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The photoelectrochemical exploration of multifunctional TiO_2 mesocrystals and its enzyme-assisted biosensing application

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ABSTRACT

Mesocrystals, as the assemblies of crystallographically oriented nanocrystals, have single-crystal-like atom structures and scattering features but with much higher porosity than single-crystalline materials, making them promising substitutes for conventional single crystals in photoelectrochemical application. As a proof-of-concept, a series of photoelectrochemical tests were investigated to understand the influence of the differences between them on photoelectrochemical activity. Expectedly, comparing with TiO_2 single crystals, TiO_2 mesocrystals demonstrated higher photoelectrochemical capability, which provides unique new opportunities for materials design in the fields of solar-energy conversion and catalysis. Therefore, an elegant photoelectrochemical biosensing platform was firstly developed by virtue of carbon nanohorns with outstanding electrical conductivity support multifunctional TiO_2 mesocrystals to accelerate the transfer of photogenerated electrons, and then horseradish peroxidase was introduced through the immune recognition reaction for enzyme-assisted in situ generating CdS QDs. The multiplex amplification strategy successfully achieved the ultrasensitive detection of α-fetoprotein antigen. Promisingly, the successful application of multiplex amplification strategy affords a rational and practical consideration for the fabrication of new and high-performance photoelectrochemical sensing devices.

1. Introduction

Photoelectrochemical (PEC) measurement, a newly emerging yet vibrantly developing analytical technique has been regarded as a rapid and high-throughput biological assay since the groundbreaking research of Fujishima and Honda (1972). Benefiting from the complete separation of excitation source (light) and detection signal (photocurrent), the undesired background signals can be significantly reduced in the PEC detection (Wang et al., 2009). Due to the performance of PEC sensors which depends intimately on the photoactive materials, enormous efforts have been devoted to explore novel materials to elevate the photoconversion efficiency (Boettcher et al., 2010; Kronawitter et al., 2011; Park et al., 2006; Garnett and Yang, 2010). Hereinto, TiO_2 has drawn considerable interest due to its photoactivity, superior chemical and physical stability, nontoxicity, earth-abundance and cost efficiency (Chen et al., 2010; Feng et al., 2008; Hartmann et al., 2010). However, it has been a challenging task to obtain high PEC properties TiO_2 materials because of their high charge recombination loss at crystal defects and grain boundaries (Zhang et al., 2007; Wang et al., 2009).

TiO_2 mesocrystals, an entirely new class of superstructure materials, consisting of ordered assemblies of aligned nanoparticles, have drawn intensive research interest in many fields (Zhou et al., 2007). Not only it possesses single-crystal-like atom structures and scattering behaviors which inherited from conventional single crystals but also it has porosity like polycrystalline materials (Wang et al., 2005, 2006; Ahmed and Ryan, 2007; Li and Estroff, 2007). The high crystallinity, subunit alignment, periodically hierarchical structure and high porosity of mesocrystals significantly improved the photoelectrochemical performance, which make it promising candidate in PEC application. More importantly, the character of mesopores making it selective incorporates various guest materials inside host framework or pore walls, leading to a high host-guest interface with improved light absorption and photoactivity (Liu et al., 2010; Pan and Lee, 2006). However, it seems that organic additives are inevitable in currently mesoscale

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transformation process, which is not conducive to explore the mechanical of TiO_2 mesocrystals in PEC sensing (Liu et al., 2009; Li and Liu, 2010). Therefore, it is highly desirable as an additive-free approach to synthesize TiO_2 mesocrystals. Herein, we proposed a simple and additive-free strategy to synthetize octahedral anatase mesocrystals (OAM) with dominant {101} facet under solvothermal (acetic acid) conditions. Simultaneously, the octahedral anatase single crystals (OAS) were also prepared under hydrothermal conditions in order to verify the excellent PEC performance of OAM.

To improve the PEC response of OAM, an enzyme-assisted signal amplified strategy for in situ forming CdS QDs was employed. Herein, the enzyme-assisted process is that horseradish peroxidase (HRP) expedited the reduction of $Na_2S_2O_3$ with H_2O_2 to generate H_2S, and then the H_2S reacted with Cd^{2+} for producing CdS QDs. Comparing with the process of pre-synthesized semiconductors which need harsh operating conditions and time-consuming fabrication process, the enzyme-assisted growth of QDs can overcome the shortcomings including high background signals, low sensitivity and increased cost of analytical assays (Zeng et al., 2014; Malashikhina et al., 2013). Besides, HRP can be introduced in the form of HRP-labeled antibody (HRP-Ab) through competitive immune recognition reaction. Motivated by these fascinating properties, a CdS QDs co-sensitized TiO_2 mesocrystals hybrid structure was designed to develop a novel and ultrasensitive PEC biosensor for α-fetoprotein antigen (AFP) detection, and this strategy could bring more convenient operation and directly display results for AFP determination. Therefore, the enzyme-assisted amplification strategy may offer a simpler, faster and cost-effective platform for fabricating the highly sensitive biosensor because of the high activity of enzyme.

To facilitate the application of this PEC biosensing platform, carbon nanohorns (CNHs) acts as a good immobile matrix because of its extremely large surface area, unique pore structure, good adhesion, and excellent physicochemical stability (Tu et al., 2009; Liu et al., 2008; Zhao et al., 2011, 2015; Zhu et al., 2014; Dai et al., 2014) was introduced. Herein, CNHs holds outstanding electronic conductivity due to its typically horn-shaped tips which are composed of single-layer graphene sheets with lots of π-conjugated electrons, enormously expediting the transfer of electrons (Zhu and Xu, 2010). Besides, the matching of energy level between CNHs, OAM and CdS QDs dramatically reduced the recombination of electron–hole pairs, which further enhanced the photocurrent in this system. More importantly, a fascinating feature of CNHs is essentially metal-free, which is instructive to explore the PEC mechanism in this sensing platform. To further improve the adhesion and membrane-forming ability of CNHs, chitosan (CS) functional CNHs was cast on the bare electrode because of the intrinsic advantages of CS including excellent high permeability toward water, good adhesion, film-forming ability, high mechanical strength and susceptibility to chemical modifications (Zhang and Gorski, 2005; Kang et al., 2009).

Herein, we compared the PEC performance between OAM and OAS by a series of PEC techniques. And then multifunctional OAM matrix supported by CNHs scaffold was used to absorb Cd^{2+} by virtue of the high porosities and large surface area for in suit generating CdS QDs with enzymatic assistance, thus realizing the determination of AFP. The results demonstrated that the co-sensitization strategy by the enzyme-assisted in situ generate narrow-bandgap QDs which will open up new avenues for future high-performance PEC sensing.

2. Experimental section

2.1. Materials and reagents

TiO_2 powder (P25) was obtained from the Degussa Co. (Germany). Potassium hydroxide (KOH), Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$), sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$), hydrogen peroxide (30%), N,N-Dimethylformamide (DMF) and acetic acid were purchased from Sinopham Chemical Reagent Co. (Shanghai, China). Glutaraldehtyde (GLD, 25% aqueous solution) and CS were purchased from Shanghai Jinshan Tingxin Chemical Plant (China) and Sigma (St. Louis, MO, USA), respectively. AFP, anti-AFP (monoclone) and bovine serum albumin (BSA, 96–99%) were purchased from Biss Inc. (Beijing, China). The phosphate buffer solution (PBS, 0.1 M, pH 7.4) as the supporting electrolyte was prepared by mixing stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting the pH. Deionized water (DI water) used for the preparation of the solution was purified using a Water purifier (China) purification system.

2.2. Synthesis fabrication of OAM, OAS and CNHs/OAM modified electrodes

Prior to modification, the bare glassy carbon electrode (GCE, with the diameter of 3 mm) was polished with 0.3 and 0.05 μm alumina slurry on chamois leather to produce a mirror-like surface, then it was washed successively with anhydrous alcohol and doubly distilled water in an ultrasonic bath and dried in air before use. With a micropipette, 4 μL of OAM and OAS solutions was dropped onto the fresh prepared GCE surface, then the OAM and OAS modified electrodes were obtained after being dried at room temperature.

The GCE/CNHs/OAM was prepared first through depositing 3 μL of CNHs solution (3 mg mL^{-1} CNHs and 0.33% CS) on the fresh prepared GCE surface and drying at room temperature. Afterwards, 4 μL of OAM solution was dropped onto the CNHs modified electrode and then the GCE/CNHs/OAM was successfully fabricated after drying at room temperature.

2.3. Construction of the PEC immunosensor

The GCE/CNHs/OAM was dipped into 50 μL of 1 mM Cd^{2+} solution for 30 min. Then the electrode was rinsed with DI water several times to remove weak absorbed Cd^{2+}. Following, AFP was immobilized covalently by GLD cross-linking between by NH_2- of CS and NH_2- of Ag in which the GCE/CNHs/OAM was soaked into GLD (5%, 30 μL) and AFP (10 ng mL^{-1}, 30 μL) solution for 50 min. Then the GCE/CNHs/OAM/AFP was immersed into 60 μL of BSA (1 wt%) for 1 h to block possible active sites. Afterwards, anti-AFP was immobilized on the sensing interface by dipping the electrode into the mixture of 15 μL of Ab and 15 μL of AFP (different concentrations) for 50 min at 4 °C. Between each step, the electrode was washed thoroughly with DI water to remove the non-specificity absorption. Finally, the above modified electrode was soaked into the mixed solution including $Na_2S_2O_3$ (1.8 mM, 30 μL) and H_2O_2 (2.6 mM, 30 μL) for 15 min to obtain GCE/CNHs/OAM/AFP /HRP-Ab/CdS and then the PEC biosensor was placed in the test solution to collect the PEC response, the whole procedure of which is demonstrated in Scheme 1A.

3. Results and discussion

3.1. Comparison of characterizations between OAS and OAM

To examine the morphologies and microstructures of OAS and