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A novel signal amplification strategy of an electrochemical aptasensor for kanamycin, based on thionine functionalized graphene and hierarchical nanoporous PtCu

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ABSTRACT

An ultrasensitive electrochemical aptasensor for the quantitative detection of kanamycin antibiotic was fabricated based on a novel signal amplification strategy. This aptasensor was developed using thionine functionalized graphene (GR-TH) and hierarchical nanoporous (HNP) PtCu alloy as biosensing substrates for the first time. HNP-PtCu alloy with controllable bimodal ligament/pore distributions was successfully prepared by two-step dealloying of a well-designed PtCuAl precursor alloy combined with an annealing operation. GR-TH composite was synthesized by one-step reduction of graphene oxide (GO) in TH solution. Greatly amplified sensitivity was achieved by using GR-TH/HNP-PtCu composite owing to its large specific surface and good electron-transfer ability. Under the optimized conditions, the proposed aptasensor exhibited a high sensitivity and a wider linearity to kanamycin in the range 5×10^{-7}–5×10^{-2} μg mL^{-1} with a low detection limit of 0.42 pg mL^{-1}. This aptasensor also displayed a satisfying electrochemical performance with good stability, selectivity and reproducibility. The as-prepared aptasensor was successfully used for the determination of kanamycin in animal derived food.

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1. Introduction

Antibiotics have been used in animals for the treatment of various diseases and also as animal growth promoters (Wu et al., 2015). In China, up to 180,000 tons of antibiotics were applied in medicine and animal agriculture in 2009 (Chen et al., 2015; Zhou et al., 2013). However, antibiotics can be accumulated in the human body through food chain, which may cause many side effects, such as loss of hearing, toxicity to kidneys, and allergic reactions to drugs (Qin et al., 2015; Sun et al., 2014; Xu et al., 2014b). Kanamycin is an aminoglycoside antibiotic produced by the fermentation of streptomyces kanamyceticus (Qin et al., 2015; Ramezani et al., 2016; Sun et al., 2014). The residual amount of kanamycin in foodstuff may lead to antibiotic resistance from pathogenic bacterial strains, which can endanger the consumer (Xu et al., 2014b). European Union (EU) has established the maximum residue limits (MRLs) for kanamycin in edible tissues and milk (Qin et al., 2015; Xu et al., 2014b). Therefore, sensitive and selective techniques for the detection of residual kanamycin in food or other products was necessary to ensure our health.

Aptamers are artificial single-stranded DNA or RNA molecules with specific 3D structures selected in vitro through SELEX (Huang et al., 2016; Qin et al., 2015; Sun et al., 2014; Yun et al., 2016). Aptamers have attracted tremendous interests as recognition probes in bioassays because of their high selectivity, simple synthesis, easy modification, and good stability (Hun et al., 2013a; Qin et al., 2015; Sun et al., 2014). Recently, a number of analytical methods, such as fluorescence (Wang et al., 2011), electrochemistry (Chen et al., 2014), surface plasma resonance (Tang et al., 2007) and chemiluminescence (Shim et al., 2014), have been reported for the detection of kanamycin. These reported bioassays showed relatively simple operation compared with the conventional labeling methods, but they involved complicated assembly processes and low sensitivity. In this paper, a highly sensitive label-free aptasensor was used for kanamycin sensing. It not only has shown significant advantages of electrochemical sensors, such as fast response, simple operation and low cost, but also inherits the high sensitivity and selectivity of aptamers (Bai et al., 2014; Qin et al., 2015; Sun et al., 2014).

With the rapid development of nanoporous metallic materials, such as Au, Ag, Cu, Pd and Pt-based (Koh and Strasser, 2007; Liu et al., 2014a; Wang et al., 2014; Xu et al., 2009; Zhang et al., 2013a) metals, it has been proven that nanoporous metals are ideal materials which can form immobilization matrices easily and amplify signals. Cu as an assistant component shows a remarkably synergistic catalytic effect for enhancing the catalytic activity of noble metals, such as Pt and Pd (Lu et al., 2014; Koh and Strasser, 2007; Wang et al., 2014; Xu et al., 2012; Zhang et al., 2013a). Dealloyed Pt/Cu alloy exhibited an excellent activity for the oxygen reduction reaction (ORR) (Koh and Strasser, 2007). NP-PdCu alloy with uniform ligament size/controllable bimetallic ratio was fabricated by a simple dealloying process in H_2SO_4 solution (Zhang et al., 2013a). Metallic PtCu and PtNiCu multi-nanorods with 5 nm diameter and 10 nm length were prepared by a polyol reduction method (Liu et al., 2014a). However, most of the reported nanoporous materials have the single-modal pore/ligament arrangement. It is noted that the fabrication of nanoporous materials with multimodal ligament/pore size by the simple dealloying strategy has been rarely studied. In this paper, a HNP-PtCu alloy with bimodal ligament/pore-size distributions was successfully fabricated, which increased the large specific surface area and provided a beneficial immobilization platform for kanamycin aptamer carrier.

The signal amplification is a key factor for the fabrication of electrochemical sensor. GR is a kind of well-known nanomaterial with unique properties of high specific surface area, great mechanical strength and promising catalytic properties (Azimzadeh et al., 2016; Kong et al., 2012; Yu et al., 2013; Zhang et al., 2016). Non-covalent functionalization is viewed as a simple but effective way to modify GR functionalities, which preserves the intrinsic properties of GR. TH molecule has a planar aromatic structure and owns two hydrophilic $-NH_2$ symmetrically distributed on each side (Hun et al., 2013b; Kong et al., 2012; Lai et al., 2014), which is easily bound to the surface of GR sheets through strong π–π stacking forces and synergistic non-covalent charge transfer (Chen et al., 2011; Hu et al., 2012). A DNA biosensor based on GR-TH nanocomposite was easily constructed (Zhu et al., 2012). A novel aptasensor based on AuNPs/TH-GR composite was developed for the determination of thrombin (Zhang et al., 2013a). Lin's groups (Lin et al., 2011) presented a sandwich structure DNA sensor based on ssDNA self-assembling onto the surface of GR by base-π stacking interactions. However, GO was often reduced by hydrazine (Zhu et al., 2012) or ascorbic acid (Kong et al., 2011), and then conjugated. Fortunately, it is found that the good reducing property of TH may serve as the reducing agent of GO (Lai et al., 2014). In this work, we tried the one-step reduction of GO in a TH solution and achieved the easy preparation of GR-TH composite. Herein, GR provided an electronically low-noise biosensing platform, and the functionalization of GR by TH improved its stability and preserved its intrinsic properties, such as high specific surface area, good electrical conductivity and biocompatibility.

In this work, a novel ultrasensitive label-free aptasensor based on thionine functionalized graphene (GR-TH) and HNP-PtCu alloy was fabricated for the detection of kanamycin residues by differential pulse voltammetry (DPV). GR-TH composite acted as a suitable charge-transfer bridge to facilitate the electrode transfer rate. Futhermore, TH performed as an electrochemical redox probe in DPV detection of the aptasensor. With the advantages of large specific surface area and excellent electrical conductivity, HNP-PtCu alloy could immobilize more aptamers and promote the electron transfer. More importantly, GR-TH/HNP-PtCu composite was first used in biosensor as well with significant signal improvement. Under optimum conditions, the prepared aptasensor offered a wider linear response range and a lower detection limit. Thus, the as-prepared aptasensor may have potential applications for the detection of residual kanamycin in the field of food analysis.

2. Materials and methods

2.1. Reagents and materials

The natural graphite powder was purchased from JingChun Co., Ltd (Shanghai, China). TH, chitosan (CS), bovine serum albumin (BSA, 96–99%), KH_2PO_4, $K_2HPO_4 \cdot 3H_2O$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6 \cdot 3H_2O$, NaCl, human chorionic gonadotropin (HCG), hormothyrin (TSH), dopamine (DA), tyrosine (Tyr), and kanamycin sulfate were purchased from Aladdin Chemical Reagent Co., Ltd. (Beijing, China). The synthetic oligonucleotide ssDNA (5′-NH_2-AGATGGGGGTTGAGGCTAAGCCGA-3′) was provided by Beijing