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A high sensitive visible light-driven photoelectrochemical aptasensor for shrimp allergen tropomyosin detection using graphitic carbon nitride-TiO_2 nanocomposite

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ABSTRACT

Herein, for the first time a visible-light-driven photoelectrochemical (PEC) aptasensor for shrimp tropomyosin determination was fabricated by using graphitic carbon nitride (g-C_3N_4) and titanium dioxide (TiO_2) as photoactive nanomaterials, ascorbic acid (AA) as electron donor and ruthenium (III) hexaammine ($Ru(NH_3)_6^{3+}$) as signal enhancer. The surface of an ITO electrode was first modified with g-C_3N_4, TiO_2, and polyethyleneimine (PEI) and then the amine terminal $aptamer_{TROP}$ probe was attached to PEI by the use of glutaraldehyde (GA) as cross-linker. After that, $Ru(NH_3)_6^{3+}$ was adsorbed on aptamer to enhance the photocurrent signal. The principle of proposed PEC aptasensor is based on the formation of a selective complex between tropomyosin and immobilized $aptamer_{TROP}$ probe on the surface of ITO/g-C_3N_4-TiO_2/PEI/$aptamer_{TROP}$-$Ru(NH3)_6^{+3}$. After the incubation of tropomyosin with TROP aptamer probe, the photocurrent signal decreased due to releasing adsorbed $Ru(NH_3)_6^{3+}$ on aptamer and preventing AA from scavenging photogenerated holes to the photoactive modified electrode. Under the optimized conditions, the fabricated PEC aptasensor was used for the determination of shrimp tropomyosin in the concentration range of 1–400 ng mL^{-1} with a limit of detection of 0.23 ng mL^{-1}. The proposed PEC aptasensor exhibited high selectivity, sensitivity, and good stability.

1. Introduction

The seafood allergies affect approximately 6.5 million people in a year (Gordon, 2006). Although most of the allergenic source materials in sea foods are deactivated by cooking, but some of them are heat stable. Tropomyosin (TROP), a kind of proteins in the muscle shellfish such as shrimp, is a major heat stable allergenic source. Commonly, antibody-based enzyme-linked immunosorbent assay (ELISA) has been applied to recognize the tropomyosin poisoning (Zhang et al., 2014). But this method suffers some disadvantages such as expensive fabrication process, instability in the antibody and enzyme, needs a lab operator with a high level of experience, limited linear response range, and time-consuming determination process. However, the biggest advantage of antibody-based biosensor is the specificity and affinity of these probes to target analytes (Amouzadeh Tabrizi et al., 2016; Teresa Fernández-Abedul et al., 2015; Wen et al., 2017; H. Zhang et al., 2016).

Due to the reduce economic losses and improve the public health and food safety, the fabrication of a sensor for the determination of tropomyosin is necessary. Photoelectrochemical (PEC) sensors are a kind of electrochemical methods which includes a light source and a suitable photoactive modified electrode. The light source excites a photoactive modified electrode to generate a photocurrent signal which it is recorded by the electrochemical device (Liu et al., 2016b).

Several photoactive nanomaterials have been used for the fabrication of PEC biosensors (Liu et al., 2015, 2016c; Shu et al., 2016; Zeng et al., 2013; L. Zhang et al. 2016b; Zhang et al. 2011a; Zhao et al., 2015, 2014). Graphitic carbon nitride (g-C_3N_4) is a novel photoactive nanomaterial consisting of carbon and nitrogen, has a visible-light-driven band gap of 2.69 eV (Chen et al., 2013; Hou et al., 2016). Among the various methods have been reported for the synthesis of graphitic carbon nitride (Jiang et al., 2014; Ong et al., 2016; Zheng et al., 2012; Zhu et al., 2014), the pyrolysis of melamine is a common method for

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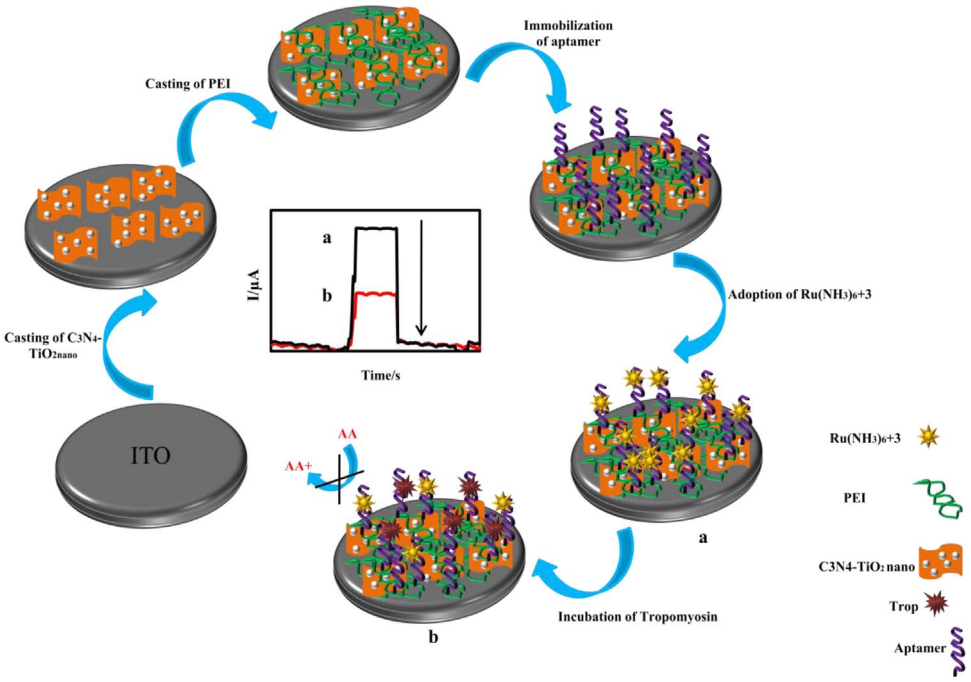

Scheme 1. The schematic illustration for fabrication of PEC aptasensor.

the synthesis of this carbon-nitrogen rich material (Amiri et al., 2016; Das et al., 2016). The g-C_3N_4 have various potential applications in many fields of science ranging such as photodegradation of water pollution components (Yan et al., 2009; Zhu et al., 2014), water splitting (Hong et al., 2017; Kong et al., 2016), carbon dioxide reduction (Walsh et al., 2016; Yu et al., 2015), bioimaging (Zhang et al., 2013), and biosensor (Fan et al., 2016; He et al., 2015; Li et al., 2016b; Liu et al., 2016a; Sun and Qi, 2016).

To the best of our knowledge, no electrochemical PEC aptasensor for the determination of TROP has been reported yet. In this study, for the first time, a PEC aptasensor was fabricated base on immobilization of the amino terminal of the amine terminal $aptamer_{TROP}$ probe via glutaraldehyde (GA) on the surface of ITO modified with graphitic carbon nitride/titanium dioxide/polyethyleneimine (g-C_3N_4-TiO_2/PEI). The proposed PEC aptasensor exhibited high analytical performance in terms of selectivity, stability, sensitivity, linear range (LR), and limit of detection (LOD).

2. Experimental section

2.1. Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Melamine, potassium chloride (KCl), potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$), potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6]$), magnesium chloride ($MgCl_2$), tris hydrochloride (Tris–HCl), potassium hydroxide (KOH) and phosphoric acid (H_4PO_4) were obtained from Merck (Darmstadt, Germany). Hexaammine ruthenium (III) chloride $(Ru(NH_3)_6^{3+})Cl_3$, polyethylene imine (PEI), titanium(IV) oxide (TiO_2), bovine serum albumin (BSA) streptavidin, and lysozyme were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tropomyosin was obtained from Medical Biology Research Center, Tehran University of Medical Sciences, Tehran, Iran. Double distilled water was used throughout. The probe aptamer was modified at the 5′-terminus with an NH_2 group and its sequence was as follow: 5′-NH_2-$(CH_2)_6$–5′-TACTAACGGTACAAGCTACCAGGCCGCCAACGTTGACCTAGAAGCA-CTGCCAGACCCGAACGTTGACCTAGAAGC-3′ (Zhang et al., 2017).

2.2. Apparatus

The Fourier Transform infrared spectra were obtained using a Bruker vector 22 Fourier transform infrared (FTIR) spectrometer. Energy Dispersive X-ray (EDS) analysis was performed with a VEGA, Model TESCAN-LMU. Transmission electron microscopy (TEM) was performed on a HITACHI H-8100 EM with an accelerating voltage of 200 kV. Atomic force microscopy (AFM) measurement was made on DME DualScope Scanner DS95-200 (Herlev, Denmark). The photoelectrochemical (PEC) and cyclic voltammetry (CV) studies were performed using an Autolab potentiostat-galvanostat model PGSTAT30 (Autolab, Netherlands). A three-electrode system was employed with an Ag|AgCl (saturated KCl) electrode as a reference electrode, a Pt wire as a counter electrode and the ITO/g-C_3N_4-TiO_2/PEI/$aptamer_{TROP}$ as a working electrode. A 150 W Xe lamp was used as an irradiation source. The ultrasonication process was carried out using an ultrasonic cleaner (Elma-E30H, Powerful cleaning with 37 kHz cavitation).

2.3. Fabrication of g-C_3N_4

The g-C_3N_4 was synthesized according to a procedure described in a previous literature (Amiri et al., 2016). In brief, an amount of 2.0 g of white melamine powder was transferred into an oven and the temperature of the oven was then increased to 520 °C and held for 4.0 h under argon condition with a ramp rate of about 3 °C/min. The obtained yellow powder was grounded and used for further characterizations (Fig. S1, Supplementary data). Finally, 5 mg of g-C_3N_4 was dispersed in 1 mL of isopropanol alcohol with ultrasonic agitation for 2 h to achieve a well-dispersed suspension.

2.4. Fabrication of aptasensor

The ITO electrode was ultrasonicated for 10 min in ethanol, acetone and, distilled water, respectively. An amount of 2.0 mg of g-C_3N_4 and 1.0 mg of TiO_2 was dispersed in 1 mL of isopropanol alcohol for 5 h. Then, 5.0 μL of g-C_3N_4-TiO_2 (5 mg mL^{-1}) solution was dropped onto the surface of ITO electrode and allowed to dry at ambient temperature. Next, 3.0 μL of an aqueous solution of PEI (1%) was cast on the