Chinese Chemical Letters 25 (2014) 29–34

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Electrochemical DNA nano-biosensor for the detection of genotoxins in water samples

Hong-Bo Xu [a,b], Ran-Feng Ye [b], Shang-Yue Yang [b], Rui Li [b], Xu Yang [b,*]

[a] Wuhan No. 11 High School, Wuhan 430030, China
[b] Lab of Environmental Biomedicine, Central China Normal University, Wuhan 430079, China

ARTICLE INFO

Article history:
Received 4 June 2013
Received in revised form 18 August 2013
Accepted 16 September 2013
Available online 12 November 2013

Keywords:
Electrochemical DNA biosensor
Au nanoparticles
Genotoxic detection
DNA damage

ABSTRACT

In the present study, a disposable electrochemical DNA nano-biosensor is proposed for the rapid detection of genotoxic compounds and bio-analysis of water pollution. The DNA nano-biosensor is prepared by immobilizing DNA on Au nanoparticles and a self-assembled monolayer of cysteamine modified Au electrode. The assembly processes of cysteamine, Au nanoparticles and DNA were characterized by cyclic voltammetry (CV). The Au nanoparticles enhanced DNA immobilization resulting in an increased guanine signal. The interaction of the analyte with the immobilized DNA was measured through the variation of the electrochemical signal of guanine by square wave voltammetry (SWV). The biosensor was able to detect the known genotoxic compounds: 2-anthramine, acridine orange and 2-naphthylamine with detection limits of 2, 3 and 50 nmol/L, respectively. The biosensor was also used to test actual water samples to evaluate the contamination level. Additionally, the comparison of results from the classical genotoxicity bioassay has confirmed the applicability of the method for real samples.

1. Introduction

More and more pollutants exhibiting genotoxicity have invaded the environment in recent decades, which are threatening human health. Genotoxic contaminants from industrial effluents are often discharged into surface water, and then later used downstream for agricultural irrigation, or even as sources of drinking water. Many studies have shown the presence of several organic mutagens and carcinogens in drinking waters, and epidemiological studies have highlighted the correlation between genotoxicity of drinking water and increased risks of cancer [1–4]. Therefore, it is essential to establish simple and rapid screening techniques to determine the presence of genotoxins and evaluate the effects of water quality on human health.

Currently, the pollutants can be determined by gas chromatography, high performance liquid chromatography or spectroscopy, which offer the possibility of identifying and quantifying specific compounds with high resolution and good precision. However, these chemical measurements cannot reflect the real effects of genotoxicity because genotoxicity is a biological response. Only bioassays are able to evaluate the combined action from potentially hazardous compounds as complex mixtures in environment. To date, several classic bioassays have been established for genotoxicity analysis, including the *Salmonella typhimurium* mutagenicity test with strains TA98 and/or TA100 (Ames test) [5,6], single-cell gel electrophoretic (SCGE or Comet) assay [7,8], micronucleus assay [9] and so on. The genotoxicity bioassays can determine the effects of hazardous compounds on nuclear DNA, providing direct and appropriate measurements of genotoxicity, but they are time-consuming, can be costly and analyses can require several days or weeks. Compared with these methods, the electrochemical DNA biosensor may be an ideal candidate for screening of these pollutants, as they require a small amount of sample and are based on biological interaction [10].

The most critical step in the preparation of DNA electrochemical biosensors is the immobilization of DNA strands on the surface of an electrode. The amount of immobilized DNA directly affects the accuracy, sensitivity and selectivity of the DNA electrochemical sensors [11]. In recent years, there has been great progress in the application of nanomaterials in biosensors to elevate sensitivity and enhance electrochemical performance [12–15]. Additionally, the high surface-to-volume ratio of Au nanoparticles can assemble larger volumes of DNA on the electrode surface [16,17].

The present study aimed at developing a more sensitive electrochemical DNA biosensor for the detection of genotoxins in water samples. A monolayer of cysteamine was self-assembled on the Au electrode surface first, and then Au nanoparticles were assembled on it. After the thiolated ssDNA were immobilized onto Au nanoparticles to form the ssDNA/Nano/Au, the dsDNA/Nano/Au biosensor was constructed by the hybridization of complementary

* Corresponding author.
E-mail address: yangxu@mail.ccnu.edu.cn (X. Yang).

1001-8417/$ – see front matter
http://dx.doi.org/10.1016/j.cclet.2013.10.011

DNA onto the thiolated ssDNA. Then, the degree of oxidative damage caused to DNA was monitored by the change of the peak current as a consequence of guanine oxidation. After comparison between the performances of the ssDNA/Nano/Au and the dsDNA/Nano/Au on standard 2-anthramine, the dsDNA/Nano/Au was used as the DNA nano-biosensor to test actual water samples.

2. Experimental

The synthetic DNA oligonucleiotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China). The base sequences are as follows [18]: thiol-terminated DNA probe: 5′-SH–$(CH_2)_6$–CAG GCG GCC GCA CAC GCC TCC A-3′, complementary target: 5′-TGG AGG ACG TGT GCG GCC GCC TG-3′. Stock solutions of the oligonucleotides were dissolved in 20 mmol/L Tris(hydroxymethyl)aminomethane (tris)–HCl (pH 8.0) containing 100 mmol/L $MgCl_2$, and kept refrigerated at −20 °C. De-ionized water was used throughout all experiments.

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4{\cdot}3H_2O$) (99.9%), trisodium citrate, 2-anthramine, acridine orange and 2-naphthylamine were obtained from Sigma–Aldrich Company (USA). All the reagents were analytical grade and were used as received.

A JEM-2100 transmission electron microscope (TEM) (JEOL, Japan) operating at 200 kV was used to size the Au nanoparticles. Electrochemical experiments were performed on A CHI 660 C Electrochemical Workstation (Shanghai CH Instrument Company, China) with a three-electrode system using the modified Au electrode as the working electrode, a platinum wire as the counter electrode and a saturated calomel reference electrode (SCE) as the reference electrode. All the potentials were referred to the SCE reference electrode.

Water samples (X1-3) were collected at three sites from a village in Henan Province, China. Each water sample (3 L) was firstly filtered under vacuum using a glass fiber filter with a 0.22 μm pore size. The filtered water samples were then acidified (pH 3.0) with 20% acetic acid and passed through pre-conditioned Oasis HLB cartridges (200 mg/6cc, 30 μm partial size, Waters Corporation, USA) at a rate of approximately 3 mL/min. Each sample was eluted with acetone (11 mL), and then evaporated to dryness under a gentle nitrogen stream at 37 °C. The residue was immediately dissolved in 1 mL dimethylsulfoxide (DMSO) as stock extract solution. Three dilutions were made from the stock extract solutions for genotoxicity analysis, in which each 1 mL was equivalent to 25, 50 or 100 mL of the original water source, respectively (the dosages were described as 25×, 50× or 100×).

2.1. Au nanoparticles preparation

The Au nanoparticles were prepared by the citrate reduction of $HAuCl_4$ according to the literature [19]. Briefly, 10 mL of 38.8 mmol/L trisodium citrate was added to 100 mL of boiling 1.0 mmol/L $HAuCl_4$ solution and stirred for 15 min at the boiling point. The solution color turned to wine red, indicating the formation of Au nanoparticles. Then, the solution was allowed to cool to room temperature and stored in a dark bottle at 4 °C. The prepared Au nanoparticles have an average diameter of approximately 15 nm measured by TEM (Fig. 1).

2.2. Preparation and characterization of the modified electrode

The Au electrode surface was polished with 1 μm, 0.3 μm and 0.05 μm α-Al_2O_3 powder, and washed with de-ionized water and 95% ethanol. Before surface modification, the bare Au electrode was scanned in 0.5 mol/L H_2SO_4 between 0.3 V and 1.5 V until a reproducible cyclic voltammogram was achieved. Following that, the electrode was washed with ultra pure water and dried under a pure nitrogen stream.

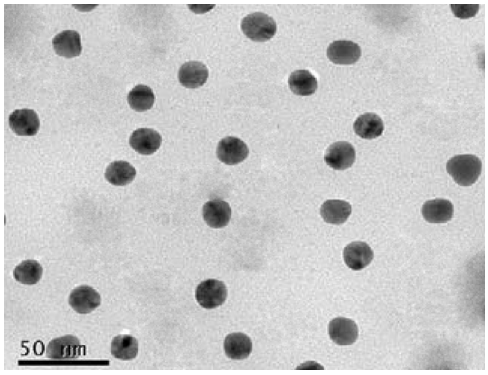

Fig. 1. The TEM image of the Au nanoparticles.

The procedures for assembling the Au nanoparticles onto the Au electrode surface were according to the literature [17] with only slight modification. Briefly, the cleaned electrode was initially immersed into 10 mmol/L cysteamine for 2 h under ambient conditions to allow the self-assembly of cysteamine, followed by immersion into the Au nanoparticle solution for 10 h. After this period, the electrode was washed with water to remove unbounded Au nanoparticles.

The steps for DNA immobilization and DNA hybridization were according to the previous literature with a minor modification [18]. The electrode modified with Au nanoparticles was immersed in 15 μL of 2 μmol/L thiolated ssDNA at 4 °C for 6 h to obtain the ssDNA/Nano/Au. Then the ssDNA/nano/Au was rinsed with water to avoid the physical absorption. The dsDNA/Nano/Au was prepared by pipetting 15 μL of 2 μmol/L complementary DNA onto the ssDNA/Nano/Au, and kept for 2 h to perform DNA hybridization at room temperature. Subsequently, the obtained dsDNA/Nano/Au was washed with water and stored at 4 °C until use.

The modified electrodes were characterized by electrochemical scanning in 2.5 mmol/L $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 mol/L KCl. Cyclic voltammetry (CV) measurements were swept from 0 V to 0.6 V with a sweeping rate of 0.1 Vs.

For comparison with and without Au nanoparticles modification, the cleaned Au electrode was immersed in 15 μL of 2 μmol/L thiolated ssDNA solution under the same condition to obtain the ssDNA/Au. The dsDNA/Au was also prepared by pipetting 15 μL of 2 μmol/L complementary DNA onto the ssDNA/Au.

2.3. Detection of genotoxic compounds with DNA-nano biosensor

The peak current of guanine was used as the transduction signal for recognizing DNA interacting agents, and the current signal was measured by square wave voltammetry (SWV) in 0.5 mol/L acetate buffer solution (pH 4.7, containing 10 mmol/L of sodium chloride) at room temperature. For SWV measurements, a potential range between +0.5 V and +1.2 V, frequency of 200 Hz, step potential of 15 mV and amplitude of 40 mV were applied. The volume of the electrochemical cell used in the study was 20 mL.

As the result of interaction of DNA with a genotoxic compound, a decrease of guanine peak current was observed. The decrease percentage of guanine peak current (D_{pc}) was used to evaluate the potentially toxic analytes: $D_{pc} = [1 - (I_s/I_b)] \times 100\%$, where I_b is the peak current of DNA-nano-biosensor scanned in acetate buffer before treatment with the analyte, and I_s is the peak current of the DNA-nano biosensor after incubated with the analyte solution for 20 min.