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A novel photoelectrochemical sensor based on photocathode of PbS quantum dots utilizing catalase mimetics of bio-bar-coded platinum nanoparticles/G-quadruplex/hemin for signal amplification

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ABSTRACT

Photocathode based on p-type PbS quantum dots (QDs) combing a novel signal amplification strategy utilizing catalase (CAT) mimetics was designed and utilized for sensitive photoelectrochemical (PEC) detection of DNA. The bio-bar-coded Pt nanoparticles (NPs)/G-quadruplex/hemin exhibited high CAT-like activity following the Michaelis–Menten model for decomposing H_2O_2 to water and oxygen, whose activity even slightly exceeded that of natural CAT. The bio-bar-code as a catalytic label was conjugated onto the surface of PbS QDs modified electrodes through the formed sandwich-type structure due to DNA hybridization. Oxygen in situ generated by the CAT mimetics of the bio-bar-code of Pt NPs/G-quadruplex/hemin acted as an efficient electron acceptor of illuminated PbS QDs, promoting charge separation and enhancing cathodic photocurrent. Under optimal conditions, the developed PEC biosensor for target DNA exhibited a dynamic range of 0.2 pmol/L to 1.0 nmol/L with a low detection limit of 0.08 pmol/L. The high sensitivity of the method was resulted from the sensitive response of PbS QDs to oxygen and the highly efficient CAT-like catalytic activity of the bio-bar-coded Pt NPs/G-quadruplex/hemin.

1. Introduction

Photoelectrochemical (PEC) analysis is a newly emerging and dynamically developing analytical method. It has the merits of simple equipment, cost-effectiveness, suitableness for miniaturization, high sensitivity and rapidness for detection (Wang et al., 2009a, 2009b; Zhao et al., 2014). The photoelectrochemically active materials largely determine the performance of the PEC sensors because the PEC detection is based on the interaction between the excited photoelectrochemically active materials and the analytes (Wang et al., 2010; Shi et al., 2011). The extensively studied PEC sensors based on photoanodes made by n-type semiconductors (such as sensitized TiO_2 or CdS quantum dots (QDs)) showed sensitive response to a variety of reductive substances, such as ascorbic acid (Wang et al., 2009a, 2009b), H_2O_2 (An et al., 2010; Tu et al., 2012; P.P. Wang et al., 2013), nicotinamide adenine dinucleotide (NADH) (Schubert et al., 2010), and thio compounds (Long et al., 2011; Zhao et al., 2012). This was because the photogenerated holes of n-type semiconductors would transfer to their surface and react with the reductive substances (acting as electron donors). However, these PEC sensors based on photoanodes are obsessed by a relatively poor selectivity in biological samples because many reductive agents coexist in biological fluids (Zachariou and Hearn, 2000; Qian et al., 2012; Kaya and Volkan, 2012; Beitollahi et al., 2008; Mazloum-Ardakani et al., 2011, 2012). Luckily, we recently found that photocathode made by CdS QDs sensitized p-type nickel oxide hardly showed any response to reductive species (Wang et al., 2014), indicating its improved selectivity for biosensing. For p-type semiconductors, photogenerated electrons immigrate to their surface (Choudhary et al., 2012), leading to their preferable interaction with electron acceptors but not electron donors in the electrolyte solution. It seems that the exploration of photocathode in PEC analysis would broaden the scope of photoelectrochemistry in biosensing. However, at present, the PEC detection platforms based on photocathodes are still very limited (Wang et al., 2014).

As a narrow band gap (bulk: 0.41 eV at 300 K) semiconductor,

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PbS is of particular interest because of its large exciton Bohr radius (Jankovič et al., 2010; Scholes and Rumbles, 2006; Seghaier et al., 2006), easily tuned electronic property (Kang et al., 2011) and the multiple exciton effect (Sukhovatkin et al., 2009; Sambur et al., 2010; Rabani and Baer, 2010). In addition, different from the commonly used n-type CdS QDs, PbS usually demonstrates p-type conductivity (García-Valenzuela et al., 2013). Though with these intriguing characteristics, PbS QDs have never been exploited in PEC analysis.

Enzyme-mediated signal amplification methodologies (by employing enzymes or enzyme mimetics as amplification labels) have attracted great attention for highly sensitive determination of target biomolecules. Natural enzymes including horseradish peroxidase (HRP) and alkaline phosphatase (ALP) have been widely applied in biosensors due to their unique merits: highly catalytic activity, high specificity, mild reaction conditions, and easy conjugation to biomolecules and nanomaterials (Perfezou et al., 2012). Though great successes have been made by HRP or ALP in biosensing, to develop other innovative and powerful labels based on enzymes or enzyme mimetics to achieve signal amplification is still a hot spot. Catalase (CAT) has shown great potentials in biosensing because it is more efficient and much cheaper than other popular alternatives such as HRP and ALP (Yu and Caruso, 2003; Roberto and Stevens, 2012; Zhang et al., 2013). The most prominent characteristics of CAT is its ultrahigh catalytic property, which can catalyze the decomposition of millions of hydrogen peroxide (H_2O_2) to water and oxygen in one second (Ammam and Fransaer, 2011). CAT is promising as a biocatalytic label for the construction of novel biosensors (Gao et al., 2013).

Compared to natural enzymes, artificial enzymes have several superiority (Gao et al., 2007; Xie et al., 2012; Wei and Wang, 2013). For example, G-quadruplex/hemin is a novel catalytic nucleic acid (DNAzyme) formed by hemin intercalated in guanine-rich nucleic acid sequences (Travascio et al., 2001), which possesses several unique features, such as it is less expensive to produce, relatively facile for labeling, and more resistant to hydrolysis and heat treatment (Yuan et al., 2012).

Considering the advantages of photocathode, CAT and enzyme mimetics, our motivation is to combine the ultrahigh catalytic activity of CAT mimetics with the merits of photocathode to exploit a novel PEC detection platform for DNA. Different from the commonly used peroxidase-like activity of the G-quadruplex/hemin (Deng et al., 2008; Li et al., 2011; Shimron et al., 2012), herein, we found that it demonstrated CAT-like activity for catalyzing the decomposition of H_2O_2 to water and oxygen. By combining the G-quadruplex/hemin with platinum nanoparticles (Pt NPs) (Fan et al., 2011; X.Y. Wang et al., 2013), a highly efficient CAT mimetics of bio-bar-coded Pt NPs/G-quadruplex/hemin was formed. The CAT mimetics of the bio-bar-code as an amplifying label catalyzed the decomposition of H_2O_2 to generate an efficient electron acceptor of PbS QDs–oxygen, leading to obviously magnified photocurrent of PbS QDs. This "switch-on" PEC sensor based on PbS QDs and CAT mimetics of the bio-bar-code was highly sensitive and selective for the detection of the target DNA. The methodology not only exploited the PbS QDs based photocathode in PEC sensing but also provided a powerful PEC tool based on CAT mimetics for biosensors.

2. Experimental details

2.1. Chemicals, materials and instrumentation

$Pb(NO_3)_2$, thioglycolic acid (TGA), hemin, $NaBH_4$ (96 wt%), H_2O_2 (30 wt%), $H_2PtCl_6 \cdot 6H_2O$, *N*-hydroxysuccinimide (NHS), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), $K_4Fe(CN)_6 \cdot 3H_2O$, $K_3Fe(CN)_6$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). $Na_2S \cdot 9H_2O$ was purchased from Shanghai TongYa Chemical Reagent Co., Ltd. (Shanghai, China). Catalase, 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide (EDC) and poly (diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, molecular weight=200,000–350,000) were obtained from Sigma-Aldrich (St. Louis, USA). Indium tin oxide (ITO) glass was purchased from Southern Glass Holding Co., Ltd. (type N-STN-S1-10, ITO coating 180 ± 20 nm, sheet resistance 8.1 ± 0.6 Ω/sq., Shenzhen, China). All the synthetic oligonucleotides, which were used as models of the developed DNA sensor, were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the capture probe, reporter probe, target DNA, and non-complementary DNA is listed as follows:

Capture probe: 3′-NH_2-$(CH_2)_6$-T CGC ATC CTA TCT-5′;
Reporter probe: 3′-A TAT GCC AAG CGC GGG GTA GGG CGG GTT GGG TTT-$(CH_2)_6$-SH-5′;
Target DNA: 5′-AGC GTA GGA TAG ATA TAC GGT TCG CGC-3′.

Non-complementary DNA (nonspecific control 1 and nonspecific control 2) with three or seven mismatched bases indicated in italics with underline: 5′-AGC GTA GGA TAG A*AA* *AAG* GGT TCG CGC-3′ (nonspecific control 1) and 5′-AGC GTA GGA TAG ATA *GG*C GG*G AT*G *GGT*-3′ (nonspecific control 1).

PEC measurements were performed with a homemade system utilizing a 500 W Xe lamp (NBeT, China) equipped with an ultraviolet cutoff filter ($\lambda \geq 400$ nm) as irradiation source and a CHI 800C electrochemical workstation (Shanghai Chenhua, China) for photocurrent measurements. PbS QDs modified ITO electrode with an area of 0.25 cm^2, a Pt wire and a saturated Ag/AgCl was employed as the working, counter and reference electrode, respectively. The photocurrent measurements were performed at a constant potential of 0 V (vs saturated Ag/AgCl) in 0.1 mol/L Tris–HCl (pH 7.0) as the supporting electrolyte. High resolution transmission electron microscopy (HRTEM) images were acquired on a JEOL JEM-2100 transmission electron microscope (Hitachi, Japan). The X-ray powder diffraction (XRD) was obtained using an X'Pert Philips materials research diffractometer (D8, Brook AXS, Germany) employing a scanning speed of $0.02^\circ\ s^{-1}$. UV–vis–NIR absorption spectroscopic measurements were carried out using a Shimadzu UV–vis–NIR-3600 spectrophotometer (Shimadzu, Japan). Gas detections were carried out using a GC9790 gas chromatograph (Zhejiang Fuli Analytical Instrument Co. Ltd., China). Electrochemical impedance spectroscopy (EIS) was carried out on a CHI 660D electrochemical workstation (Shanghai Chenhua, China) in 0.1 M KCl containing a redox probe of 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture with an applied voltage of 5 mV over a frequency range of 2–900 kHz. A 15% native polyacrylamide gel electrophoresis (PAGE) was prepared by using 1 × Tris-borate–EDTA (TBE) buffer. 7 μL of DNA sample was mixed with 2 μL of native loading buffer (10 ×) and loaded into 15% native polyacrylamide gel. The gel electrophoresis was run at 100 V for 120 min in 1 × TBE buffer. After that, gel was stained with ethidium bromide (EB) for 30 min. The resulting board was illuminated with UV light and photographed with a Molecular Imager Gel Doc XR (Bio-Rad, USA).

2.2. Synthesis of PbS QDs and fabrication of PbS QDs modified ITO (ITO/PbS) electrodes

Thioglycolic acid (TGA)-capped PbS QDs were synthesized according to a slightly modified procedure (Yu et al., 2012). The synthetic details and the fabrication of PbS QDs modified ITO (ITO/PbS) electrodes are shown in the supplemental information.