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Short communication

Self-assembled CNTs/CdS/dehydrogenase hybrid-based amperometric biosensor triggered by photovoltaic effect

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ABSTRACT

A novel multi-components hybrid material, self-assembled quantum dots (CdS) and glutamate dehydrogenase (GDH) onto multiwall carbon nanotubes (CNTs), was designed for amperometric biosensing system. The ζ-potential and transmission electron microscopy (TEM) analyses confirmed the uniform growth of the CdS/GDH onto carboxyl-functionalized CNTs. Compared with the single CdS, the resulting hybrid material showed more efficient generation of photocurrent upon illumination. The incident light excites CdS and generates charge carriers, and then CNTs facilitates the charge transfer. For dehydrogenase-based biosensor, normally, the cofactor of β-nicotinamide adenine dinucleotide (NAD^+) or β-nicotinamide adenine dinucleotide phosphate ($NADP^+$) is necessary. Furthermore, we found the photovoltaic effect of CNTs/CdS/GDH can trigger the dehydrogenase enzymatic reaction in the absence of the NAD^+ or $NADP^+$ cofactors. The electrochemical experiment results also demonstrate that the cofactor-independent dehydrogenase biosensing system had series attractive characteristics, such as a good sensitivity (11.9 nA/μM), lower detection limit (up to 50 nM), an acceptable reproducibility and stability. These studies aid in understanding the combination of the semiconductor nanohybrids (CNTs/QDs, etc.) and biomolecules (enzymes, etc.), which has potential for the applications in biosensor, biofuel cell, biomedical and other bioelectronics field.

1. Introduction

The organization of functional semiconductor nanoparticles (QDs) on surfaces attracts more and more research efforts in recent years (Ma et al., 2005; Sapsford et al., 2004; Daniel and Astruc, 2004). For application of the photoactive materials, photochemical excitation of QDs to generate an electron–hole pair is the fundamental process (Feeman et al., 2007). Therefore, the hybrid or composite semiconductor systems were developed to behave as the matrices, which can retard the electron–hole recombination and improve photocurrents (Zaban et al., 2000; Liu and Kamat, 1993; Subramanian et al., 2001). For instance, the preparation of semiconductor-CNTs hybrid, such as SiO_2- (Fu et al., 2002), TiO_2- (Banerjee and Wong, 2002), SnO_2- (Han and Zettl, 2003), CdSe- (Ravindran et al., 2003) and CdS-CNTs (Shi et al., 2004), has been pursued as an effective route. Of special interest is the CdS-CNTs composite, which is capable of generating photocurrent from visible light with unusually high efficiency (Shi et al., 2004; Sheeney-Haj-Ichia et al., 2005).

For developing amperometric biosensors or biofuel cell, the most fundamental principle is electrical contacting of redox-enzymes with electrodes surface (Willner and Katz, 2000; Katz et al., 1999). Different methods have been used to assemble integrated, electrically contacted enzyme electrodes. Conductive CNTs and quantum dots (QDs) usually have high values of conductivity and transmittance, which allow efficient electron transfer between the matrix electrode and redox proteins (enzymes) (Gooding, 2005; Joseph, 2005; Zhao et al., 2002; Zhou et al., 2005). Moreover, some hybrid complexes with QDs and redox proteins, such as AChE-CdS (Katz et al., 2004), formaldehyde dehydrogenase (FDH)-CdS (Vastarella and Nicastri, 2005) and hemoglobin (Hb)-TiO_2 were also employed to assay the enzyme activities and to develop different biosensors (Willner et al., 2007; Zhang et al., 2007). To our best knowledge, the use of CNTs/QDs/enzyme hybrid system application in electrochemical biosensor, however, is little explored (Liu et al., 2007).

For the dehydrogenase-based biosensors, a critical issue is that their cofactors $NAD(P)^+$ are recycled only at high overpotential at most electrodes, resulting in the loss of functionality and stability (Schmakel et al., 1975; Prieto-Simón and Fabregàs, 2006). In previous studies, semiconductor nanocrystals represent a possible route to enhance the electron/hole transfer efficiency,

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overcoming the problems of the $NAD(P)^+$-dependence (Vastarella and Nicastri, 2005; Zhang et al., 2007). Herein, we described a novel $NAD(P)^+$-independent dehydrogenase biosensor based on CNTs/CdS assembly. The hybrid system showed good generation effects upon illumination, and the photoelectrochemical response can trigger the dehydrogenase reaction without using the cofactors $NAD(P)^+$. Thus, dehydrogenase-based reagentless biosensors can be afforded by combination of the photovoltaic effect with QDs.

2. Experimental

2.1. Materials and chemicals

Glutamate dehydrogenase (GDH, EC 1.4.1.3), L-glutamate (monosodium salt, 99%) and β-nicotinamide adenine dinucleotide (NAD^+), Poly (diallyldimethylammonium chloride) (PDDA) were purchased from Sigma–Aldrich. Multiwall CNTs were obtained from Shenzhen Nanotech Port Co. Ltd. (China). The monodisperse CdS-PDDA clusters with diameter about 6 nm were synthesized as the previously described (Zhang et al., 2006). Phosphate buffer was used for immobilization and electrochemical measurements 0.2 M potassium phosphate (PBS), pH 7.4. Other regents were all of analytical grade. Doubly distilled and deionized water was used through this work.

2.2. Preparation of CNTs/CdS/GDH suspension

CNTs was pretreated by refluxing in a mixture of HNO_3/H_2SO_4 (3:1) for 4 h, followed by an extensive washing with deionized water to pH 7.0 and drying at 60 °C. The acid-functionalized CNTs were used as ligands and templates to grow CdS-PDDA and GDH via self-assembly procedure. The resultant CNTs were dispersed in deionized water with a concentration of 5 mg/mL. As a typical procedure to synthesize the CNTs/$(CdS/GDH)_n$ hybrid, the negatively charged (at pH 7.4) GDH (pI 4.83) and positively charged CdS-PDDA were alternating assembled onto the surface of CNTs, via the negative–positive charge interaction and until the desired number of layer. The obtained suspension was stored at 4 °C before use.

2.3. Fabrication of the CNTs/CdS/GDH electrodes

The tin doped indium oxide on glass (ITO; resistivity <15 Ω) was used as the substrate for electrode buildup, which was cleaned by sonication sequentially for 20 min each in acetone, 10% KOH in ethanol and doubly deionized water. After the treatment, 10 mL CNTs/CdS/GDH suspension (containing 1 mg/mL BSA and 5% glutaraldehyde) was dropped on cleaned electrode surface and dried with N_2 and then kept under dry condition at 4 °C. Here, the dry layer can be crosslinked by the glutaraldehyde and the BSA was used for sensor stabilization. The resulting electrode was denoted as CNTs/CdS/GDH-ITO.

2.4. Electrochemical and other measurements

The electrochemical and photoelectrochemical measurements were performed by using a homemade set-up, consisting of a CHI 660C workstation (CH Instruments, Chenhua Inc.) and a UV–Vis lamp with tunable wavelength. A three-electrode configuration was employed, consisting of the ITO serving as a working electrode, whereas Ag/AgCl (3 M KCl) and platinum wire served as the reference and counter electrodes, respectively. The area of an ITO electrode exposed to the solution was maintained at a constant 0.5 cm^2. The electrophoretic mobilities of the adsorbed CNTs suspension were measured using a Malvern Zetasizer 3000HS. High-resolution transmission electron microscope (HR-TEM) images were obtained using a JEOL-2100 at an acceleration voltage of 200 kV.

3. Results and discussion

3.1. LbL self-assembly CdS and GDH on carbon nanotubes

The layer-by-layer (LbL) technique based on electrostatic or other molecular forces creates an advantageous approach to construct different types of assembled materials. It has been used to construct polyelectrolyte/CNTs multilayer to improve solubility and mechanical properties and retain the enzyme molecular activity (Tang et al., 2007; Xu et al., 2007). Herein, GDH and CdS-PDDA were alternating adsorbed onto the surface of the CNTs. Fig. 1a shows weak negatively charged (uncoated) CNTs gave a ζ-potential of about −4.12 mV. Subsequent adsorption of the functional polyelectrolyte (CdS-PDDA) resulted in ζ-potential of about 27.6 mV. Then deposition GDH onto CNTs, the ζ-potential is −14.8 mV. The subsequent alternate assembly of PDDA-CdS and GDH caused a typical reversal in the sign of ζ-potentials with each deposition. The alternating negative and positive ζ-potentials suggest stepwise growth of the multilayer onto the CNTs. From the HR-TEM images (Fig. 1b, c), roughly spherical CdS nanoparticles (about 6 nm) were randomly decorated on the outermost shell of nanotubes. It could be seen that CdS-PDDA/GDH layer evenly and densely spread on almost all of the surfaces of CNTs surface. Nonuniform coating occasionally observed might be attributable to island formation during initial polymer coating or possible local precipitation of the residual polymer remaining in solution after rinsing. Such ζ-potentials and TEM provide strong evidence for the formation of CNTs/CdS/GDH hybrid.

3.2. Photovoltaic effect of CNTs/CdS/GDH hybrid

The photoelectrochemical properties of the CNTs/CdS/GDH hybrid were studied. The origin of photocurrent generation was probed by recording the photocurrent at different excitation wavelengths. The dependence of the photocurrents on the excitation wavelength is shown in the Fig. 2a. It was observed that the maximum photocurrent in these experiments was $I(\lambda 380) = 0.18\ \mu A$. Furthermore, the functionalized electrode revealed reasonable stability. CNTs/CdS/GDH-ITO system generated outstanding photocurrent upon illumination (Fig. 2b). The photocurrent increased immediately upon illumination and returned back to its original position when the light is off. As to the control experiments in the absence of electron donor triethanolamine or the CdS nanoparticles, no photocurrent was observed upon irradiation (data not shown). The results indicate that the photocurrent originates from the CdS nanoparticles and the CNTs are not photoactive in the process. Additionally, the photocurrent for the CNTs/CdS/GDH is impressively higher than single CdS. It should be noted that the incident light excites CdS and generates charge carriers, and then CNTs facilitates the charge transfer from exited CdS to ITO surface (Sheeney-Haj-Ichia et al., 2005).

Alternating assembly CdS onto the surface of CNTs helped to improve the absorption cross section and to augment the photoresponse to light. Fig. 2a (inset) summarized the impact that the number of $(CdS/GDH)_n$ exert on the photocurrent efficiencies under illumination (i.e. 380 nm). In all the cases we see overall amplification of photocurrents up to 2.0. Thus, the photocurrent generated by the system was controlled by the number of layers assembly onto the CNTs surface. From previous studies, we know that the layer

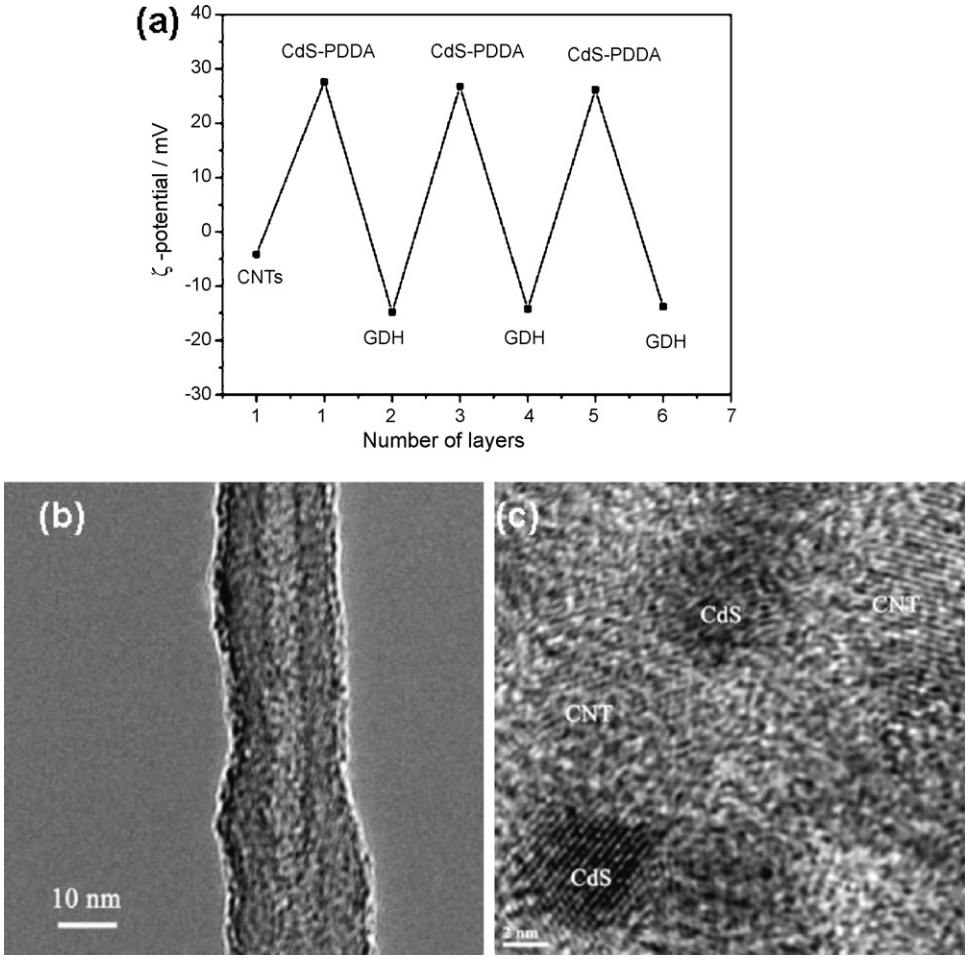

Fig. 1. The ζ-potentials changes of absorption of CdS-PDDA and GDH onto CNTs (a). The TEM and HR-TEM images of the CNTs/CdS/GDH hybrid structure (b, c).

number also has an effect on the sensitivity of the sensor (Tang et al., 2007; Xu et al., 2007). To this point, the CNTs/$(CdS/GDH)_3$-ITO electrodes were selected for the further electrochemical measurements.

3.3. Enzymatic photo-activity with CNTs/CdS/GDH hybrid

The photoexcited electron–hole pair generated in the QDs/biomolecules system stimulated the generation of photocurrents that probe the biorecognition or biocatalytic transformation. Fig. 3a showed the enzymatic photo-activity of the CNTs/CdS/GDH hybrid in the dark and under illumination. Without NAD^+ and in the dark (curve a), no any electrochemical response can be observed for CNTs/CdS/GDH-ITO electrode, whereas, upon addition of NAD^+ or light illumination, the cyclic voltammograms were characterized with well-defined anodic peaks in 0.2 V (curve b, c). Note, the response peak current under illumination was larger than in the dark. Direct comparison with the response of CNTs/GDH and CdS/GDH electrodes in 0.1 M PBS containing 0.1 mM glutamate without NAD^+ was also performed. The CNTs/GDH electrode did not yield any electrocatalytic oxidation of glutamate neither in the dark nor under illumination (380 nm). And a small anode peak was

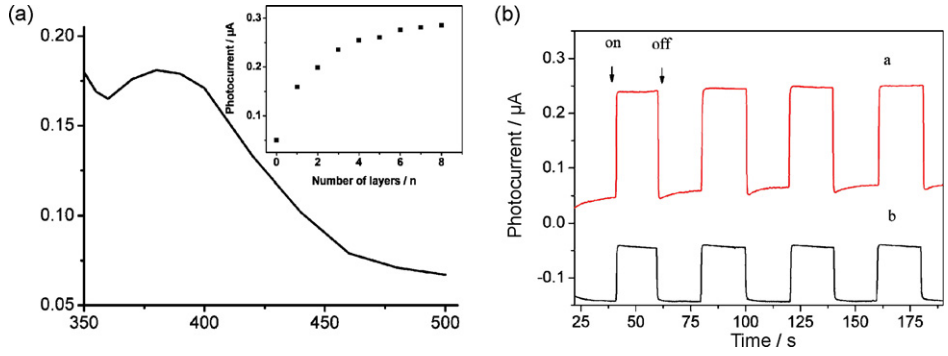

Fig. 2. Photocurrent spectrum of CNTs/CdS/GDH-ITO electrodes in 0.1 M PBS containing triethanolamine (0.005 M). Inset: Photocurrent response of variable CNTs/$(CdS-PDDA/GDH)_n$ (n: 0–8) on ITO electrodes (a). Photocurrent on–off cycles of CNTs/CdS/GDH-ITO (curve a) and CdS-ITO (curve b) exited with visible light (b).

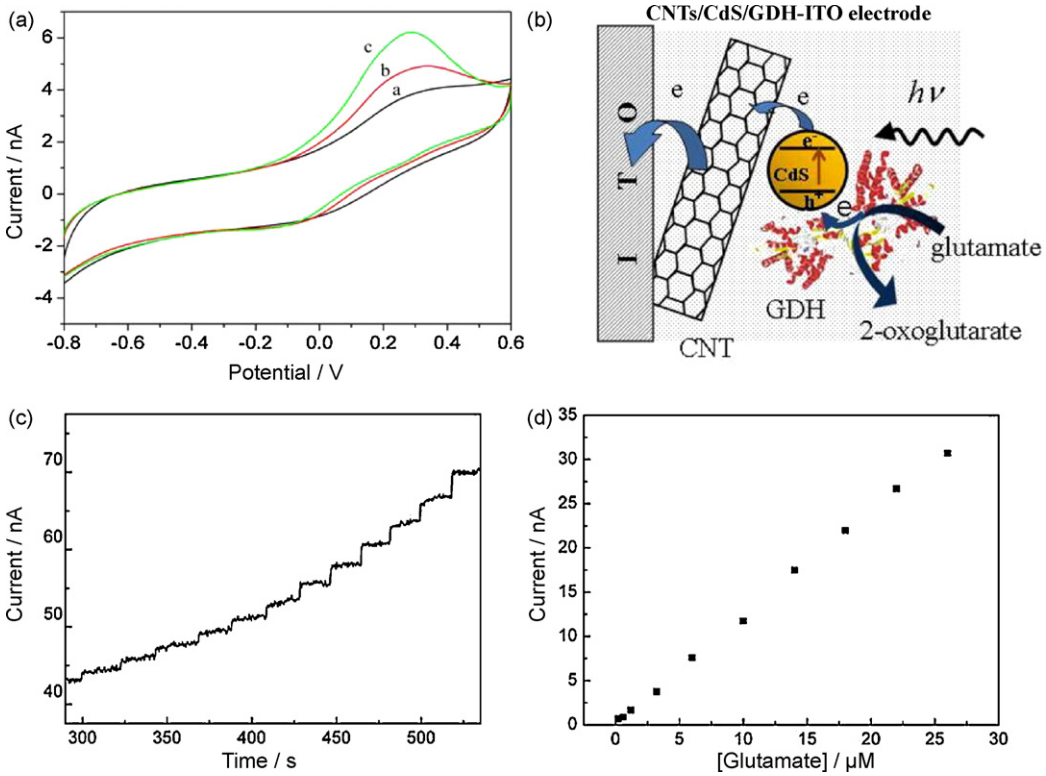

Fig. 3. (a) Cyclic voltammetry of CNTs/CdS/GDH-ITO electrodes to catalytic oxidation of glutamate in 0.1 PBS. Potential scan rate: 50 mV/s. Curve a: without NAD^+ and in the dark; curve b: in the presence of NAD^+ and in the dark; curve c: without NAD^+ and under illumination (380 nm). (b) Illustration of the electrocatalytic process of CNTs/CdS/GDH-ITO to the oxidation of glutamate under illumination (380 nm). (c) Amperometric response of the CNTs/CdS/GDH-ITO electrodes under illumination (380 nm) upon the addition of glutamate in PBS (pH 7.4) at 0.2 V vs. Ag/AgCl. (d) Corresponding calibration plot.

observed at the CdS/GDH electrode under illumination (380 nm), while no any peak in the dark (data not shown). As expected, control experiments show all electrodes nearly have no interesting anode peak in 0.2 V in the absence of glutamate. All such results indicate that nanosized CdS semiconductor should function as mediator in the dehydrogenase-based oxidation of glutamate.

Traditionally, the biosensing of glutamate using glutamate dehydrogenase is based on the following enzymatic reaction (Vastarella and Nicastri, 2005; Zhang et al., 2007).

$$\text{L-glutamate} + \text{NAD(P)}^+ + \text{H}_2\text{O} \xrightarrow{\text{GDH}} \text{2-oxoglutarate} + \text{NAD(P)H} + \text{NH}_4^+ + \text{H}^+ \quad (1)$$

According to the previous studies, the irradiation of CdS nanoparticles can light-induce the dehydrogenase enzymatic reaction upon irradiation (Katz et al., 2004; Vastarella and Nicastri, 2005). In the CNTs/CdS/GDH hybrid, CdS nanoparticles are decorated with the enzyme GDH. Upon the illumination (380 nm), electron (e) and hole (h) generated, respectively in the conduction and valence bands. Here, this injection of the conduction-band electrons into the electrode yields the photocurrent, whereas the valence-band holes was capture by the enzymatic reaction, meanwhile complete the photocurrent generation cycle (Katz et al., 2004; Zhang et al., 2007). To date, it is not very clear how the photovoltaic effect works with the enzymatic reaction. A preliminary explanation can be addressed as the Fig. 3b. The primary processes presented as follows.

$$\text{CdS} + \text{h}\nu \rightarrow \text{CdS(h} + \text{e)} \quad (2)$$

$$\text{CdS(h)} + \text{L-glutamate} + \text{H}_2\text{O} \xrightarrow{\text{GDH}} \text{2-oxoglutarate} + \text{NH}_4^+ + \text{H}^+ \quad (3)$$

$$\text{CdS(e)} + \text{CNTs} \rightarrow \text{CdS} + \text{CNTs(e)} \quad (4)$$

$$\text{CNTs(e) or CdS(e)} \rightarrow \text{ITO(e)} \quad (5)$$

Furthermore, the CdS (h^+) can generate superoxide (O_2^-) and hydroxyl (OH^-) radicals in aqueous solutions, which may have an effect on the enzyme activity as well as some amino aside residues of protein framework (Zhang et al., 2007). Thus, the enzymatic reaction without cofactor was accomplished by the photovoltaic effect of CdS nanoparticles.

3.4. Performance of the CNTs/CdS/GDH hybrid based on biosensor

Amperometric biosensors fabricated with these hybrid nanomaterials were characterized electrochemically (Fig. 3c and d). It displays the amperometric response of the CNTs/CdS/GDH-modified ITO electrodes under illumination upon the addition of glutamate solution. The results show that the response is linear for a glutamate concentration between 0.1 μM and 25 μM ($R^2 = 0.9991$, $n = 10$) with a sensitivity of 11.9 nA/μM. The response time and detection limit (S/N = 3) are determined to be 5 s and 50 nM, respectively.

The stability of the CNTs/CdS/GDH hybrid has also evaluated by examining the response of the enzyme electrode. The reproducibility of the current signal for 10 repeated injections of glutamate was within 5% (R.S.D.). We kept the enzyme electrode under 4 °C for 20 days, and it remained 95% of its initial current response. Furthermore, the interference experiments show the CNTs/CdS/GDH hybrid system has good anti-interferent ability. All of results indicted these matters coexisting in the sample matrix did not affect its determination, the resulting biosensor have good selectivity and is suitable to practical applications.

4. Conclusions

In summary, a novel hybrid nanostructure was successfully prepared by LbL self-assembly GDH and CdS onto surface of CNTs. The CNTs/CdS/GDH hybrid system shows an efficient photovoltaic effect under visible light irradiation, which can trigger the dehydrogenase-based enzymatic reaction without the $NAD(P)^+$. The resultant biosensor behaved good electrochemical performance to glutamate with well sensibility, low detect limit. Thus, the overall procedure is shown to successfully combine the efficient light-harvesting properties of CdS-CNTs system with dehydrogenase-based amperometric biosensor, which has potential for applications in biosensors, biofuel cells, photoelectrical and other bioelectrochemical devices.

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