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Simultaneous detection of guanine, adenine, thymine and cytosine at choline monolayer supported multiwalled carbon nanotubes film

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ABSTRACT

A rapid, convenient and accurate method for the simultaneous detection of guanine (G), adenine (A), thymine (T) and cytosine (C) was developed at a multiwalled carbon nanotube (MWCNT)/choline (Ch) monolayer-modified glassy carbon electrode (GCE). X-ray photoelectron spectroscopy data demonstrated that Ch was covalently immobilised on the surface of GCE through oxygen atom. The Ch monolayer provides a positively charged surface with $-N^{+}(CH_3)_3$ polar groups, so that it can attract negatively charged MWCNTs to the surface. Consequently, the MWCNT/Ch film exhibited remarkable electrocatalytic activities towards the oxidation of G, A, T and C due to the advantages of high electrode activity, large surface area, prominent antifouling property, and high electron transfer kinetics. All purine and pyrimidine bases showed well-defined catalytic oxidation peaks at MWCNT/Ch/GCE. The peak separations between G and A, A and T, and T and C are 270, 200, and 190 mV, respectively, which are sufficiently large for their potential recognition and simultaneous detection in mixture. Under the optimum conditions, the designed MWCNT/Ch/GCE exhibited low detection limit, high sensitivity and wide linear range for simultaneous detection of G, A, T and C. Moreover, the proposed method was successfully applied to the assessment of G, A, T and C contents in a herring sperm DNA sample with satisfactory results.

1. Introduction

Deoxyribonucleic acid (DNA) is an important biological macromolecule that plays crucial roles in the storage of genetic information and protein biosynthesis (Shen and Wang, 2009). Guanine (G), adenine (A), thymine (T) and cytosine (C) are purine and pyrimidine bases found in DNA molecular structure, which are involved in cellular energy transduction and signalling mediated by enzymatic oxidation reactions (Oliveira-Brett et al., 2002). Abnormal changes of DNA bases in organism suggest the deficiency of the immunity system and may indicate the presence of various diseases including cancer, epilepsy and HIV infection (Cekan and Sigurdsson, 2009; Pietrzyk et al., 2010). Therefore, the detection of DNA bases is of great significance for clinical diagnosis as well as insight into fundamental mechanisms of genetic information (Jelen et al., 2009; Abbaspour and Ghaffarinejad, 2010). In recent years, several analytical methods have been developed to detect DNA bases, such as microchip capillary electrophoresis (Wang et al., 2004), flow injection chemiluminescence (Liu and Xue, 2006), ion-pairing liquid chromatography (Ganzera et al., 2006), laser-induced fluorescence detection (Wang et al., 2008), and micellar electrokinetic chromatography (Chen et al., 2002). Although these methods exhibit some merits and advantages, expensive instruments, complicated operations, or time-consuming sample pretreatments are usually involved (Sun et al., 2009).

Electrochemical techniques are promising for the analysis of DNA bases due to their advantages of rapidity, convenience, low cost and ease of miniaturization for small volume samples (Kato et al., 2008). The electrocatalytic oxidation of purine bases has been extensively investigated in literature (Ye and Ju, 2005; Shen and Wang, 2009; Zhu et al., 2010; Ferancová et al., 2010). However, the electrochemical detection of pyrimidine bases was rarely studied due to the following two challenges. First, it is difficult to obtain accurate oxidation signals of pyrimidine bases because of their extremely positive oxidation potentials and slow electron transfer kinetics (Ivandini et al., 2007; Zhou et al., 2009). The high oxidation potentials cause large background currents in blank solutions, which severely mask the peak currents and greatly influence their sensitive detection (Oliveira-Brett et al., 2004; Boussicault and Robert, 2008). Second, the electrochemical oxidation of pyrimidine bases is irreversible (Singhal and Kuhr, 1997). The traditional solid electrodes often suffer from fouling effects due to accumulation of oxidized products on the electrode surface, resulting in rather poor sensitivity and reproducibility (Singhal and Kuhr, 1997; Wang et al., 2006). In order to overcome these limitations, modified electrodes with a wide potential window, high electrocatalytic activity and excellent antifouling property are highly required.

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Multiwalled carbon nanotubes (MWCNTs) have attracted considerable attention in electrochemical field because of their unique physical and chemical properties (McQueen and Goldsmith, 2009; Khalap et al., 2010). For example, the electrocatalytic reactivity of MWCNT is due to edge plane defects occurring at the open-ends of the nanotube and around the tube walls where one of the concentric tubes terminates (Banks et al., 2004, 2005; Banks and Compton, 2005). Interestingly, the catalytic performance of MWCNTs has been shown to be closely related to the structure of their local microenvironment (Tasis et al., 2006; Valcárcel et al., 2007). In turn, the local microenvironment is known to be greatly influenced by the supporting material on which they are deposited (Welch and Compton, 2006). Moreover, the distribution status and stability of MWCNTs are markedly dependent on the structure and property of their substrates (Tasis et al., 2006; Kakade et al., 2008). Therefore, it is very imperative and important to select a suitable supporting material for the assembly of MWCNTs.

Choline ($HOCH_2CH_2N^+(CH_3)_3Cl^-$; Ch) is an essential nutrient required by the body for healthy cell membrane function (Rahman et al., 2004). It contains –OH and $–N^+(CH_3)_3$ groups. The –OH group of Ch makes it easy to be immobilised on a carbon electrode surface through covalent linkage. Furthermore, the $–N^+(CH_3)_3$ polar head group of Ch can provide a positively charged monolayer surface with a suitable density, which can not only facilitate the assembly of MWCNTs through electrostatic interaction with the negatively charged carboxylic groups of MWCNTs, but also decrease the resistance of the electron transfer between MWCNTs and electrode surface. The MWCNT-immobilised Ch monolayer exhibits excellent physicochemical characteristics of wide potential window, high electrode activity, prominent antifouling property, and high electron transfer kinetics, allowing sensitive detection of purine and pyrimidine bases.

Herein, a promising electrochemical device for sensitive detection of DNA bases was constructed by immobilising MWCNTs on a Ch monolayer-modified glassy carbon electrode (GCE). X-ray photoelectron spectroscopy (XPS), field emission scanning electron microscopy (FE-SEM) and electrochemical techniques were used for the surface characterization of the modified electrode. It was demonstrated that Ch was covalently immobilised on the GCE surface forming a positively charged monolayer, which could provide a suitable supporting substrate and favorable local microenvironment for the construction of MWCNTs. As a result, the MWCNT/Ch/GCE exhibited extraordinary electrocatalytic activities towards the oxidation of DNA bases with obvious reduction of overpotentials. Moreover, the proposed method was successfully applied in the determination of G, A, T and C contents in herring sperm DNA sample, and the obtained results were consistent with reference values.

2. Experimental

2.1. Chemicals and reagents

G, A, T and C were purchased from Sigma (USA). Herring sperm DNA and Ch were obtained from Shanghai Chemical Co. Ltd. (Shanghai, China). They were used without further purification. MWCNTs (purity >95%) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Prior to use, the MWCNTs were purified by refluxing the as-received MWCNTs in 2.6 M nitric acid for 5 h followed by centrifugation, resuspension, filtration, and air-drying to evaporate the solvent (Lin et al., 2009). The purified MWCNTs were further heated under vacuum at 400 °C for 2 h. Phosphate buffered saline (PBS; 0.1 M) of different pH were prepared by mixing stock solutions of 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4, and the pH adjusted by 0.1 M H_3PO_4 or NaOH (Beijing Chemical Reagent Company, Beijing, China).

All other chemicals were of analytical reagent grade, unless stated otherwise. Aqueous solutions were prepared with doubly distilled water at ambient temperature.

2.2. Apparatus and measurements

Electrochemical experiments including cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out using a CHI 760C electrochemical workstation (Chenhua, Shanghai, China). A conventional three-electrode electrochemical system was used for all electrochemical experiments, which consisted of a working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode. A 3-mm diameter glassy carbon disc electrode, modified by a procedure reported in Section 2.3, was used as a working electrode (Chenhua, Shanghai, China).

EIS measurements were carried out in 10 mM $K_3[Fe(CN)_6]$:$K_4[Fe(CN)_6]$ (1:1) mixture in the range of 100 kHz to 0.01 Hz with an amplitude of 0.005 V. The DC potential was selected as 0.23 V, which was the formal potential of the redox couple.

FE-scanning electron micrographs were obtained on a JSM-6700F field emission scanning electron microanalyzer (JEOL, Japan). XPS was performed on an ESCALAB spectrometer (VG Co., UK) equipped with the Mg Kα X-ray radiation as the source for excitation at a pressure of less than 10^{-9} Torr in the chamber.

2.3. Electrode preparation and modification

Prior to use, GCE was sequentially polished with 1.0, 0.3 and 0.05 μm alumina powder and then ultrasonically washed in doubly distilled water and ethanol for 10 min, respectively. The cleaned GCE was dried in nitrogen atmosphere for the next modification. Ch monolayer modified GCE (Ch/GCE) was prepared by immersing the GCE in 0.1 M pH 7.0 PBS containing 2.0 mM Ch and scanning between −1.70 and 1.80 V for 6 cycles at the scan rate of 25 mV s^{-1}. The obtained Ch/GCE was rinsed with distilled water and sonicated for 10 min to remove the physically adsorbed materials. In order to immobilise MWCNTs on Ch/GCE, 0.50 mg mL^{-1} MWCNTs were dispersed into N,N-dimethylformamide (DMF), and the mixture was sonicated to give a homogeneous dispersion. The MWCNT/Ch/GCE was prepared by applying 10 μL of the MWCNTs-DMF suspension to the Ch/GCE surface and dried under an infrared lamp.

For comparison, Ch/GCE and MWCNT/GCE were also prepared under the same conditions.

2.4. DNA sample preparation

DNA sample was hydrolyzed as follows for the quantification of G, A, T and C. In brief, accurate amount of herring sperm DNA (12.0 mg) was hydrolyzed in 600 μL of 88% (w/w) formic acid at 170 °C for 30 min in a sealed glass tube as described by Ivandini et al. (2007). The hydrolysate was then adjusted to neutrality with 2.0 M NaOH. Next, the obtained sample solution was diluted with doubly distilled water. Finally, 100 μL of the pretreatment sample was added into 0.1 M pH 7.0 PBS for detection.

3. Results and discussion

3.1. Construction and characterization of MWCNT/Ch/GCE

The construction mechanism of the MWCNT/Ch/GCE is shown in Scheme 1. The bare GCE was oxidized to generate cationic radicals on the surface (Jin and Lin, 2004). The Ch was then covalently