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A facile label-free colorimetric aptasensor for acetamiprid based on the peroxidase-like activity of hemin-functionalized reduced graphene oxide

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ABSTRACT

A facile aptasensor has been developed for the colorimetric detection of acetamiprid by using the hemin-functionalized reduced graphene oxide (hemin-rGO) composites. The as-prepared hemin-rGO composites possessed both the ability of rGO to physically adsorb the aptamers and the peroxidase-like activity of hemin that could catalyse 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H_2O_2, to produce a solution with blue color. The well-dispersed hemin-rGO composites coagulated completely at the proper salt concentration; however, the coagulation of hemin-rGO was vanished when abundant aptamers were adsorbed on its surface because the attached negatively charged DNA backbone increased individual hemin-rGO electrostatic repulsion. In the detection scheme, acetamiprid with different concentrations was firstly incubated with the same amount of aptamer. The more acetamiprid in the tested solution, the less free aptamers were absorbed on the hemin-rGO surface, making the composites coagulate to a higher degree in the presence of the optimum NaCl concentration. As a consequence, the content of hemin-rGO in the supernatant was decreased after centrifugation, which catalysed oxidation of TMB in the presence of H_2O_2 to produce light blue color with a low absorbance. The color variation relavant to the acetamiprid concentration can be judged by the naked eyes and easily monitored by the inexpensive UV–vis spectrometer. Such designed aptasensor displayed a linear response for acetamiprid in the range from 100 nM to 10 μM with a detection limit of 40 nM ($S/N=3$). This colorimetric aptasensing platform offers great advantages including the simple operation process, low-cost portable instrument, and user-friendly applications.

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1. Introduction

Aptamers are single-stranded oligonucleotides that are capable of binding tightly and specifically to targets ranging from metal ions (Kawakami et al., 2000), molecules (Zuo et al., 2007), proteins (Bang et al., 2013), toxins (Wang et al., 2011), even to cells (Wang et al., 2010). In contrast to antibodies production which involve *in vivo* immunization of animals, aptamers are obtained from random-sequence DNA pools through *in vitro* selection technique known as systematic evolution of ligands by exponential enrichment (SELEX) (Bai et al., 2014; Alsager et al., 2014). Moreover, aptamers are known to offer a series of advantages over antibodies such as easy storage, simple synthesis, commercial availability, and enough stable in terms of temperature and biological activity (Yang et al., 2014). As promising alternative recognition elements, aptamers have been used extensively as affinity ligands instead of antibodies for many target molecules on the basis of different signal transducers such as fluorescence (Ozaki et al., 2006), electrochemiluminescence (Fang et al., 2008), and electrochemistry (Schoukroun-Barnes et al., 2014). Most of these strategies need aptamers to be labeled with signal materials; however, the introduction of a labelling substance makes these assays more complicated, time consuming, and laborious (Li et al., 2009). Besides, the multi-step labeling procedure affects the bioaffinity between the aptamers and their targets to a certain degree (Hayat et al., 2013). Therefore, there is an urgent need to develop a facile, rapid, and label-free detection strategy for aptasensing.

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Colorimetric assays for real-time naked-eye detection have attracted much attention due to its low cost, label-freeness, and simplicity (Kim and Jurng, 2013). Since the color changes can be read by the naked eyes, these assays only need cheap instrument or simple design, and can be performed without the presence of trained technician, make it very attractive for field analysis. Currently, the tremendous development in nanotechnology has produced many unique nano-materials with excellent peroxidase-like activity. Carbon nanodots (Shi et al., 2011), Fe_3O_4 nanoparticles (NPs) (Gao et al., 2007), and cubic Pt nanocrystals (Ma et al., 2011) are all reported to possess peroxidase-like activity, which can catalyse the oxidation of 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H_2O_2, to produce a solution with blue color. This kind of nanomaterials has been employed in the colorimetric sensing of some routine analytes such as glucose and H_2O_2 (Shi et al., 2011). Lately, efforts toward the construction of colorimetric biosensors have been realized by using graphene derivatives with their intrinsic peroxidase-like activity (Guo et al., 2011; Tao et al., 2013; Wei et al., 2013). For example, Guo et al. (2011) have prepared hemin-graphene nanosheets successfully through the π–π interactions. The resulting nanosheets possess not only the ability of rGO to distinguish single-stranded DNA (ss-DNA) and double-stranded DNA (ds-DNA), but also the peroxidase-like activity of hemin that can catalyse the reaction of peroxidase substrate. This attractive material has been used to develop a label-free colorimetric detection system for single-nucleotide polymorphism in disease-associated DNA. Very recently, Wei et al. (2013) have developed a colorimetric method for detection of ss-DNA and damage DNA by exploiting the same material.

Acetamiprid, a kind of systemic broad-spectrum insecticide, has been widely used for controlling various insect pests (Wanatabe et al., 2001). Despite the relatively low mammalian toxicity and special acting characteristics, acetamiprid can generate potential health risk of human beings, who are exposed to the environments polluted by acetamiprid (Imamura et al., 2010). Some conventional analysis methods including gas chromatography (Zhang et al., 2008), liquid chromatography (Seccia et al., 2008), high performance liquid chromatography (Obana et al., 2002), and enzyme-linked immunosorbent assays (Wanatabe et al., 2001), have been applied for the acetamiprid determinatrion. Although these analytical methods are sensitive and accurate, their practical applications have been hindered by some disadvantages, such as complicated sample pretreatment steps, high operation cost, usage of expensive instruments, time-consuming process, and high-skilled personnel (Xu et al., 2011). As a consequence, developing a facile sensing strategy for acetamiprid detection is of great significance. Recently, Fan et al. (2013) have developed an electrochemical aptasensors for acetamiprid by immobilizing thiol-terminated aptamer on the Au NPs deposited electrode surface. This label-free aptasensor based on electrochemical impedance spectroscopy (EIS) can efficiently overcome the complex and laborious aptamers labeling process. Based on the aptamer specific to acetamiprid and the color variation from the redispersion or aggregation of Au NPs, Shi et al. (2013) have established a highly sensitive and selective colorimetric assay for convenient acetamiprid detection. Though some progress has been made, the development of label-free colorimetric aptasensor for acetamiprid based on the peroxidase-like activity of nanomaterials has not been reported.

In light of above considerations, we sought to develop a facile label-free colorimetric aptasensor for acetamiprid based on the peroxidase-like activity of the hemin-functionalized reduced graphene oxide (hemin-rGO) composites. Under the proper salt concentration, the as-prepared hemin-rGO composites coagulated completely and the absorbance of the supernatant solution was very low. In the detection procedure, acetamiprid with different concentrations was firstly incubated with the same amount of aptamer. The conformation of aptamer was changed after binding with acetamiprid, which was caused by the formation of aptamer–acetamiprid complexes. The free aptamer strands left in the tested solution could be stably adsorbed on the hemin-rGO surface due to the π–π stacking interactions, which enhanced individual hemin-rGO electrostatic repulsion and resisted the salt-induced coagulation. Based on this, a selective colorimetric aptasensor was developed for convenient acetamiprid detection.

2. Experimental

2.1. Reagents

Graphite was purchased from Qingdao Tianhe Graphite Co., Ltd. Hemin, ammonium hydroxide (25%), hydrazine hydrate (85%), TMB, and H_2O_2 (30%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Aptamer with the sequence of 5′-TGT AAT TTG TCT GCA GCG GTT CTT GAT CGC TGA CAC CAT ATT ATG AAG A-3′ was chosen according to the previously reported literature (He et al., 2011) and purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Acetamiprid, chlorpyrifos, parathion-methyl, and pentachlorophenol were purchased from Aladdin Chemistry Co., Ltd. Other reagents were of analytical grade and used as received without further purification. Double-distilled water was used throughout the study.

Graphene oxide (GO) was prepared using modified Hummers method from graphite powders (Hummers and Offeman, 1958). Phosphate buffer solution (PBS) was prepared by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4. Aptamer stock solution was prepared with 25 mM Tris–HCl buffer solution (pH 7.4) and stored at 4 °C in dark.

2.2. Apparatus

The UV–vis absorption and Raman spectra were surveyed with a UV-2450 spectrophotometer (Shimadzu, Japan) and microscopic confocal Raman spectrometer (RM 2000, England), respectively. The scanning electron microscopy (SEM) was operated with FE-SEM (Hitachi S4800, Japan). An attached energy dispersive spectrometer (EDS) in the FE-SEM was applied to chemical composition analysis. Cyclic voltammetry (CV) experiments were performed on a CHI-660B electrochemical workstation (Chenhua Instruments Co., Shanghai, China), which was connected to a conventional three-electrode cell using glassy carbon electrode (GCE) as a working electrode, saturated calomel electrode (SCE) as a reference electrode, and Pt wire as a counter electrode. EIS was performed in a 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-/4-}$ with a frequency range from 0.01 Hz to 10 kHz at 0.25 V, and the amplitude of the applied sine wave potential in each case was 5 mV.

2.3. Preparation of hemin-rGO composites

The hemin-rGO composites were synthesized according to Guo's method (Guo et al., 2011) but with some modification. Briefly, 10 mg of GO was added into 20 mL of water and sonicated for 1 h to obtain a homogeneous dispersion. The resulting dispersion was mixed with 20 mL of 0.5 mg mL^{-1} hemin aqueous solution in a round-bottomed flask, followed by the addition of 30 μL of hydrazine hydrate and 200 μL of ammonium hydroxide. After stirring for 1 h, the above solution was heated to 60 °C with vigorous stirring for another 3.5 h. After being cooled to room temperature, the solution was filtered with 0.22 μm millipore

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