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Voltammetry of the Anticancer Drug Mitoxantrone and DNA

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The interaction of mitoxantrone (MTX) with calf thymus double-stranded DNA (dsDNA) and calf thymus single-stranded DNA (ssDNA) was studied electrochemically by using differential pulse voltammetry (DPV) and cyclic voltammetry (CV) at a carbon paste electrode (CPE). The changes in the experimental parameters (the concentration of MTX, the concentration of DNA and the accumulation time of MTX) were studied by DPV; in addition, the detection limit and the reproducibility were determined. It was observed for the interaction of MTX with dsDNA and ssDNA that the signal of the bare electrode was higher than the signal of the dsDNA modified CPE. The signals in the cyclic voltammograms were found to be decreasing in the following order: bare CPE, ssDNA modified and dsDNA modified CPE.

Key Words: Mitoxantrone, Biosensors, DNA, Voltammetry, Intercalation

Introduction

There have been intensive studies to apply modern voltammetric methods in nucleic acid research and DNA analysis[1−12] since the electroactivity of deoxyribonucleic acid (DNA) was first discovered[1]. DNA biosensor technologies are currently under intense investigation owing to their great promise for rapid and low-cost detection of specific DNA sequences in human, viral and bacterial nucleic acids.

The binding of small molecules to deoxyribonucleic acid (DNA) occurs through primarily in three modes: electrostatic interactions with the negative-charged nucleic sugar-phosphate structure, binding interactions with two grooves of DNA double helix and intercalation between the stacked base pairs of native DNA [13].

The interactions of some anticancer drugs with DNA have been studied with a variety of techniques [14−17] and, in recent years, there is a growing interest in the electrochemical investigations of interactions between anticancer drugs and other DNA-targeted molecules and DNA [4,7−9,18−28].

A quantitative understanding of such factors that determine recognition of DNA sites would be valuable in the rational design of new DNA targeted molecules for application in chemotherapy and in the development of tools for biotechnology based on DNA hybridization.

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In this article, the results obtained for the electrochemical oxidation of mitoxantrone (MTX) using a carbon paste electrode (CPE) are presented. MTX is an anticancer drug and has a planar heterocyclic ring structure The basic side groups are critical for intercalation into DNA and the quinone prosthetic group, which together could confer chemical reactivity. The positively charged, nitrogen-containing side chains project out words from the molecule, and stabilize the ring between base pairs by intercalating with the negatively charged phosphate backbone of DNA[18].

In a previous electrochemical study with MTX, Oliveira Brett et al. [18] reported an electrochemical procedure for the determination the interaction between MTX and dsDNA or ssDNA in an aqueous medium or on an electrode surface by using a glassy carbon electrode (GCE).

In this study, we used the DNA-modified carbon paste electrode (CPE) in combination with cyclic voltammetry (CV) and especially differential pulse voltammetry (DPV) to obtain information about the interaction of MTX with double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA). The changes in the experimental parameters (the concentration of MTX, the concentration of DNA and the accumulation time of MTX) were studied by using DPV; in addition, the detection limit and the reproducibility were determined. The features of the of method were discussed and compared with those of methods previously reported for similar compounds in the literature.

Experimental

Apparatus

DPV was performed with a Metrohm 626 Polarecord Analyzer (Switzerland). A PowerLab voltammetric unit (ADInstruments, Australia) in connection with an EG& G PAR (Princeton, USA) 264A potentiostat was used to obtain the cyclic voltammograms. The three-electrode system consists of the CPE, the reference electrode (Ag/AgCl) and a platinum wire as the auxiliary electrode. The body of the working electrode was a glass tube (3 mm i.d.) tightly packed with carbon paste. The electrical contact was provided by a copper wire inserted into the carbon paste. Carbon paste was prepared in the usual way by hand-mixing graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The surface was polished on weighing paper to a smooth finish before use. The convective transport was provided by a magnetic stirrer.

Chemicals

Single-stranded calf thymus DNA (ssDNA, lyophilized powder, Catalogue No. D8899) and double-stranded calf thymus DNA (dsDNA, activated and lyophilized, Catalog No. 4522) were purchased from Sigma.

All DNA stock solutions (1000 mg / L) were prepared with TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and kept frozen. More dilute solutions were prepared with 0.5 M acetate buffer (pH 4.8).

The MTX used was a synthetic sample solution of Novantrone that was supplied by Lederle Laboratories, UK. Stock standard solutions of MTX were prepared by dissolving in 0.50 M acetate buffer (pH 4.8) and were kept away from light to avoid photodecomposition. Other chemicals were of analytical reagent grade. Sterilized and deionized water was used in all solutions.

Procedure for voltammetric assay

Each measurement involved the immobilization/detection cycle at a fresh carbon paste surface.

All the experiments were performed at room temperature (25.0 ± 0.5°C.

Differential pulse voltammetry (DPV): The CPE was pretreated by applying +1.70 V for 1 min in 0.05 M phosphate buffer solution (pH 7.4) without stirring. The dsDNA was immobilized on a pretreated CPE by applying a potential of +0.50 V for 5 min in 5 mg / L dsDNA solution with 200 rpm stirring. The electrode was then rinsed with distilled water for 10 seconds. The dsDNA-modified CPE was then immersed into a 0.50 M acetate buffer (pH 4.8) containing 4.5 μM MTX with 200 rpm stirring for 3 min by applying a potential of +0.2 V. After the accumulation of MTX, the electrode was rinsed with 0.50 M acetate buffer (pH 4.8) for 10 seconds. The accumulated MTX was then measured by using differential pulse voltammetry (DPV: 10 mV pulse amplitude).

Repetitive measurements were carried out by renewing the surface and repeating the above assay format. Except when stated otherwise, the reported voltammetric signals represent the differences in MTX magnitudes at the bare CPE and dsDNA-modified CPE.

Cyclic voltammetry: In a typical experiment, the CPE was pretreated by applying +1.70 V for 1 min in 0.05 M phosphate buffer solution (pH 7.4) without stirring. The dsDNA was immobilized on a pretreated CPE by applying a potential of +0.50 V for 5 min in 5 mg / L dsDNA solution with 200 rpm stirring. The electrode was then rinsed with sterilized and deoinized water for 10 seconds. The dsDNA-modified CPE was then immersed into 0.50 M acetate buffer (pH 4.8) containing 0.45 mM MTX with 200 rpm stirring for 3 min by applying a potential of +0.2 V. After accumulation of MTX, the electrode was rinsed with 0.50 M acetate buffer (pH 4.8) for 10 seconds. The dsDNA-modified CPE was then immersed into 0.50 M acetate buffer (pH 4.8) and was scanned at 50 mV / s from +0.00 V to at least +1.20 V beyond the redox couple of MTX.

The same protocol was also applied to the ssDNA-modified CPE.

Results and Discussion

The changes in the differential pulse voltammetry (DPV) peak currents of MTX with the bare and dsDNA-modified CPE are shown in Figure 1. We found two oxidation peak potential values of MTX: 500 mV for the first one and 660 mV for the second one. For both peaks, currents with the bare electrode were higher than those obtained with the dsDNA modified CPE. Due to MTX intercalating into dsDNA, the peak current of MTX observed with dsDNA modified CPE decreased to about 28.7% and 56.4% for the first and second peaks, respectively.

A series of three repetitive measurements of the interaction of MTX with dsDNA resulted in reproducible results for the first peak at 500 mV (for bare CPE and dsDNA modified CPE, respectively; mean hybridization response of 1054 nA and 754 nA with a relative standard deviation of 12.2 and 10.0%; the detection limits estimated from S/N=3 correspond to 56.2 nM and 80.4 nM for MTX; 5 min accumulation time).

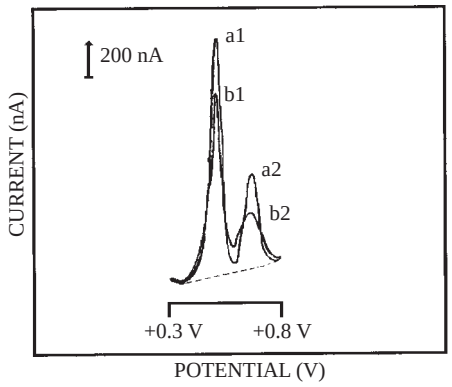

Figure 1. Differential pulse voltammograms, 10 mV amplitude, (DPV), for 4.5 μM MTX in 0.50 M acetate buffer (pH 4.8) at 5 mV/s at **(a)** bare CPE and **(b)** dsDNA-modified CPE. CPE pretreatment, 1 min at +1.70 V in 0.05 M phosphate buffer (pH 7.4); 5 ppm dsDNA immobilization, 5 min at +0.50 V in 0.50 M acetate buffer (pH 4.8), MTX accumulation, 3 min at +0.20 V in 0.50 M acetate buffer (pH 4.8), measurement, scanning at 5 mV / s between +0.30 V and +0.80 V in 0.50 M acetate buffer (pH 4.8). The protocol above was applied to bare CPE and no DNA was immobilized at bare CPE. Dashed lines denote the voltammetric response of the bare CPE in 0.50 M acetate buffer (pH 4.8) at 5 mV / s.

In the cyclic voltammetry (CV) study, the changes in the currents of MTX with bare, dsDNA-modified CPE and ssDNA modified CPE are shown in Figure 2. It was observed for the interaction of MTX with dsDNA and ssDNA that the signal of the bare electrode was higher than the signal of the dsDNA modified CPE. The observed signals caused by the interaction of MTX with DNA were, in order of decreasing signals, CPE, ssDNA modified CPE, and dsDNA modified CPE.

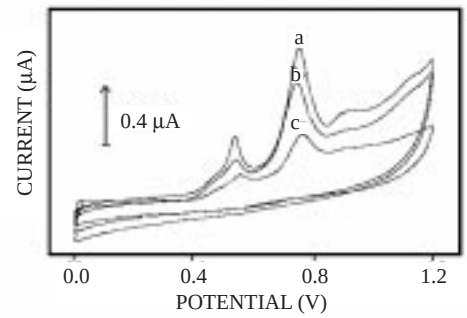

Figure 2. Cyclic voltammograms of MTX in 0.50 M acetate buffer (pH 4.8) at 50 mV / s at **(a)** bare CPE, **(b)** ssDNA-modified CPE and **(c)** dsDNA-modified CPE. CPE pretreatment, 1 min at +1.70 V in 0.05 M phosphate buffer (pH 7.4); 5 mg / L ssDNA immobilization, 5 min at +0.50 V in 0.50 M acetate buffer (pH 4.8), MTX accumulation, 0.45 mM MTX, 3 min at +0.20 V in 0.50 M acetate buffer (pH 4.8) with 20 mM NaCl, measurement, scanning at 50 mV / s between +0.00 V and +1.20 V in blank 0.50 M acetate buffer (pH 4.8). The protocol above was applied to dsDNA-modified CPE and no DNA was immobilized at bare CPE.

Our results are in parallel to those of Oliveira Brett et al.[18], who used by using square wave voltammetry (SWV). They observed that the addition of an excess of dsDNA or ssDNA in MTX solution caused changes in the peak currents of the oxidation wave of MTX. They also reported that in the presence of ds-

DNA only two small peaks (E_{p1}= +0.48 V and E_{p2}= +0.66 V) appeared, because the MTX groups involved in bonding with DNA are not available for oxidation.

The concentration of MTX has a pronounced effect on its interaction with dsDNA. In both bare and dsDNA-modified CPEs, the response of MTX increases sharply with concentration up to 9.0 μM in Figure 3. Since the highest difference between these signals is obtained at 4.5 μM, this optimum MTX concentration was used in all experiments.

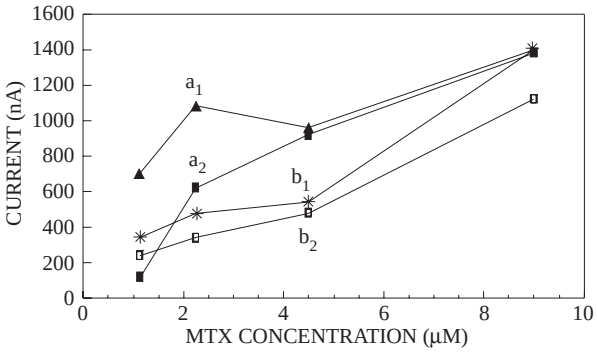

Figure 3. Effect of concentration upon MTX oxidation peak current; (1) for the first oxidation peak at 500 mV and (2) for the second oxidation peak at 660 mV obtained at **(a)** bare CPE and **(b)** dsDNA-modified CPE with different concentration of MTX; 1.125 μM , 2.250 μM, 4.5 μM and 9.0 μM MTX in 0.50 M acetate buffer. Other conditions are as in Figure 1.

The dsDNA concentration was decreased from 10 mg / L to 2.5 mg / L (not shown) and the optimum dsDNA concentration was then determined to be 5 mg / L. Our results are consistent with those of Xia et al.[13] and Plambeck et al.[27].

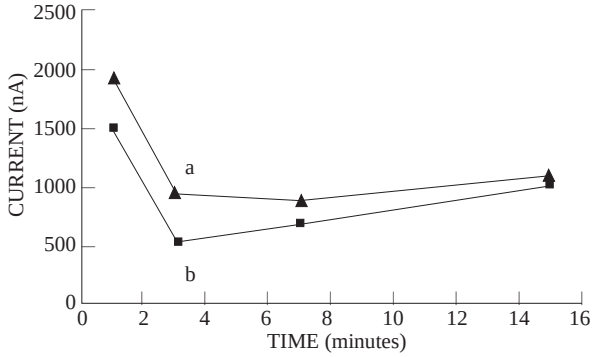

Figure 4a. Effect of interaction time of MTX with dsDNA upon the first oxidation peak current of MTX at 500 mV obtained at **(a)** bare CPE and **(b)** dsDNA-modified CPE in different intercation time; 1 min, 3 min, 7 min and 15 min. Other conditions are as in Figure 1.

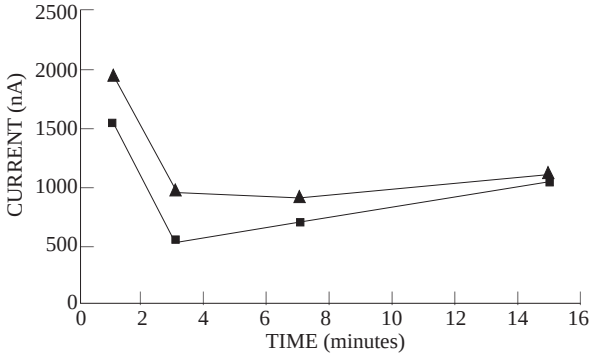

Figure 4b. Effect of interaction time of MTX with dsDNA upon the second oxidation peak current of MTX at 660 mV obtained at **(a)** bare CPE and **(b)** dsDNA-modified CPE in different intercation time; 1 min., 3 min., 7 min and 15 min. Other conditions are as in Figure 1.

The binding of MTX to the surface of the bare electrode and dsDNA-modified electrode was carried out after the transfer of the electrode into acetate buffer containing MTX. Conditions affecting the MTX intercalation into dsDNA and detection were assessed and obtained in the range from 1 min to 15 min (Figure 4a for the first peak at 500 mV and Figure 4b for the second peak at 660 mV). The optimum MTX

binding time was determined as 3 min for both peaks. Oliveira Brett et al.[18] found by using square wave voltammetry (SWV) that 2 min was the optimum time for MTX's binding to dsDNA in aqueous medium and they reported that the currents for the peaks corresponding to the oxidation of MTX increased due to the increase concentration near the electrode surface of MTX that intercalated with DNA.

Conclusion

The DNA-modified carbon paste electrode (CPE) was used in combination with cyclic voltammetry (CV) and especially differential pulse voltammetry (DPV) to obtain information about the interaction of MTX with double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA), and changes were observed in the MTX signals caused by the interaction of MTX with DNA as decreasing signals.

This method is experimentally convenient and sensitive so that it requires only small amounts of materials. In principle, it can be applied to a wide range of intercalating drugs[4,13,18,25,26,28], provided they bear an electrochemically active moiety.

In conclusion, we described in detail the variation of voltammetric behaviour of MTX in aqueous medium at a DNA modified CPE to modify a promising DNA biosensor for a novel anticancer drug which has a chemical structure similar to that of MTX.

DNA biosensors eliminate the need for radioisotopes, and require significantly shorter hybridization times. The determination of hybridization indicators that recognize DNA would be valuable in the design of sequence-specific DNA binding molecules for application in chemotherapy and in the development of tools for the point-of-care tests and diagnosis based on DNA.

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