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A sensitive electrochemical aptasensor for highly specific detection of streptomycin based on the porous carbon nanorods and multifunctional graphene nanocomposites for signal amplification

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ABSTRACT

Quantitative detection of antibiotic residues in animal food stuffs is of great significance. In this work, a highly sensitive electrochemical aptasensor for the sensitive detection of streptomycin antibiotic was fabricated based on a novel signal amplification strategy. Specifically, this aptasensor was constructed utilizing porous carbon nanorods (PCNR) formed by porous carbon nanosphere and multifunctional graphene composite (GR–Fe_3O_4–AuNPs) as biosensing substrate. PCNR samples with large specific pore volume and high specific surface area were successfully prepared by hydrothermal and chemical activation treatment for the first time. GR–Fe_3O_4–AuNPs was served as labels to achieve a high sensitivity and low limit of detection (LOD). Under the optimized conditions, the proposed aptasensor exhibited a high sensitivity and a wider linearity to streptomycin in the range 0.05–200 ng/mL with a low detection limit of 0.028 ng/mL. The proposed aptasensor displayed an excellent analytical performance with great reproducibility, high selectivity and stability. In addition, the as–prepared aptasensor was successfully utilized for the determination of streptomycin in real samples.

1. Introduction

Streptomycin is an aminoglycoside antibiotic which is produced by Streptomyces griseus [1] and has been widely utilized in veterinary and human for treatment of gram-negative infectious disease [2,3]. Incorrect and uncontrolled application of streptomycin could result in the presence of antibiotic in foodstuffs and serious side effects on human health, such as nephrotoxicity and ototoxicity [4]. To date, various methods and strategies have been applied for the quantitative detection of streptomycin. Microbial inhibition assay, enzyme immunoassay and enzyme linked immuno-sorbent assay (ELISA) are commonly employed as screening tests but have cross-reactions with other substances in biological sample analysis [5]. Liquid chromatography-mass spectrometry (LC–MS) [6] and high performance liquid chromatography (HPLC) [7,8] have been conventionally described for confirmatory analysis. Although the chromatographic techniques are sensitive and specific, they are restricted to confirmatory analysis being very laborious and expensive [9]. The sensitive determination of streptomycin with low cost is still a challenge in the practical applications. Therefore, it is highly desired to develop anaccurate and sensitive appraisal system to track the residual streptomycin.

Aptamers are short single-stranded DNA (ssDNA) or RNA molecules, obtained by an in vitro process called systematic evolution of ligands by exponential enrichment (SELEX) [10–12]. In addition, aptamers are able to specifically and selectively bind to their targets, ranging from small molecules to proteins and even cells [13,14]. Compared with traditional antibodies, aptamers possess the intrinsic advantages of its low cost, ease of synthesis and modification, excellent thermal stability and lack of immunogenicity and toxicity [15–17]. Owing to these advantages, numerous electrochemical aptasensors have been proposed and applied in the fields of food safety and clinical diagnosis. However, the reported aptamers specific to streptomycin are still very limited. In this work, an ssDNA aptamer [18] that binds to streptomycin with high affinity are introduced.

In addition, the signal amplification is a key factor for the fabrication of aptasensors. Recently, enormous efforts have been devoted to the development of porous materials for signal amplification. As one kind of novel carbon material, ordered mesoporous carbon (OMC), has been receiving much attention in both scientific

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researches and practical applications owing to the extremely well-ordered pore structure, high specific pore volume, high specific surface area, and tunable pore diameters in the mesopore range. Besides, high thermal stability and chemical inertness make them suitable for applications in sensing, catalysis, bioreactor construction, energystorage and capacitors, etc. [19]. Traditionally, porous carbon is prepared with SBA–15 mesoporous silicates as template, which is complicated and high cost [20]. However, in this paper, we conveniently prepared porous carbon nanosphere by the chemical activation with $ZnCl_2$. Interestingly, some nanospheres are fused together to form a pearl necklace and such structures could be described as "porous carbon nanorods (PCNR)" made of porous carbon nanospheres. This phenomenon should be due to the presence of $[Fe(NH_4)_2(SO_4)_2]$, which leads to the formation of rod–like carbon nanostructures instead of spheresby catalyzed hydrothermal heating in a sealed vessel [21]. Contrary to most poroussilica–based materials (for example, SBA–15) that are electronic semiconductors, the mesoporous carbons are intrinsical conductors [20].The high electrocatalytic activity observed at PCNR may attributed to the presence of a large number of edge plane graphite sites within the rod, since researchers have demonstrated that electrochemical reactions may proceed on carbon with spatial non–uniformity, and edge plane graphite sites/defects may generally show much more reactive than those at the basal–plane graphite toward electron transfer [22–24]. Hence, PCNR may have more interests and potential advantages for many advanced applications than other porous materials. Despite such potential capability of PCNR, there have been no studies on the electroanalytical applications for aptasensor.

Currently, different signal amplification strategies have been created to improve the sensitivity and decrease the limit of detection (LOD) of the aptasensor. As a result, a wide variety of multifunctional nanomaterials have been designed as labels for different signal amplification strategies [25,26]. GR, a single–atom–thick sheet of sp^2–bonded carbon atoms, is often used as a substrate and GR-based composite materials have received increasing attention due to the synergistic contribution of two or more functional components and their potential applications [27]. Recently, metal and metal oxide nanoparticles have been widely applied to fabricate nanocomposites due to large surface-to-volume ratio, great electrical properties, strong adsorption ability, high surface reaction activity, small particle size and great surface properties [28], which are helpful for the immobilization of biomolecules.A series of nanomaterials based on Fe_3O_4 NPs have been designed for signal amplification strategy because of its great biocompatibility and electrocatalytic properties toward the reduction of hydrogenperoxide (H_2O_2) [29].In addition, gold nanoparticles (AuNPs) have been widely used in many applications because of their unique optical, physical and chemical properties [30–33]. What's more, AuNPs have good conductivity and biocompatibility and they can also form covalent bonds and combine with materials containing many functional groups, such as —CN, —NH3, or —SH [34]. Thus, in this work, novel multifunctional grapheme nanocomposites (GR–Fe_3O_4–AuNPs) were constructed to achieve dual signal amplification strategy for the fabrication of aptasensor. In addition, the excellent performance of GR–Fe_3O_4–AuNPs is mainly due to these reasons: (1) Graphene was introduced to combine with Fe_3O_4 NPs by chemical reaction and the obtained magnetic graphene nanocomposites (MGN) have a better electron transfer capability; (2) AuNPs were employed to functionalize the MGN to produce synergetic effect, which could result in the increasing of electrotransfer properties of the nanocomposites; (3) The introduction of AuNPs was beneficial to promote the biocompatibility of nanomaterials and increase the conjunction with aptamer.

In present work, a novel electrochemical aptasensor based on PCNR/GR–Fe_3O_4–AuNPs was constructed for sensitive detection of streptomycin. Compared with the predecessors report, the prepared aptasensor offered several advantages: (1) It was facile for aptamers to convert streptomycin into physically detect able electrochemical signals with high affinity and specificity; (2) PCNR with high electrocatalytic activity was synthesized simply and firstly applied for electroanalytical aptasensor; (3) The introduction of PCNR and GR nanocomposites greatly reduced the cost of the aptasensor; (4) Aptamers was immobilized on the electrode by Au-SH covalent bond rather than π-π weak interaction between aptamer and the surface of GR, which makes the aptasensor more stable. In addition, under the optimum conditions, the prepared aptasensor had a wider linear response range and a lower detection limit, which proved that the proposed aptasensor is sensitive and highly specific. More importantly, the as–prepared aptasensor could be used to determine streptomycin in milk. Thus, it may have potential applications for the detection of residual streptomycin in the field of food analysis.

2. Materials and methods

2.1. Reagents and materials

The streptomycin aptamer (Apt), 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′ was synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Ferric chloride ($FeCl_3{\cdot}6H_2O$) was purchased from Damao Chemical Reagent Tianjin Co., Ltd., China. Chloroauric acid ($HAuCl_4{\cdot}3H_2O$) was purchased from Sinopharm Chemical Reagent Shanghai Co., Ltd., China. Chitosan (CS), bovine serum albumin (BSA), glucose, HCl, trisodium citrate, ferrous ammonium sulfate hexahydrate ($FeSO_4(NH_4)_2SO_4{\cdot}6H_2O$) and zinc chloride ($ZnCl_2$) were purchased from Aladdin Chemical Reagent Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and received from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Distilled water was used throughout the experiment.

2.2. Apparatus

Electrochemical experiments of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with a CHI 760E electrochemical workstation (Chenhua Instruments Co., Shanghai, China). Electrochemical impedance measurement (EIS) was performed with a Zennium electrochemical workstation (Zahner, Germany). Powder X–ray diffraction (XRD) data was obtained on a Bruker D_8 advanced X–ray diffractometer using Cu Kα radiation at a scan of 0.02°/s.The morphologies and energy-dispersive X–ray spectroscopy (EDS) of the samples were characterized by a QUANTA PEG 250 field emissionscanning electron microscope (SEM). N_2 adsorption–desorption isotherms was carried out at 196 °C using a micromeritics ASAP 2020 analyzer. Before adsorption, the samples were out-gassed at 120 °C for 12 h. The specific surface area (S_{BET}) was evaluated using the Brunauer–Emmett–Teller (BET) method, and the mesopore volume was calculated according to the Barrett–Joyner–Halenda (BJH) formula and t–plot method, respectively.

2.3. Synthesis of PCNR

PCNR was synthesized with glucose as carbon source by an improved and controllable hydrothermal synthetic route [21,35]. Typically, 4.0 g of glucose and 2.4 g $FeSO_4(NH_4)_2SO_4{\cdot}6H_2O$ were dissolved in 40 mL of distilled water to form a clear solution, and then the solution was transferred into a Teflon–sealed autoclave and maintained at 180 °C for 10 h. Subsequently, the products were impregnated in $ZnCl_2$ (0.25 M) solution for 6 h. Finally, the material