Sensors and Actuators B 240 (2017) 785–792

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A sensitive photoelectrochemical aptasensor for oxytetracycline based on a signal "switch off-on" strategy

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ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form 5 September 2016
Accepted 8 September 2016
Available online 9 September 2016

Keywords:
Aptasensor
Oxytetracycline
Photoelectrochemical
Visible light
Switch off-on

ABSTRACT

A sensitive photoelectrochemical (PEC) aptasensor has been successfully constructed for oxytetracycline (OTC) biosensing based on a signal "switch off-on" strategy. When the conjugates of hairpin DNA probe and CdTe QD (H-DNA@QD) was immobilized on the ITO/TiO_2 electrode by Au—S bonds to form an ITO/TiO_2/H-DNA@QD system, CdTe QDs at 3′ end of hairpin DNA close to the electrode surface led to the relatively strong photocurrent under visible light irradiation due to the effective electron transfer. After hybridized with aptamer of OTC, the hairpin DNA probe changed to a rigid, rod-like double helix, and CdTe QDs was far away from the electrode surface, resulting in significantly decrease of photocurrent. The photocurrent signal of the ITO/TiO_2/H-DNA@QD/aptamer would recovery when immersing in the OTC solution, the recovery value increased with the increase of OTC concentration. Therefore, the aptasensor for OTC based on signal "switch off-on" mode have successfully been constructed, which exhibited a satisfying linear range (2–300 nM), low limit of detection (0.19 nM), good reproducibility and stability, and could offer an analytical application in complex food or environmental samples.

1. Introduction

Antibiotic is a class of compounds with antibacterial activity, which residue in foods of animal origin is one of the typical food safety issues and is deemed as an important health hazard owing to abuse and increasing antimicrobial resistance [1,2]. Oxytetracycline (OTC), a member of the tetracycline family [3,4], is a common antibiotic with a broad range of activity and widely used as both a human and veterinary drug in the treatment of various diseases caused by gram positive and gram negative bacteria and pathogenic Rickettsia [5–8]. However, excessive residual of OTC in the environment can cause antibiotic resistance and seriously affects the human health [9,10], which has been listed as pharmaceutical and personal care products (PPCPs) by USA environmental protection agency (EPA) [11], and the WHO suggested the temporary acceptable daily intake (ADI) and a maximum residue limit (MRL) is 0.15 $\mu g\,g^{-1}$ and 0.1 $\mu g\,g^{-1}$ respectively [12]. Therefore, it is essential to establish a sensitive and simple method for the quantitative determination of OTC in food and environment samples.

In recent years, several methods including high performance liquid chromatography [13–17], electrochemical analysis [18–21], and spectrofluorimetry [22,23] have been reported for OTC determination. Due to the unique advantages with high specificity and long-term stability compared with antibody [24], aptamers have been applied in biosensing for OTC [25–28]. However, to our knowledge, there has few studies [29] for OTC detection based on the combination of aptamer and photoelectrochemical (PEC) technique. PEC analysis method has attracted much attention due to higher sensitivity and selectivity compared with conventional methods because of total separation of excitation signal and detection signal [30–39]. As a semiconductor, TiO_2 has been extensively applied in the PEC sensors owing to its excellent photochemical property, biocompatibility and chemical inertness [40], however, only ultraviolet light can excite TiO_2 due to its large energy band gap [41], limiting its some PEC applications. Various functional materials, such as quantum dots (QDs) [42,43], metal or metal oxides [44,45] have been combined with TiO_2 to extend its PEC biosensing application. In our previous work [46], a PEC aptasensor for MUC1 has been constructed which was based on the effective photoelec-

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http://dx.doi.org/10.1016/j.snb.2016.09.042
0925-4005/

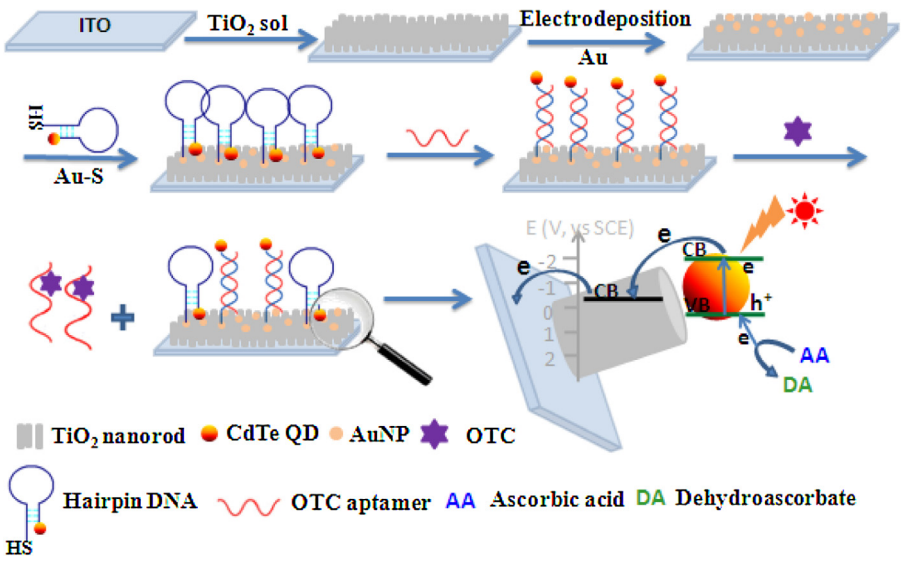

Fig. 1. Fabrication process of the ITO/TiO_2/H-DNA@QD/aptamer aptasensor for OTC based on a PEC signal "switch off-on" mode.

tron transfer from CdTe QDs to TiO_2 nanotube arrays through DNA chains.

In the present work, we developed a sensitive aptasensor for OTC determination based on a PEC signal "switch off-on" strategy. In details, as shown in Fig. 1, TiO_2 nanorods were uniformly prepared on the indium tin oxide (ITO) conducting glass to form a TiO_2 film by sol method, then, gold nanoparticles (AuNPs) were electrodeposited on the surface of TiO_2 film, followed by the conjugates of hairpin DNA and CdTe QD (H-DNA@QD) were immobilized on the ITO/TiO_2 electrode by Au—S bonds. In such situation, it could generate a relatively strong photocurrent under visible light irradiation. After immersed in OTC aptamer solution, due to the specific binding between OTC aptamer and hairpin DNA, the photocurrent of ITO/TiO_2/H-DNA@QD significantly decreased, the decrease value varied with the hairpin DNA chain length. When the ITO/TiO_2/H-DNA@QD/aptamer system was immersed in the target OTC solution, the photocurrent signal would recovery, the recovery value increased with the increase of OTC concentration. Therefore, a sensitive aptasensors for OTC would have successfully been fabricated by PEC signal "switch off-on" mode.

2. Experimental

2.1. Apparatus

The scanning electron micrographs were recorded with a field emission scanning electron microscopy (FESEM, Hitachi S-4800, Japan). Electrochemical impedance and photoelectrochemical measurements were carried out on a CHI 660E electrochemical workstation (Chenhua, China). A 500 W Xe lamp was used as an irradiation source fitted with a 420 nm UV filter (Zolix, China) and a mechanical shutter was used to control the light on and off.

2.2. Construction of the PEC ITO/TiO_2/H-DNA@QD/aptamer aptasensor for OTC

Before construction of the PEC aptasensor, ITO slices (0.5 × 1.5 cm) were well cleaned in acetone, ethanol and deionized water each for 30 min ultrasonically. Fabrication of TiO_2 nanorods film on ITO slice was carried out by sol method. In details, 2.5 mL ethanol was mixed with 0.25 mL acetic acid, and adjusted pH to 2 by concentrated nitric acid. Titanium butoxide was added into the mixture under vigorous stirring for 2 h in a sealed flask. Then deionized water was added dropwise, followed by stirring vigorously for 2 h at room temperature (RT). So a yellowish transparent sol appeared. The TiO_2 sol was coated on ITO slices by doctor-blading (coating area is ca. 0.5 cm^2), and was annealed at 450 °C for 2 h to form ITO/TiO_2 electrode. AuNPs were electrodeposited on the surface of TiO_2 film by cyclic voltammetry in a 5-mL cell containing 3 mL of 0.1 mM $HAuCl_4$ solution with a potential window of 0.5 to −1.0 V at a scan rate of 50 mV s^{-1}, the corresponding scheme diagram was shown in Fig. S1.

For construction of a PEC aptasensor for OTC, its aptamer was selected according to the literature [47]. And the complementary hairpin DNAs (H-DNAs) were listed in Table 1, which were heated to 90 °C for 5 min, and slowly cooled down to room temperature before usage. The conjugates of thiolated hairpin DNAs and CdTe QDs (H-DNA@QDs) was prepared and described in Supporting information. And the ITO/TiO_2/Au electrode was immersed in 200 μL of the prepared H-DNA@QDs solution and incubating for 6 h at RT to form a self-assembled DNA monolayer on the electrode surface. Then the ITO/TiO_2/H-DNA@QD electrode was washed with 10 mM pH 7.4 PBS and incubated in 1 mM 6-mercaptohexanol solution for 1 h and rinsed with water, follow by such electrode was immersed in 200 μL of OTC aptamer solution (1.0 μM in 10 mM pH 7.4 PBS), and kept for 2 h at RT to obtain an ITO/TiO_2/H-DNA@QD/aptamer aptasensor. Finally the prepared aptasensor was washed with 10 mM pH 7.4 PBS for several times and stored at 4 °C for subsequent use.

2.3. Determination of OTC in real samples

To evaluate analytical reliability and application potential of the proposed aptasensor, the concentration of OTC in food sample, such as raw milk, chicken, and environmental water samples, such as tap water, and lake water has been determined. For milk or chicken sample, 1.0 mL raw milk or 1.0 g chopped chicken was placed into a 15-mL centrifuge tube and diluted with water to 10 mL, then 2 mL of 10% trichloroacetic acid and 2 mL chloroform were added and mixed under vortex for 10 min to deposit protein and dissolve fat and other organic substances in the sample matrix. After centrifuged at 13,000 rpm for 10 min, the supernatant was centrifuged at 10,000 rpm for 10 min to remove the deposit once again and the final solution was used for OTC detection. For water samples, tap water and lake water samples were filtered through a 0.2-μm nitrocellulose membrane filter immediately after sampling.