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Interaction of sulfadiazine with DNA on a MWCNT modified glassy carbon electrode: Determination of DNA

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ABSTRACT

The interactions of sulfadiazine (SD), an antimicrobial drug, with double-stranded calf thymus DNA on the multi-walled carbon nanotubes modified glassy carbon electrode (MWCNT-GCE) have been studied by cyclic voltammetry and UV–vis spectroscopy. In the presence of DNA, the oxidation peak current of SD decreases and the peak potential shifts to a positive potential which indicates the interaction of SD with DNA. The binding of SD with DNA shows both electrostatic and intercalative modes. The binding of SD with DNA, when analyzed in terms of the cooperative Hill model, yields the binding constant, $K_a = 2.87 \times 10^3\ M^{-1}$ and a Hill coefficient $m = 1.9$ in Britton–Robinson (B-R) buffer solution, pH = 6.5. This electrochemical method was further applied to the determination of DNA. Under the selected conditions, two linear calibration curves for DNA detection were obtained in the concentration ranges from 0.03 to 0.13 $\mu g\ mL^{-1}$ and 0.60–3.50 $\mu g\ mL^{-1}$ with detection limit 0.03 $\mu g\ mL^{-1}$. The method was also applied to the determination of DNA in human blood plasma sample.

1. Introduction

Sulfonamides are the most widely employed antibacterial agents in both human and veterinary medicine, because of their low cost and high efficiency in treatment of bacterial diseases. Sulfonamides' mechanism of action is based on inhibiting the conversion of p-aminobenzoic acid and interrupting the bacterial utilization of this compound in the synthesis of folic acid and ultimately in synthesis of purine and DNA [1]. As a systematic antibacterial agent, sulfadiazine (4-amino-N-2-pyrimidinylbenzenesulfonamide) has several applications. For instance, in ophthalmology, it is used in treatment of trachoma and ocular toxoplasmosis.

DNA contains all of the genetic information as well as it plays a very important role in life processes. Thus, in order to obtain the biochemical mechanisms of prevention and treatment of human diseases, great efforts have been made to investigate the interaction between DNA and drug molecules [2,3]. Moreover, nucleic acids interaction with drugs is a fundamental issue in life phenomena. This process is associated with molecular recognition in DNA hybridization, gene mutations, sensing of bioactive agents such as anticancer drugs, and action mechanism of some DNA-targeted drugs [4–6].

Many methods such as resonance light scattering [7], fluorescence [8], spectroscopy [9] and voltammetry methods [10,11] have been developed for determination of drug–DNA interactions. DNA immobilization has attracted great attention and is considered as a fundamental methodology in construction of DNA biosensors. The DNA-based biosensor is a device that incorporates immobilized DNA, as molecular recognition element in the biological active layer on the surface, and measures specific binding processes with DNA mainly electrochemical transducers. These sensors have a rapid response time and should be quantitative, sensitive, suitable for automation and cost effective. Considering these applications, DNA immobilization has been proved to be a useful complement compared to other analytical methods [12,13].

In recent years, different studies have evaluated the interaction of various biological molecules with DNA using electrochemical methods [14,15]. For instance, the interaction between buzepide and DNA in solution at bare GCE and at the surface of GCE using DNA biosensor was studied by Seetharamappa et al. [16]. Tian et al. [17] studied the interaction of rutin with DNA. Salimi et al. [18] reported simultaneous detection of DNA nucleic bases using SiC nanoparticles modified glassy carbon electrode. Lin et al. [19] used voltammetric methods to probe the interaction of metronidazole with DNA for calculating the binding constant. Voltammetric studies of the interaction of berberin with DNA immobilized on GCE have been reported [20]. The interaction of DNA with curcumin in the presence of Cu(II) has been investigated by differential pulse adsorptive voltammetry [21]. Nevertheless, to the best of our knowledge, there is no report on the interaction of SD with

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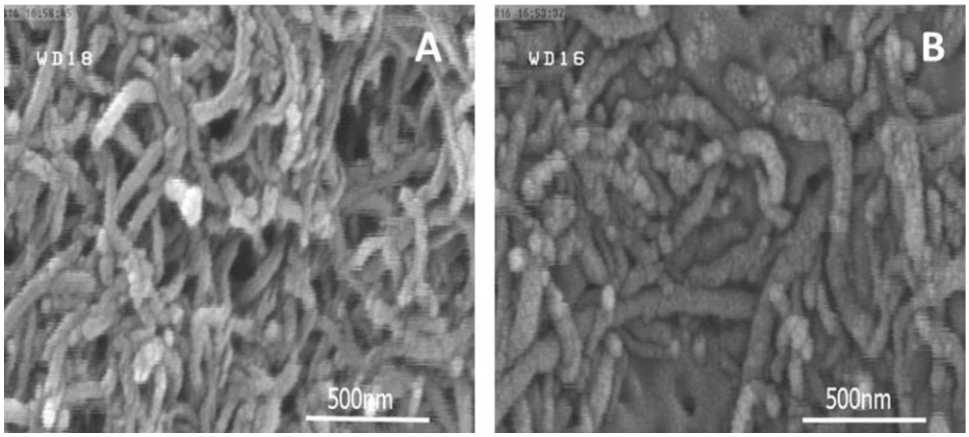

Fig. 1. SEM images of (A) MWCNT/GCE and (B) MWCNT-DNA-GCE.

DNA at a MWCNT modified electrode. Following our recent studies [22–25], we describe the interaction of SD with DNA, both in solution and DNA immobilized on the MWCNT-DNA-GCE using cyclic voltammetry and UV spectroscopy.

2. Experimental

2.1. Apparatus

Electrochemical measurements were carried out with a Metrohm model 746 VA trace analyzer connected to a 747 VA stand. The working electrode was a glassy carbon electrode (2 mm diameter). A platinum wire and a commercial Ag/AgCl saturated KCl electrode from Metrohm were used as auxiliary and reference electrodes, respectively.

Absorption spectra were measured on a UV-vis spectrophotometer Lambda 35-Perkin Elmer.

S-4160, Etaji (Japan) Scanning Electron Microscope (SEM) was used for surface image measurements.

2.2. Reagents

Double-stranded calf thymus DNA was purchased from Sigma. Calf thymus DNA solutions were prepared with Britton–Robinson (B-R) buffer (0.04 M, pH = 6.5).

Sulfadiazine was obtained from Alfa Aesar. All other reagents used were of analytical grade without further purification. Multiwalled carbon nanotubes with purity 95% (10–30 nm diameter and 5 μm length) were obtained from io-li-tec, Ionic Liquid Technologies. A stock Britton–Robinson (B-R) buffer solution 0.04 M with respect to boric acid, orthophosphoric acid and acetic acid were prepared from proanalysis reagents. From this stock buffer, solutions with various values of pHs were prepared by the addition of 0.2 M sodium hydroxide solution. The stock solution of DNA (10^3 μg mL^{-1}) was prepared in B-R buffer solution (0.04 M, pH = 6.5) and stored at 4 °C. Dilution was done just prior to use. The concentration of DNA was calculated according to the absorbance at 260 nm by using ε_{DNA} = 6600 M^{-1} cm^{-1}. All electrochemical experiments were carried out at room temperature.

2.3. Preparation of the MWCNT-GCE suspension

MWCNT (4 mg) was added to 1 mL DMF. A homogeneous and stable suspension of 4 mg mL^{-1} MWCNT was achieved with the aid of ultrasonic agitation for about 30 min. Then 20 μL of the black suspension of MWCNT was added to the 20 μL of 400 mg L^{-1} DNA and the resulting suspension was kept in an ice-water bath and sonicated for 20 s. A homogeneous well-distributed suspension of MWCNT-DNA was then obtained.

2.4. Fabrication of MWCNT-GCE and MWCNT-DNA-GCE

Prior to the immobilization, glassy carbon electrode was sanded using ultrafine sand paper, polished with 10 μm alumina slurry in sequence and sonicated in water for 10 min. The MWCNT-GCE was prepared by casting 3 μL of the suspension of MWCNT on the surface of a GCE, which was dried in air for 30 min at room temperature. The immobilization of DNA was performed by casting the GCE with 6 μL of the MWCNT-DNA suspension and allowed to dry in air for 30 min at room temperature. The resulting electrode is called MWCNT-DNA-GCE.

3. Results and discussion

3.1. Morphology of modified electrodes

Fig. 1 shows the SEM images of MWCNT-GCE and MWCNT-DNA-GCE. SEM image of MWCNT-GCE shows that MWCNT with average diameters of 10–30 nm almost homogeneously distributed on the electrode surface forming a cover/layer (thin layer) without aggregation (Fig. 1A). When DNA was immobilized on the surface of electrode the clear MWCNT image became dim and the surface morphology changed (Fig. 1B) with slightly increasing the diameters of MWCNT (40–60 nm). It demonstrated that MWCNTs were partially wrapped by DNA, so MWCNT-DNA existed as bundles, which are entangled with one another.

3.2. Interaction of SD with DNA

The cyclic voltammograms of SD at MWCNT-GCE in the presence and absence of 400 μg mL^{-1} DNA in B-R buffer solution (0.04 M, pH = 6.5) are shown in Fig. 2. The SD has an irreversible anodic peak current of 18 μA at 0.85 V (Fig. 2, curve a), which is related to the electrode oxidation of the –NH_2 group to hydroxylamine [26,27] (Scheme 1). The MWCNT-GCE exhibited excellent electrocatalytic behavior for oxidation of SD, as evidenced by the enhancement of peak current and the shift in the oxidation potential to less positive values in comparison with a bare GCE [24]. By adding 400 mg L^{-1} DNA, a decrease in the peak current and a positive shift in the peak potential were observed for anodic peak of SD (Fig. 2, curve b), which indicates the interaction of SD and DNA. Cyclic voltammograms of SD in B-R buffer solution (0.04 M, pH = 6.5) at MWCNT-DNA-GCE yielded results similar to those obtained for DNA in the solution (Fig. 2, curve c). Cyclic voltammograms of 1 mM