increasing glucose at -0.26 V (Figure 4b), which means that they can avoid the interfering oxidation at such low potential from many other interferences. As a result, the linear dependence of current response with glucose concentration gives rise from 0.01 µM to 100 µM with a low detection limit of 1 nM (Figure 4c). Compared with the linear range of 0.04-46 mM for Pd/Ag based non-enzymatic glucose sensor, 1.5-12 mM for Pt/Pd based non-enzymatic glucose biosensor, 0.08-9.26 mM for Au/Cu alloy NPs on CNTs based non-enzymatic glucose sensor, 0.062-14.07 mM for non-enzymatic glucose detection on Pt/Pd NPs/carbon vesicle modified electrodes, the linear range of our proposed enzyme-free glucose sensor is more superior. The detection limit is 1 nm at signal-to-noise ratio of 3 which is lower than 70 nM for CuO nanellipsoids based non-enzymatic glucose sensor and 7 nM for using Pd/Ni alloy/carbon fiber nanocomposites based enzyme-free glucose sensor. Pt nanomaterials with mesoporous structures are expected to show high electrocatalytic performance toward glucose due to the large electrochemical active surface area (ECSA) and the resulted high electrocatalytic activity. Here the ECSA value of APME is calculated to be 34.7 m$^2$g$^{-1}$, which are about 1.7 times larger than the ECSAs of commercial Pt/C (20.5 m$^2$g$^{-1}$) and 1.14 times larger than the ones of commercial Pd/C, respectively. So the ultra-high sensitivity is attributed to the unique nanodendrites structure of Au/Pt NPs. Meanwhile, the surface Pt content, and the uniform dispersion of nanodendrites in the electrode surface should increase the electron transport and mass transfer, contributing to high sensitivity. Our proposed sensor offers a promising way to determine glucose concentration with high selectivity.

For amperometric sensing applications, electrodes could be also generally evaluated by measuring current response at a fixed potential and within a certain time after adding the analytes and possible interfering species. The real-time amperometric response to glucose oxidation on APME was further evaluated by successively adding glucose with solution stirring at -0.35 V with successive addition of the glucose concentration in increments from 0.1 to 10 µM (Figure S1a). When an aliquot of glucose is added, a remarkable oxidation current signal was raised to a steady-state value within good response time (2 s), indicating sensitive and significant rapid detection of glucose by APME (Figure S1a). The corresponding calibration curve under optimized condition shows a linear range from 0.1 to 10 µM ($R^2$= 0.99916) and the detection limit is 40 nM (S/N=3) (Figure S1b). The capability of resisting the normal electroactive interferences in biological fluid is
extremely important for non-enzyme glucose biosensor. The normal blood glucose level of human is in the range from 4 to 7 mM, whereas the concentration of uric acid (UA) and ascorbic acid (AA) are normally only up to 0.15 mM and 0.3 mM, respectively. Since AA and UA, the common substrates in the biological fluids, have similar electrochemical activity to glucose, they could be oxidized on the electrode surface with glucose simultaneously, yielding difficulties in glucose determination. As many used metal based electrodes of glucose sensors are susceptible to AA and UA, the antifouling ability of a sensor needs to be considered when people evaluate its performance. Figure 5 shows the amperometric response of APME associated with adding 0.5 μM glucose, 0.1 mM AA and 0.1 mM UA consequently into the stirring solution at the potential of -0.35 V. No significant current jump was observed after adding UA and AA whereas absolute current increase occurred by adding glucose. Moreover, the second addition of glucose obtained smaller current signal than the first addition. The declining current response is due to the accumulation of intermediates, which were involved in glucose oxidation on the electrode surface and fouled the electroactive sites. These results suggest that this glucose sensor exhibited favour for glucose detection and resisting the interferences of AA and UA. Since the electrochemical oxidation of glucose is a kinetic controlled reaction, its oxidation rate will be increased with the increasing of electrochemical active surface area. While the electrochemical responses of the common interfering species of AA and UA will not be changed significantly, indicated that bimetallic Au/Pt nanodendrites are good enough for high selective detection of glucose. Another possibility for the good selectivity of the APME against AA and UA can also be hypothesized as the repelling effect. Au/Pt nanodendrites were prepared by AA reduction, therefore bimetallic Au/Pt nanodendrites would be negatively charged in 0.1 M NaOH solution (pH~13.5). AA and UA are also negatively charged in 0.1 M NaOH solution due to the loss of proton. Consequently, the negatively charged bimetallic Au/Pt nanodendrites surface could strongly repel the same charged of interfering species, thus resisting the negligible electrochemical oxidation of AA and UA on the electrode surface. Hence, this glucose sensor is capable of sensing low concentration glucose without being interfered by the common indiscernible oxidation from AA and UA.

In addition, the long-time stability of APME should be another focus for glucose sensing. These modified electrodes were stored at room temperature. The stability of the APME was investigated by measuring the CV curves to 0.1 mM glucose in NaOH for each 12 hours. Figure 6 shows that the oxidation current responses kept more than 90% of the initial value after 72 hours for testing, indicating that the APME has good stability.

The feasibility was performed by determining the glucose concentration in the human serum samples from local hospital. The results are presented in Table 1. The results tested using APME are in well agreement with the value obtained by the commercial glucose meter, indicating that as-prepared APME can be utilized for clinical sample testing with good accuracy.

<table>
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<tr>
<th>Sample</th>
<th>Clinical test (mM)</th>
<th>RSD (n=3)%</th>
<th>Measured by APME (mM)</th>
<th>RSD (n=3)%</th>
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</table>

4. Conclusions
We have successfully prepared mesoporous Au/Pt nanodendrites using a mild one-step process. The Au/Pt nanodendrites modified electrodes have strong and sensitive responses to glucose. They are capable of sensing glucose amperometrically at -0.26 V (Ag/AgCl), where the interferences from the oxidation of common interfering species such as AA and UA are effectively avoided. High electroactive surface area combined with the unique structure of Au/Pt nanodendrites are considered to be the key factors responsible for the excellent performance of the Au/Pt nanodendrites modified electrodes. Thus, mesoporous Au/Pt nanodendrites offer a novel way to develop high performance non-enzymatic glucose sensors.

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References