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ORIGINAL ARTICLE

Characterization of a sensitive biosensor based on an unmodified DNA and gold nanoparticle composite and its application in diquat determination

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Abstract DNA usually adsorbs gold nanoparticles by virtue of mercapto or amino groups at one end of a DNA molecule. However, in this paper, we report a sensitive biosensor constructed using unmodified DNA molecules with consecutive adenines (CA DNA) and gold nanoparticles (GNPs). The CA DNA–GNP composite was fabricated on gold electrodes and characterized by using of scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and the electrochemical method. Using an electrochemical quartz crystal microbalance (EQCM), the mechanism by which the CA DNA and GNPs combined was also studied. The modified electrode exhibited an ultrasensitive response to diquat. Differential pulse voltammetry (DPV) was used to study the linear relationships between concentrations and reduction peak currents, ranging from 1.0×10^{-9} M to 1.2×10^{-6} M. The detection limit of it is 2.0×10^{-10} M. The feasibility of the proposed assay for use in human urine and grain was investigated, and the satisfactory results were obtained.

1. Introduction

Diquat (1,1′-dimethyl-4, 4′-bipyridilium dibromide) is one of the most widely used herbicides, and holds the largest share of the global herbicide market until recently overtaken by glyphosate (Mhammedi et al., 2009). However, DQ possesses undesirable characteristics those are highly toxic to human health, the repeated exposures may cause skin irritation, sensitization, or ulcerations on contact (Vanholder et al., 1981).

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With a remarkable persistence in the environment, DQ represents a potential danger to the natural waters due to its high solubility (about 620 g L^{-1} at 25 °C) (Bethesda, 1988). From above, whether the DQ can be accurately quantified in samples has become important. Therefore, there is an increasing need for rapid reliable method to measure DQ concentrations in the real samples.

Nowadays, a numerous of analytical techniques have been applied for the analysis of DQ, such as fluorescence (Pérez-Ruiz et al., 1991; Carrillo-Carrión et al., 2011), chromatography (Shōzi et al., 1984; Cherukury et al., 1995) and chromatography/mass spectrometry (Rafael and Mauricio, 2007; Hao et al., 2013). Although chromatography is a conventional method, it often requires a concentration step to improve the detection limits, resulting in sample destruction (Walcarius and Lamberts, 1996). Moreover, other methods are comparatively complicated and require expensive equipments, highly qualified technicians and specialized laboratories. By contrast, electrochemistry is an interesting and convenient alternative that simplifies detection processes and enhances the response signals of the analytes (Walcarius and Lamberts, 1996).

In recent years, the development of DNA biosensor has received much attention because of its extreme importance in numerous fields. As is well known, the immobilization of DNA onto electrode surfaces is one of the key steps toward DNA sensor development (Zhang et al., 2010). Usually, by virtue of the mercapto or amino groups at one end of DNA molecules, they can be anchored on gold nanoparticles (GNPs) because GNPs have large specific surface area, high surface free energy, better biocompatibility, and suitability for constructing DNA biosensors (Zhang et al., 2010; Lin et al., 2010). Nevertheless, some of the deficiencies for this strategic assembly are the imprecise controls of the orientation and conformation of surface-tethered DNA. In addition, the coulomb and electromagnetic interactions between nanoparticles are prominent effects on the distribution of GNPs (Yao and Yaomura, 2013). Thus, investigating a new fabrication strategy to better control the interaction forces between GNPs and finding a new method to assemble DNAs are necessary.

The study on the affinity of DNA for combining with GNPs revealed that the four nucleotides display high affinities, whereas adenine interacts much more strongly with the gold surface compared with the other nucleotides (Storhoff et al., 2002). Fan and co-workers demonstrated that the affinities of consecutive adenines (CA, which means continuous arrangement of adenine bases,) were even comparable to the strength of an Au−S chemical bond. Furthermore, the DNA monolayer fabricated with CA blocks (CA DNA) showed better order and the upright conformation of it increased advantages in target DNA hybridization or detection (Pei et al., 2012). In addition, the method offers a more cost-effective alternative, considering that the thiol or amino fabrications can be > 90% of the total cost of DNA synthesis (for example, $0.30/bp for gene synthesis while $50 for DNA modification for Sangon Biotech Co., Ltd.) (Zhang et al., 2012).

Our previous report has presented a biosensor constructed by three-dimensional (3D) GNPs and studied the simultaneous determination of dopamine, uric acid, adenine and guanine (Niu et al., 2013). However, the 3D GNPs were constructed by unmodified double-strand DNA (ds-DNA) and the lesser exposure of bases and the rigid structure of ds-DNA make them exhibit less interaction with GNPs (An and Jin, 2012). This paper aimed to report a novel DNA fabrication method by virtue of the CA DNA. With the aid of CA blocks, CA DNAs could be fabricated on GNPs in a highly order and upright structure. The sensitivity of the presented biosensor was consequently improved. The electrostatic interactions between DQ and all of the CA DNAs, are the main reaction to interpret. The CA DNA–GNP composite was successfully applied in human urine and grain determination with satisfactory results.

2. Experimental

2.1. Reagents

4-Mercaptobenzoic acid, 4-aminothiophenol, N-(3-dimethyla minopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and DQ were purchased from Sigma. Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4 \cdot 3H_2O$) and sodium citrate were obtained from the Sinopharm Chemical Reagent Co., Ltd. and were used as received.

Human urine samples were obtained from The Second Hospital of Hebei Medical University. Phosphate buffer solutions (PBS, 0.1 M) of different pH values were prepared by mixing stock solutions of 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4 (Shanghai Chemical Reagent Company). The pH was adjusted using 0.1 M H_3PO_4 or NaOH (Shanghai Chemical Reagent Company). Citrate buffer (0.01 M, pH 3.0) was prepared by mixing 0.01 M citric acid and 0.01 M sodium citrate. $K_3Fe(CN)_6$ and KCl were also obtained from the Shanghai Chemical Reagent Company. All chemicals used were of analytical-reagent grade. Water (> 18 MΩ cm) was obtained from a SMART ultra-pure water system.

Various oligonucleotides were purchased from the Shanghai Sangon Bioengineering Technology and Services Co., Ltd. The solutions (0.1 mM) were prepared with citrate buffer (pH 3.0) and stored at 4 °C. Their sequences were as follows:

Probe ss-DNA (1): 5′-AAA TAC GCC ACC AGC TCC-3′
Target ss-DNA (2): 5′-AAA GGA GCT GGT GGC GTA-3′

2.2. Apparatus

Electrochemical impedance spectroscopy (EIS) was performed on a CHI 650D and electrochemical experiments were carried out on a CHI 440 electrochemical workstation (Chen Hua Instruments Co., Shanghai, China) with a three-electrode system, which includes the working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. The electrochemical quartz crystal microbalance (EQCM) experiments were performed on a CHI 440 electrochemical workstation with a 7.995 MHz AT-cut quartz crystal. A polished Au/Cr-coated AT-cut quartz crystal was used as the working electrode. The reference and the counter electrodes were similar to those used in the electrochemical studies. All potentials were provided with respect to the Ag/AgCl electrode (saturated KCl).

The sizes of GNPs were measured using a transmission electron microscopy (TEM, HITACHI-600, HITACHI Co., Japan) and gold colloid concentration was determined with