Electrochimica Acta 153 (2015) 190–199

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Electrochemical Behavior of Paraquat on a Highly Ordered Biosensor Based on an Unmodified DNA-3D Gold Nanoparticle Composite and Its Application

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ARTICLE INFO

Article history:
Received 25 August 2014
Received in revised form 16 November 2014
Accepted 29 November 2014
Available online 2 December 2014

Keywords:
unmodified DNA
gold nanoparticles
paraquat

ABSTRACT

DNA usually adsorbs gold nanoparticle by virtue of mercapto or amino group at one end of a DNA molecule. However, in this study, we report a highly ordered biosensor constructed using unmodified DNA molecules with consecutive adenines and three-dimensional gold nanoparticles (3D GNPs). The unmodified DNA-3D GNP composite was fabricated on gold electrodes and characterized through the use of scanning electron microscopy (SEM), atomic force microscopy (AFM), and electrochemical methods. Using an electrochemical quartz crystal microbalance (EQCM), the mechanism by which the unmodified DNA and GNPs combined was also studied. The modified electrode exhibited an ultrasensitive response to paraquat. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to study the linear relationships between the concentrations and the reduction peak currents. The linear relationship for DPV is 7.0×10^{-9} M to 1.5×10^{-6} M with a detection limit of 2.0×10^{-10} M. The redox mechanism of paraquat on this modified electrode was also elucidated. The feasibility of the proposed assay for use in human serum, human urine, and natural samples was investigated, and satisfactory results were obtained.

1. Introduction

Paraquat (1,1′-dimethyl-4-4′-bipyridinium) (PQ) is widely used as a nonselective herbicide or desiccant for various crops in over 130 countries [1–3]. However, PQ possesses undesirable characteristics making it highly toxic to plants and aquatic organisms [4]. With a remarkable persistence in the environment, PQ represents a potential danger to the health of entire ecosystems because of its high solubility in water (approximately 620 g L^{-1} at 25 °C) [1,5]. More importantly, PQ is extremely toxic to humans (LD50 = 35 mg kg^{-1}) and animals (LD50 = 110–150 mg kg^{-1}) because of its rapid reduction [3]. Ingesting PQ can cause such symptoms as liver, lung, heart, and kidney failure within several days up to several weeks, which can lead to death up to 30 days after ingestion. People who suffer high exposure to PQ are unlikely to survive. Some studies even showed a link between PQ use and Parkinson's disease in farm workers because PQ increases the production of certain oxygen derivatives that may harm cellular structures [6]. Whether the PQ can be timely eliminated and accurately quantified in an individual's body have become crucial factors in determining the survival and recoveries of patients. Therefore, a research to find a rapid and accurate procedure for PQ determination is urgently needed.

Currently, a wide range of analytical techniques have been applied for the analysis of PQ, such as spectrophotometry [4,7,8], liquid chromatography [9–11], capillary electrophoresis [12] and gas chromatography/mass spectrometry [13]. Although spectrometry and liquid chromatography are conventional methods, they often require a concentration step to improve the detection limits, resulting in destruction of the samples [14]. 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 [15,16], particularly when using surface electrochemical techniques [17].

In recent years, the development of a DNA biosensor has received much attention because of its extreme importance in

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http://dx.doi.org/10.1016/j.electacta.2014.11.191
0013-4686/

numerous fields. As is well known, the immobilization of DNA onto electrode surfaces is one of the key steps toward the development of a DNA sensor [18]. Usually, by virtue of the mercapto or amino groups at one end of the DNA molecules, they can be anchored onto gold nanoparticles (GNPs) because GNPs have large specific surface areas, high surface free energies, high biocompatibility, and high suitability for constructing DNA biosensors [17–22]. Nevertheless, several of the deficiencies of this strategic assembly are the imprecise control of the orientation and conformation of the surface-tethered DNA. In addition, the coulombic and electromagnetic interactions between nanoparticles have prominent effects on the distribution of the GNPs [23]. Thus, investigating a new fabrication strategy to better control the forces of interaction between the GNPs and finding a new method to assemble the tethered DNA molecules are necessary.

The study on the interaction of DNA 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 [24]. Fan and co-workers demonstrated that the affinities of consecutive adenines (CA) were even comparable to the strength of an Au—S chemical bond. Furthermore, the DNA monolayer fabricated with CA blocks showed better order, and the upright conformation of it increased advantages in the target DNA hybridization or detection [25]. In addition, the method offers a more cost-effective alternative, considering that 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 by the Sangon Biotech Co., Ltd.) [26].

Our previous report described a biosensor constructed with three-dimensional (3D) GNPs and studied the simultaneous determination of dopamine, uric acid, adenine and guanine [27]. However, the 3D GNPs were constructed by unmodified double-strand DNA (ds-DNA), and the smaller exposure of bases and the rigid structure of ds-DNA make them interact less with the GNPs [28]. This paper aims to report a novel DNA fabrication method by virtue of CA blocks of unmodified DNA molecules. Using the unmodified DNA molecules as cross-linkers (CL DNA), highly ordered and 3D-distributed GNPs were constructed. Compared with poorly-ordered DNAs, well-ordered DNAs can anchor more GNPs and make a good preparation for the next fabrication step. The sensitivity of the presented biosensor was consequently improved. To further increase the sensitivity of the biosensors, hairpin DNAs (H-DNAs) have been utilized as the terminal monolayer in the fabrication process. Compared to other DNA sensors, it is well known that the H-DNA probe displays an extraordinary stability, better selectivity, higher sensitivity, better reproducibility, faster speed, and greater convenience [29,30]. In the present paper, H-DNAs were also synthesized with CA blocks to be fabricated onto GNPs. Due to the orderliness of the CA blocks, each GNP provided more space to anchor more H-DNAs. Consequently, the sensitivity of the presented biosensor was further enhanced. Based on the electrostatic interactions between PQ and all of the DNA molecules, the determination and reaction mechanism of PQ were investigated. The unmodified DNA-3D GNP composite was successfully applied for the determination of PQ in body fluids and natural samples with satisfactory results. Compared with other methods, such as reference [1], the presented method in this paper showed a wider linear range and a better sensitivity.

2. Experimental

2.1. Reagents

4-Mercaptobenzoic acid, 4-aminothiophenol, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and PQ 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 blood 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 of the 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:

ss-DNA (1): 5′-AAA TAC GCC ACC AGC TCC-3′

Complementary ss-DNA (2): 5′-AAA GGA GCT GGT GGC GTA-3′

H-DNA (3): 5′-AAA CTG ACG TCA GCC ACC TAC TCG AAG TAT GGC TGA CGT CAG-3′

ss-DNA (4): SH-5′-TAC GCC ACC AGC TCC-3′

Complementary ss-DNA (5): SH -5′ -GGA GCT GGT GGC GTA-3′

H-DNA (6): SH-5′-CTG ACG TCA GCC ACC TAC TCG AAG TAT GGC TGA CGT CAG-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 of the potentials were provided with respect to the Ag/AgCl electrode (saturated KCl).

The sizes of the GNPs were measured using a transmission electron microscopy (TEM, HITACHI-600, HITACHI Co., Japan), and the gold colloidal solution concentration was determined with a UV–vis spectrophotometer (UV-7500, Keda Instrument Co., Ltd.). Scanning electron microscopy (SEM) images were obtained using a HITACHI S-4800 instrument operated at 0 and 3 kV (HITACHI S-4800, HITACHI Co., Japan). Atomic force microscopy (AFM) images were obtained using Nanoscope R (III) instrument (Digital Instruments Veeco Metrology Group). A SMART ultra-pure water system (Heal Force SMART-N, Heal Force Development Ltd.) was also used. All of the electrochemical experiments were carried out under the protection of high-purity nitrogen.

2.3. H-DNA/GNP/CL DNA/GNP/thiol composite preparation

According to previous works [25,26], a gold colloidal solution with a 13 nm dimension was selected for use in the following research. Gold colloidal solutions were prepared according to the method of Frens [31] with slight modifications. Briefly, 50.0 mL of 0.2 g L^{-1} $HAuCl_4$ was brought to a rolling boil with vigorous stirring in a 100 mL round-bottom flask. The rapid addition of 4.0 mL of 10 g L^{-1} sodium citrate to the vortex of the solution resulted in a color change from pale yellow to orange red. Boiling was continued