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A novel electrochemical aptasensor for highly sensitive and quantitative detection of the streptomycin antibiotic

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ABSTRACT

In the present study, we report a facile approach to employ gold nanoparticle (AuNPs) and thiol graphene quantum dots (GQD-SH) as the nanomaterial for ultrasensitive detection of streptomycin (STR). Based on this strategy, a GQD-SH was immobilized onto the surface of a glassy carbon electrode (GCE). AuNPs have been immobilized on SH groups of GQDs through bonding formation of Au—S and Apt have been loaded on the electrode surface through the interaction between thiol group of aptamer. By incubating STR as a target onto the surface of the prepared Apt/AuNPs/GQD-SH/GCE as a proposed nanoaptasensor, the Apt/STR complex was formed and the changes of the electrochemical signal were evaluated with the EIS technique. The proposed nanoaptasensor showed wide linear range from 0.1 to 700 pg ml^{-1}. Finally, the proposed nanoaptasensor was successfully applied for the determination of STR in real samples and satisfactory results were obtained.

1. Introduction

Streptomycin (STR), an aminoglycoside antibiotic, is used in human and veterinary for treatment of gram-negative infections [1–3]. However, incorrect and uncontrolled application of STR can cause contamination along food chains and also serious side effects on human health due to allergic reactions, loss of hearing and kidney toxicity [4–6]. Toxic effects could occur when the plasma concentration of STR reaches above 35 μg/ml. According to commission, maximum acceptable residues of STR in milk is 200 μg/ml [7,8]. Thus, development of sensitive and selective methods for detection of STR residue in serum and animal derived foods are in great demand both in the field of environmental analysis and food safety control. As listed in Table 1, several analytical approaches on applying aptamers have been introduced for the detection of STR accurately. Therefore, we attempted to develop an aptasensor for the precise detection of STR. Nanomaterials are promising candidates as basic building blocks and signaling elements for fabrication of biosensors with great sensitivity. In the past few years, different nanomaterials have been integrated into aptamer-based biosensors [9–12]. Graphene quantum dots (GQDs) are a unique class of nanoparticles (NPs). Due to such unique properties such as suitable conductivity, large surface to volume ratio, good biocompatibility, chemical stability, and low-toxicity, GQDs have been demonstrated to be efficient in biosensing applications [13–16]. Noble metal nanoparticles such as gold nanoparticles, platinum nanoparticle, and silver nanoparticle, are the kinds of nanomaterials that have been developed to improve the sensitivity and performance of electrochemical immunosensor and aptasensor [17–19]. Of the various metals, gold (Au) due to its unique properties such as high electrical conductivity, biological compatibility, conductive coating for biological specimens and applicability in medicine and biosensing is a suitable choice. It seems that utilizing AuNPs along with GQDs as a specific platform can be a good choice in designing electrochemical sensors and aptasensors [20,21]. In this study, we developed, a novel and highly selective aptasensor on a glassy carbon electrode (GCE) for the ultrasensitive detection of STR. AuNPs can strongly interact with thiol functionalized GQD (GQD-SH) and can be utilized as an efficient nanocomposite to immobilize aptamer in the construction of aptasensor. Moreover, large amounts of aptamer can be immobilized on the GQD-SH due to its large specific surface area and good biocompatibility. In the proposed method, thiol-aptamer (SH-Apt) was covalently attached to the AuNPs which was attached to the surface of the GCE modified by the GQD-SH.

2. Experimental

2.1. Materials and chemicals

The aptamer (Apt) were custom-synthesized by Bioneer (South Korea), and its sequence are given as follows: 5′-TAG GGA ATT CGT CGA CGG ATC CGG GGT CTG GTG TTC TGC TTT GTT CTG TCG GGT CGT CTG CAG GTC GAC GCA TGC GCC G-Thiol-3′. All chemicals used in the study were of analytical reagent grade with high purity. Streptomycin, dimethylformamide (DMF), dicyclohexylcarbodiimide (DCC), multi-walled carbon nanotubes (MWCNTs), $Na_3C_6H_5O_7 \cdot 2H_2O$, tetrachloroa uric acid ($HAuCl_4$) and all other reagents were ordered from

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Table 1
Analytical parameters for aptasensors have been recently used for streptomycin detection.

Method	LOD	Linear range	Year	References
DPV	11.4 nM	30–1500 nM	2015	[29]
fluorescence	54.5 nM	NR	2016	[30]
DPV	0.036 ng ml^{-1}	0.05–300 ng ml^{-1}	2016	[28]
EIS	0.033 pg ml^{-1}	0.1–700 pg ml^{-1}	This study	

commercial suppliers (Sigma-Aldrich, Merck or Fluka Company). All substance solutions with various concentrations were prepared by direct dissolution in 0.1 M phosphate buffer (PB) solution (in pH 7.4) that was prepared using Na_2HPO_4. In addition, a solution containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ at a ratio of 1:1 and 0.1 M KCl for the experiments was applied. All experiments were performed at room temperature.

2.2. Instruments and apparatus

Spectroscopic characterizations of GQD-SH were studied by Fourier transform infrared (FTIR) spectroscopy (VERTEX 70 FT-IR spectrometer) and UV–Vis spectroscopy (VARIAN 300 Bio CARY). Transmission electron microscopy (TEM) investigations were performed using an electron microscope (Hitachi H-800). The fluorescence spectra were obtained through a Carry Eclipse (Agilent, Fluorescence and Phosphorescence spectrometer). Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) measurements were carried out with a μ-AUTOLAB electrochemical system type III in conjunction with FRA2 board computer controlled Potentiostat/Galvanostat (Eco-Chemio, Switzerland) driven with NOVA software. A modified GCE was used as the working electrode, Ag/AgCl electrode was used as the reference, and a Pt wire was used as the counter electrode. The DPV measurements were performed in 0.1 M KCl solution containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ with 50 ms pulse period, 0.5 s pulse width, 25 mV step height, and 9 mV potential steps. The EIS measurements were recorded within the frequency range of 0.1 to 10 kHz with 5 mV amplitude.

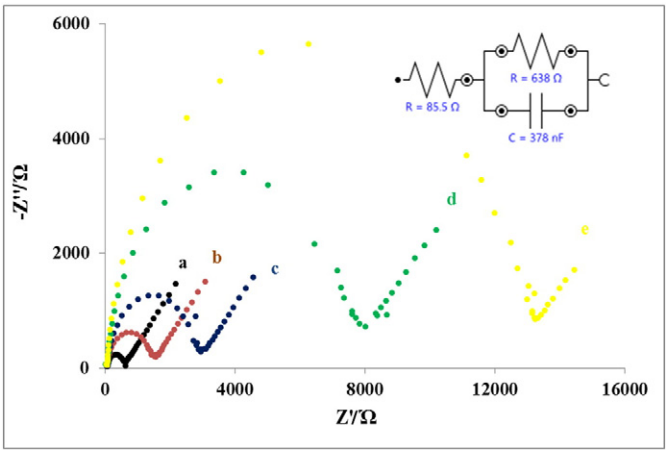
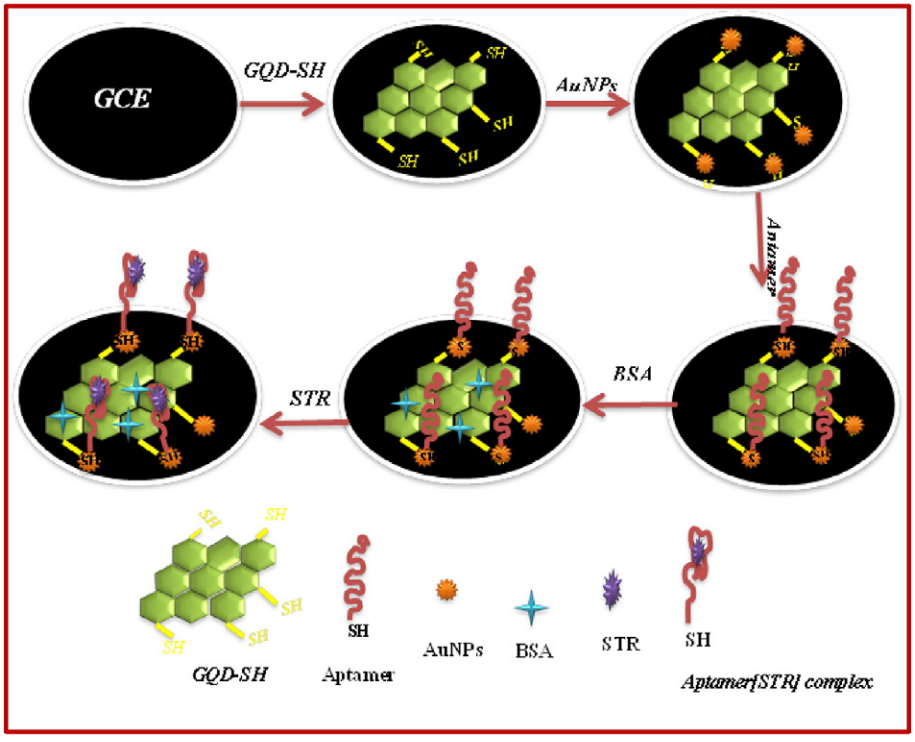


Fig. 1. Nyquist curves of the recorded modified electrode for each steps in: GCE (curve a), GQD-SH/GCE (curve b), AuNPs/GQD-SH/GCE (curve c), Apt/AuNPs/GQD-SH/GCE (curve d), BSA/Apt/AuNPs/GQD-SH/GCE (curve e), Inset is the equivalent circuit. at the frequency range of 0.1 Hz–10 kHz.

2.3. The synthesized of GQD-SH

The preparation of the GQD-SH was carried out according to the literature [22], but with some modifications. Briefly, 0.1 g multi walled carbon nanotubes (MWCNTs) powder was added to 10 ml of HNO_3/H_2SO_4 mixture at a ratio of 1:3 and was sonicated for 6 h at 40 °C to achieve oxidized multi walled carbon nanotubes (ox-MWCNTs). The obtained black suspension containing ox-MWCNTs was diluted with distillated water and allowed to stand overnight for precipitation. Then the supernatant was removed and the suspension of ox-CNT was diluted with deionized water and filtered with a filter membrane (0.05 μm) under vacuum. In order to form stable colloidal suspensions, the ox-CNT were dissolved in plenty of solvents including water, ethanol and dimethylformamide. An amount of 1.00 ml of 1.00 mM



Schematic 1. Schematic illustration of the proposed electrochemical aptasensor for streptomycin detection.