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Study of interactions between DNA and aflatoxin B1 using electrochemical and fluorescence methods

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ABSTRACT

In this study, a carbon paste electrode modified with *N*-butylpyridinium hexafluorophosphate ($BPPF_6$) ionic liquid and DNA was introduced as an electrochemical biosensor to study the interaction between DNA and aflatoxin B1 molecules. For this purpose, variations in oxidation peak current of guanine in various concentrations of aflatoxin B1 were measured by using the differential pulse voltammetry (DPV) method. According to this study, the binding constant of DNA–aflatoxin B1 was found to be $3.5 \times 10^6\ M^{-1}$. This modified electrode was also used for determination of low concentrations of aflatoxin B1 by using differential pulse voltammetry. A linear dynamic range from 8.00×10^{-8} to 5.91×10^{-7} M and a limit of detection of 2.00×10^{-8} M resulted from DPV measurements. To confirm our results, a fluorescence study was also performed. It resulted in a binding constant of $2.8 \times 10^6\ M^{-1}$, which is in good agreement with that obtained from electrochemical study.

The study of interactions between small molecules and DNA is of importance because these interactions are the basis of carcinogenic and therapeutic properties of many carcinogenic species and antitumor and antivirus drugs [1–4]. Electrostatic interactions with sugar phosphate backbone, binding interactions with minor and major grooves of DNA double helix, and intercalation between two adjacent base pairs of DNA double helix are three modes of interaction between DNA and small molecules [5]. Electrochemical [6,7] and spectroscopic [8,9] methods have been used for studying the interaction between DNA and small molecules.

In addition to the cases where the small molecules are electroactive [10], as a result of electroactivity of DNA molecules, it is possible to accomplish electrochemical study of these interactions based on electrochemical signals of DNA [11]. These signals are due to the oxidation and reduction of nucleotides that have been studied mainly on carbon- and mercury-based electrodes, respectively [12].

The electrochemical study of DNA–small molecule interactions can be done in three different ways: DNA-modified electrodes, small-molecule-modified electrodes, and interaction in solutions [13]. In DNA-modified electrodes, the immobilization step plays a major role in overall performance of electrode. As a result of firm and irreversible adsorption of DNA at the surface of carbon electrodes, it is possible to modify the surface of carbon-based electrodes simply by immersing the electrode into a DNA solution. These modified electrodes are very suitable as an electrochemical biosensor for studying the interaction of DNA–small molecules because these interactions can influence the electrochemical oxidation peaks of DNA. They can also be used for analytical applications for the quantitative determination of toxins and drugs. Because of the lower oxidation potentials of guanine and adenine relative to cytosine and thymine, their oxidation peaks often have been used for these purposes. The electrochemical oxidation of guanine is shown in Fig. 1. Because of the slow direct electron transfers in oxidations of adenine and guanine residues on the bare working electrodes, application of modified electrodes in nucleic acid electrochemistry has been attracting attention [14–16].

Carbon paste electrodes (CPEs)[1] have been widely used in electrochemical investigations due to their ease of fabrication and modification, low cost, good sensitivity of detection, and renewable surface. The replacement of nonconductive mineral oil, which weakens the electrochemical response of these electrodes, with an immiscible ionic liquid as a binder can improve the conductivity and electrocatalytic activity of them [17]. Application of these ionic liquid–modified carbon paste electrodes (IL–CPEs) in the electro-

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[1] *Abbreviations used:* CPE, carbon paste electrode; IL–CPE, ionic liquid–modified carbon paste electrode; UV–Vis, ultraviolet–visible; CD, circular dichroism; ds-DNA, double-stranded DNA; BPPF6, *N*-butylpyridinium hexafluorophosphate; DPV, differential pulse voltammetry.

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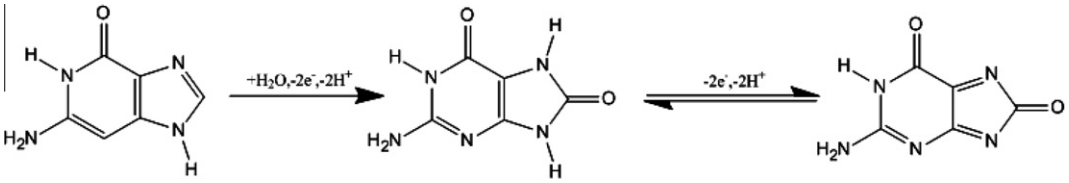

Fig.1. Electrochemical oxidation of guanine.

chemical study of nucleic acids has been shown to have superior results relative to traditional CPEs [14,18].

There are also many studies on the interaction of small molecules with DNA based on spectroscopic methods such as ultraviolet–visible (UV–Vis) absorption, fluorescence, and circular dichroism (CD) [19–21]. The fluorescence method has high sensitivity and is often used for the quantitative analysis of nucleic acids [22]. Although DNA has a natural fluorescence, the intensity is weak at room temperature, so that it is not suitable for studying the interaction of small molecules with DNA [23]. The direct use of natural fluorescence of DNA is limited to studying its properties [24]. In cases where the interacting small molecules have good fluorescent properties themselves, the study can be accomplished based on it [25]. The binding of these molecules to DNA can affect their fluorescence spectra through blue or red shift and/or fluorescence efficiency [19,20]. In other cases, sensitive fluorescence probes such as lanthanide complexes and fluorescent dyes may be used [23,26]. Because fluorescence species should have a planar structure for efficient fluorescence emission, most of them bind to DNA through intercalation.

In this work, we studied the interaction of aflatoxin B1 with immobilized double-stranded DNA (ds-DNA) on CPE modified with *N*-butylpyridinium hexafluorophosphate ($BPPF_6$) by using differential pulse voltammetry (DPV). Spectroscopic study of DNA–aflatoxin B1 interaction was also carried out by using the fluorescence technique based on fluorescent property of aflatoxin B1. The resulting binding constant is in relatively good agreement with that obtained from electrochemical investigation.

Materials and methods

Materials and reagents

Carbon powder (<20 μm), calf thymus DNA salt (purity > 99%) with high molecular weight, and aflatoxin B1 (purity > 99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The stock solution of aflatoxin B1 (10 mg L^{-1}) was prepared in ethanol. Dilution of stock solutions was performed using 0.1 M phosphate buffer (pH 7.4). $BPPF_6$ ionic liquid (99%) was purchased from Io-Li-Tec (China). The doubly distilled deionized water was used throughout the experiments. Other chemicals were of analytical reagent grade and used without any purification.

Apparatus

All voltammetric experiments were carried out in a conventional three-electrode cell using a μAutolab Type III electrochemical system. A three-electrode system was used with an Ag/AgCl (3 M KCl) electrode as a reference electrode, a platinum rod as an auxiliary electrode, and a homemade IL–CPE as a working electrode whose surface was modified with a layer of adsorbed DNA. The pHs of buffer solutions were measured using a digital WTW Metrohm 827 ion analyzer (Switzerland) equipped with a combined glass–calomel electrode. Concentrations of DNA solutions were determined using a Raylight UV–Vis spectrometer (model UV-2100). Fluorescence studies were carried out using a Varian Cary Eclipse fluorescence spectrophotometer.

Working electrode preparation

The graphite powder and $BPPF_6$ were mixed thoroughly in an agar mortar in a ratio of 3:1 to become completely uniform. Then the mixture were heated at 80 °C for 1 h and mixed in the agar mortar again. By using this carbon paste mixture, one end of a Teflon tube (φ = 3 mm) was filled and electrical contact was established with a copper rod that was inserted through the opposite end of the Teflon tube and was completely fixed in the hole of the tube. To obtain more reproducible results by using this electrode, compaction of carbon paste was performed by insertion of pressure through the copper rod while the electrode was placed on a flat surface. Then the electrode surface was polished on a piece of polishing paper and was washed with deionized water.

Before any modification of electrode with DNA, the electrode was first pretreated by applying a relatively high potential of +1.3 V for 2 min in a 0.1 M phosphate buffer solution. Then the electrode was rinsed with water.

Immobilization of DNA on the surface of electrode was performed by an adsorption process through insertion of a concentrated droplet of DNA solution in water with known concentration (3 mg/ml) and volume (7 μl) on the surface of electrode in a fixed time (20 min). A high concentration of DNA solution was used to obtain maximum immobilization of DNA molecules at the surface of electrode in the least time. Finally, the electrode was rinsed with water for 10 s and then used for experiments.

Electrochemical study of interaction between ds-DNA and aflatoxin B1

To study the interaction of immobilized calf thymus ds-DNA with aflatoxin B1, different amounts of 10 mg L^{-1} aflatoxin B1 solution in ethanol were added to the 0.1 M phosphate buffer solution (pH 7.4) and differential pulse voltammograms were obtained by using modified CPE. Before each voltammetric measurement, purging of solution with nitrogen was accomplished for 15 min and the modified electrodes were placed in contact with a solution containing aflatoxin B1 for 10 min to attain equilibrium conditions. The changes in guanine oxidation peak can be attributed to aflatoxin B1 concentration. The experimental conditions for DPV were as follows: deposition potential of 0.4 V for 160 s, equilibration time of 3 s, potential range from 0.4 to 1.1 V, and scan rate of 5 mV/s.

Fluorescence study of interaction between ds-DNA and aflatoxin B1

A fluorescence study was performed based on aflatoxin B1 fluorescence spectra (λ_{ex} = 365 nm) in a 0.1 M phosphate buffer solution at room temperature. Spectra were recorded in a range from 370 to 560 nm (λ_{em} = 440 nm) at a constant concentration of aflatoxin B1 and various concentrations of ds-DNA.