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Aptasensor for electrochemical sensing of angiogenin based on electrode modified by cationic polyelectrolyte-functionalized graphene/gold nanoparticles composites

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ABSTRACT

Herein, a label-free and highly sensitive electrochemical aptasensor for the detection of angiogenin was proposed based on a conformational change of aptamer and amplification by poly(diallyldimethyl ammonium chloride) (PDDA)-functionalized graphene/gold nanoparticles (AuNPs) composites-modified electrode. PDDA-functionalized graphene (P-GR) nanosheets as the building block in the self-assembly of GR nanosheets/AuNPs heterostructure enhanced the electrochemical detection performance. The electrochemical aptasensor has an extraordinarily sensitive response to angiogenin in a linear range from 0.1 pM to 5 nM with a detection limit of 0.064 pM. The developed sensor provides a promising strategy for the cancer diagnosis in medical application in the future.

1. Introduction

Angiogenin, a 14.4-kDa polypeptide, a member of the pancreatic ribonuclease family, was originally isolated solely based on its ability to induce angiogenesis (Fett et al., 1985). It can strongly stimulate blood vessel formation and its concentration in serum is elevated in patients affected by various types of cancers (Yoshioka et al., 2006). Targeting angiogenin may find a potential therapeutic approach in human malignant melanoma. Thus, sensitive detection of angiogenin has become a very important issue. Serum concentration of angiogenin is commonly detected with antibody-based enzyme-linked immunosorbant assay (Katona et al., 2005). In 1998, AL6, DNA aptamers of angiogenin, was generated by *in vitro* selection process (Nobile et al., 1998). To date, very few studies have been carried out on detection of angiogenin. At present, enzyme-linked immunosorbent assay (ELISA) is the most widely used immunoassay method in the detection of angiogenin (Zhao et al., 2005; Kishimoto et al., 2005). The fluorescence detection of angiogenin using molecular labels is generally used in most of the cases (Li et al., 2007). However, the poor limit of detection, long assay time, and photo-bleaching effect often limit its potential applications.

Recently, electrochemical aptamer-based (E-AB) sensors have emerged as a promising and versatile new biosensor platform (Zhang et al., 2009; Jin et al., 2007). The electrochemical methods play important roles in the development of aptasensors due to their high sensitivity, fast response, simple instrumentation, low production cost, and portability. Among all the voltammetric techniques, SWV has many advantages, such as excellent sensitivity and high speed. This high speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal-to-noise ratio. Compared to both linear sweep and cyclic voltammetry (CV), SWV has a much broader dynamic range and lower limit of detection because of its efficient discrimination of capacitance current (Li et al., 2010, 2011).

As a novel affinity reagent, aptamers can provide the specificity lacking in many extraction matrixes. Aptamers are single-stranded oligonucleotides that bind target molecules with very high affinity in a manner similar to antibodies. However, aptamers possess more advantages over antibodies such as chemical synthesis, selection through the systematic evolution of ligands by exponential enrichment (SELEX) process, easy modification, high stability, target versatility, easy-to-stock, and resistant to denaturation and degradation. All of these unique properties increase the likelihood that aptamers will outperform other affinity reagents (Sefah et al., 2009).

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Graphene, a one-atom thick and two-dimensional closely packed honeycomb lattice, has become a fast rising star among carbon materials, triggering a gold rush to exploit its possible applications in both experimental and theoretical studies, since its discovery by Novoselov and Geim in 2004 (Novoselov et al., 2004). Graphene has remarkably high electron mobility under ambient conditions with reported values in excess of 15,000 $cm^2/(V\,s)$ (Zhang et al., 2005). Furthermore, graphene has a very large specific surface area (theoretical value 2600 m^2/g) with low manufacturing cost. These unique properties provide potential applications in synthesizing nanocomposites (Stankovich et al., 2006; Xu et al., 2008; Williarris et al., 2008) and fabricating various microelectrical devices, such as battery (Guo et al., 2009), field-effect transistors (Gilje et al., 2007), electromechanical resonators (Bunch et al., 2007), and ultrasensitive sensors (Schedin et al., 2007), etc. Research on electrical biomolecule detection using graphene and graphene-like materials has gradually increased over the past few years, however, the integration of graphene with noble metal nanoparticles such as gold nanoparticles (AuNPs) (Baby et al., 2010; Hong et al., 2010), is relatively new in biosensor applications. As we know, directly taking advantage of graphene nanosheets' negative charges in the process of self-assembly of these metal nanoparticles has two main difficulties: (1) graphene nanosheets are apt to aggregate, which are caused by strong van der Waals' and π–π interactions between graphene nanosheets; and (2) the negative charge is too weak to assemble AuNPs directly. To overcome the above two obstacles, we employ poly(diallyldimethyl ammonium chloride) (PDDA) as the modifier onto the graphene nanosheets, The positively charged PDDA protected P-GR colloids were obtained via the in situ reduction of GO with abundant oxygen functionalities in the presence of PDDA. The use of PDDA not only alters the electrostatic charges of graphene, but also provides a convenient self-assembly approach for the hybridization of graphene. Owing to the oxygenation of the GR nanosheets in GO, it can weaken the van der Waals interactions between the layers of GO and make them strongly hydrophilic, which thus facilitates their hydration and exfoliation in aqueous media. The reduction process of GO removes oxygen functionalities with partial restoration of aromatic grapheme network (sp^2) (Gilje et al., 2007), leading to hydrophobic GR sheets. After GR sheets were modified with positively charged PDDA, the obtained PDDA-functionalized graphene (P-GR) nanosheets were hydrophilic and soluble in the presence of a static repulsion force.

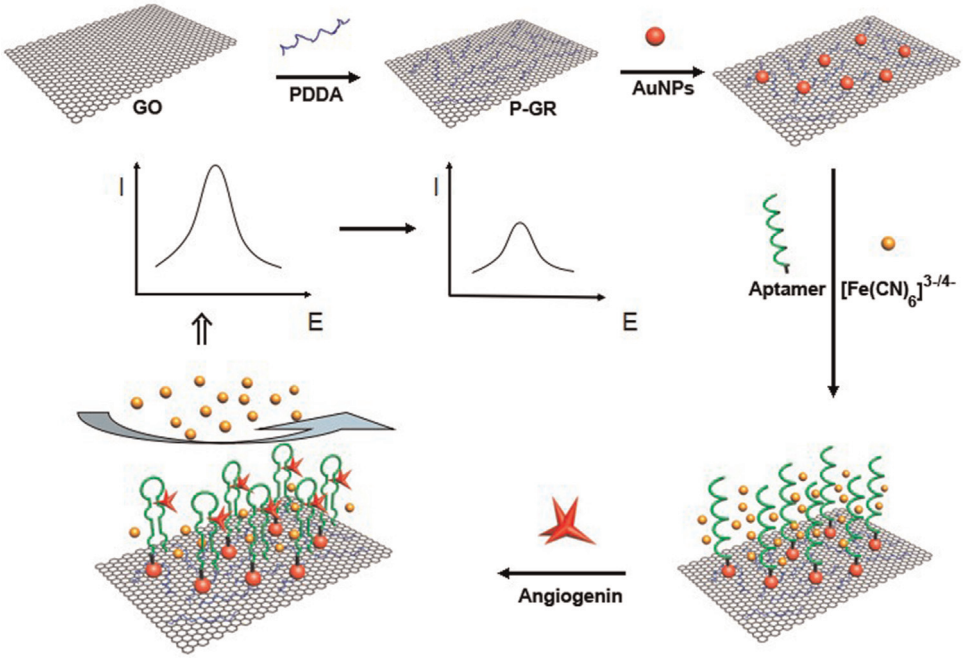
Herein, as shown in Scheme 1, we present a label-free and highly sensitive electrochemical aptasensor for angiogenin detection based on P-GR nanosheets/AuNPs composites-modified glassy carbon electrode (GCE). The transduction principle is based on electron transfer resistances in the presence of an $[Fe(CN)_6]^{3-/4-}$ redox couple. Angiogenin can promote the conversion of the DNA sequence from a loose random coil into the secondary stem-loop structure (Li et al., 2008) (Fig. S1), and leads to an increase in the electron transfer resistance, repelling the $[Fe(CN)_6]^{3-/4-}$ redox couple to approach the electrode. Thus, it resulted in a substantial decrease in square-wave voltammetry (SWV) current. The proposed electrochemical aptasensor for angiogenin is very simple, cost-effective, highly sensitive. Of note, it is lable-free, and requires no external modification on the biomolecules. The developed sensor provides a promising strategy for the cancer diagnosis in medical application in the future.

2. Experimental section

2.1. Reagents and chemicals

The aptamer of angiogenin used in this study was synthesized by TaKaRa Biotechnology (Dalian, China) Co., Ltd. The sequence of oligonucleotide employed is ferrocene-5′-CGG ACG AAT GCT TTG ATG TTG TGC TGG ATC CAG CGT TCA TTC TCA-$(CH_2)_6$-(SH)-3′. Sodium tetrachloroaurate (III) ($HAuCl_4$) and sodium citrate were purchased from Sigma-Aldrich (USA). Tris-base was purchased from Sigma-Aldrich. Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Alfa Aesar. 6-Mercapto-1-hexanol (MCH) was purchased from J&K Chemical Ltd.

All other reagents are of analytical reagent grade. All solutions were prepared with doubly distilled water. Immobilization buffer



Scheme 1. Schematic representation of the overall detection of angiogenin based on a conformational change of aptamer and amplification by P-GR/AuNPs composites-modified electrode.