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An electrochemical sensor based on single-stranded DNA–poly(sulfosalicylic acid) composite film for simultaneous determination of adenine, guanine, and thymine

Li-Jun Feng, Xiu-Hua Zhang *, Ping Liu, Hua-Yu Xiong, Sheng-Fu Wang

Ministry of Education, Key Laboratory for the Synthesis and Application of Organic Functional Molecules, and College of Chemistry and Chemical Engineering, Hubei University, Wuhan 430062, People's Republic of China

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ABSTRACT

Poly(sulfosalicylic acid) and single-stranded DNA composite (PSSA–ssDNA)-modified glassy carbon electrode (GCE) was prepared by electropolymerization and then successfully used to simultaneously determine adenine (A), guanine (G), and thymine (T). The characterization of electrochemically synthesized PSSA–ssDNA film was investigated by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS). The modified electrode exhibited enhanced electrocatalytic behavior and good stability for the simultaneous determination of A, G, and T in 0.1 M phosphate buffer solution (PBS, pH 7.0). Well-separated voltammetric peaks were obtained among A, G, and T presented in the analyte mixture. Under the optimal conditions, the peak currents for A, G, and T increased linearly with the increase of analyte mixture concentration in the ranges of 6.5×10^{-8} to 1.1×10^{-6}, 6.5×10^{-8} to 1.1×10^{-6}, and 4.1×10^{-6} to 2.7×10^{-5} M, respectively. The detection limits (signal/noise = 3) for A, G, and T were 2.2×10^{-8}, 2.2×10^{-8}, and 1.4×10^{-6} M, respectively.

The detection and quantitation of nucleotides, nucleosides, and their bases have become increasingly important in the field of biomedical research. The presence of nucleic acid components in physiological fluids, tissues, and cells results from catabolism of nucleic acid, enzymatic degradation of tissues, dietary habits, and various salvage pathways. Changes of the concentration of these components may reflect substantial alterations in the activity of catabolic, anabolic, and interconversion enzymes and may be used to indicate the presence of various disease states that cause alterations in the normal purine and pyrimidine metabolic pathways [1]. Hence, determination of individual concentrations of adenine (A)[1], guanine (G), and thymine (T) or their ratio in DNA is important for the measurement of nucleic acid concentration itself [2]. So far, a large number of methods have been established for their determination, including mass spectrometry (MS) [3], high-performance liquid chromatography (HPLC) [4], ion-pairing liquid chromatography (IPIC) [5], capillary electrophoresis (CE) [6], flow injection–chemiluminescence (CL) [7], and spectroscopic methods [8,9]. However, some of these mentioned methods have the disadvantages of time-consuming, high cost, low sensitivity, and complicated pretreatment. Electrochemical methods provide an easy and fast way to detect A, G, and T. Wang and coworkers [10] reported a β-cyclodextrin/multiwalled nanotube (MWNT)-modified electrode for simultaneous determination of G and A with detection limits of 6.6×10^{-7} and 1.5×10^{-6} M, respectively. Tang and Chen [11] investigated a composite film that contained MWNTs along with the incorporation of poly(new fuchsin) (PNF) for the simultaneous determination of A, G, and T. They found that the sensitivity of the composite film toward A, G, and T in the differential pulse voltammetry (DPV) technique was higher than that in the MWNT film. Diamond electrode is another topical and well-known alternative that shows good properties such as low detection limit and good reproducibility. However, for some coexisting electrochemical active systems, the diamond electrode is not suitable for simultaneous determination because of its low electrocatalytic activity and selective quantitation.

Polymer-modified electrodes have attracted enormous interest in the past few years due to their good stability, reproducibility, more active sites, homogeneity in electrochemical deposition, and strong adherence to electrode surface [12]. Fabrication of conducting polymer film is flexible and controlled; hence, it provides an attractive means of overcoming the problems caused by the solvent evaporation method [13]. Among the different methods for preparing polymeric-modified electrode, electropolymerization is a good approach to immobilize polymers on electrodes because adjusting the electrochemical parameters can

* Corresponding author. Fax: +86 27 88663043.
E-mail address: zhanganal@yahoo.com.cn (X.-H. Zhang).

[1] *Abbreviations used:* A, adenine; G, guanine; T, thymine; MS, mass spectrometry; HPLC, high-performance liquid chromatography; IPIC, ion-pairing liquid chromatography; CE, capillary electrophoresis; CL, chemiluminescence; MWNT, multiwalled nanotube; PNF, poly(new fuchsin); DPV, differential pulse voltammetry; GCE, glassy carbon electrode; C, cytosine; ssDNA, single-stranded DNA; PSSA, poly(sulfosalicylic acid); PBS, phosphate buffer solution; dsDNA, double-stranded DNA; SCE, saturated calomel electrode; EIS, electrochemical impedance spectroscopy; SEM, scanning electron microscopy; CV, cyclic voltammetry; LSV, linear sweep voltammetry; S/N, signal/noise.

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control film thickness, permeation, and charge transport characteristics [14]. The chemically modified electrodes by polyaniline [15], polythiophene [16], or polypyrrole [17] have been used to detect electroactive materials such as dopamine, ascorbic acid, nitrate, norepinephrine, hemoglobin, and so on [18]. As far as we know, there have been very few reports about poly(sulfosalicylic acid) film-modified glassy carbon electrode (GCE).

DNA carries heritage information and instructs the biological synthesis of proteins and enzyme through the process of replication and transcription of genetic information, so it plays a major role in the life process. DNA is quite often the main cellular target for studies with smaller molecules of biological importance such as carcinogens, steroids, and several classes of drugs [19–22]. It is a natural polymer consisting of three parts: a phosphate acid group, a basic group (A, G, T, or cytosine [C]), and a sugar unit. The structure consists of two molecular chains [23]. Experimental results indicate that the single-stranded DNA (ssDNA) can be adsorbed on the surface of the GCE and exhibit an enhanced electrochemical oxidation with the increase of the peak current of G, A, and T.

In this work, the advantages of poly(sulfosalicylic acid) and ssDNA composite-modified GCE (PSSA–ssDNA/GCE) were illustrated by comparison with the PSSA and bare GCE for electrochemical sensing. The electrochemical behaviors of A, G, and T were investigated using DPVs at the prepared composite-modified electrode. Separation of the oxidation peak potentials were 289 and 262 mV between G-A and A-T in pH 7.0 phosphate buffer solution (PBS), making it suitable for simultaneous determination of these compounds.

Materials and methods

Chemicals and apparatus

A, G, and T were obtained from Sinopharm Chemical Reagent (Beijing, China). Double-stranded DNA (dsDNA) from calf thymus was obtained from Sigma. ssDNA was produced by heating a native dsDNA solution in a 100 °C water bath for approximately 15 min and then rapidly cooling in an ice bath. Sulfosalicylic acid (>99.0%) was purchased from Anhai Chemical (Hefei, China). Phosphate buffer was prepared by 0.1 M Na_2HPO_4–KH_2PO_4. The pH value was adjusted with NaOH and H_3PO_4. All other chemicals were of analytical reagent grade. All solutions were prepared with double-distilled water.

Electrochemical measurements were performed by a model CHI660C electrochemical workstation (CH Instruments, Chenhua, Shanghai, China) controlled by a personal computer. A three-electrode system was used in the measurements, with a bare GCE (2 mm in diameter) or modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. All following potentials reported in this work were against the SCE. The electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl and plotted in the form of complex plane diagrams (Nyquist plots). They were recorded with a frequency range of 0.1 Hz to 100 kHz. The amplitude of the applied sine wave potential is 5 mV, with a formal potential of 0.2 V. The observations of the morphology of samples were performed using scanning electron microscopy (SEM) with a JSM-5510 LV (Jeol).

Fabrication of PSSA–ssDNA biosensor

The bare GCE was carefully polished with 0.05 μm of gamma alumina powder (γ-Al_2O_3) on chamois leather and then washed ultrasonically in water and then ethanol. The aqueous micellar medium was prepared by dissolving 10 mM sulfosalicylic acid in an aqueous solution containing 0.1 mg/ml ssDNA. The PSSA–ssDNA/GCE was formed in the prepared mixture solution by cyclic voltammetry (CV) with a potential scanning range of −1.0 to +2.0 V at 100 mV s^{-1} for 10 scans. The modified electrode was electroactivated by CV from −0.2 to +0.6 V at 100 mV s^{-1} in PBS (pH 7.0). The PSSA/GCE was prepared at the same mixture solution as mentioned above without adding ssDNA. All resulting electrodes were washed with water and stored in PBS at room temperature when not in measurements.

Results and discussion

Electrochemical impedance characterization of PSSA–ssDNA composite film

To investigate the ssDNA loading on the surface of GCE, the EIS was used to monitor the change of the electrode surface during the modification process. The EIS includes a semicircular part and a linear part. The semicircular part at higher frequencies corresponds to the electron transfer limited process, and the diameter is equivalent to the electron transfer resistance (R_{et}), which reflects the electron transfer kinetics of the redox probe at the electrode interface. Fig. 1 shows the Nyquist plots of the EIS obtained in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution at the bare GCE, PSSA/GCE, and PSSA-ssDNA/GCE. The EIS response for bare GCE formed nearly a straight line (Fig. 1, curve a), indicating that there was almost no heterogeneous charge transfer resistance. The R_{et} value for PSSA/GCE was 4100 Ω (Fig. 1, curve b). However, the R_{et} value for PSSA–ssDNA/GCE was increased to 9400 Ω (Fig. 1, curve c), most likely due to the binding of DNA to the conductive support leading to dielectric behavior for the interfacial electron transfer process [24]. The change of the R_{et} value during the modification process clearly suggests that the ssDNA is tightly assembled onto the PSSA/GCE.

Characterization of PSSA–ssDNA/GCE surface

The morphologies of PSSA and PSSA–ssDNA were characterized by SEM. As shown in Fig. 2A, it was very clear that small particles (size < 500 nm) were observed on the surface of the film; this should be attributed to the GCE surface being covered by PSSA. The surface of PSSA–ssDNA composite film (Fig. 2B) was quite uniform and exhibited a curly morphology consisting of a dendritic

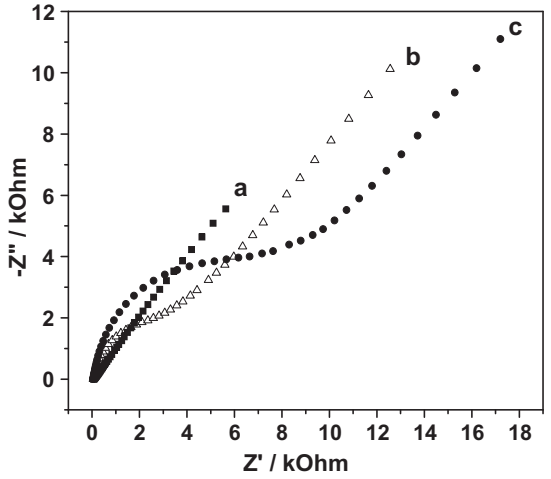

Fig.1. Nyquist plots of different electrodes in 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl with frequency of 0.1 Hz to 100 kHz for bare GCE (a), PSSA/GCE (b), and PSSA–ssDNA/GCE (c).