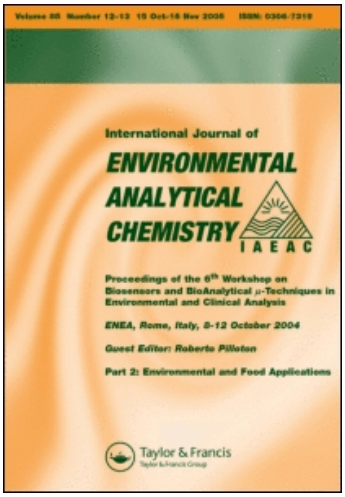

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Ioanna Ch. Gherghi[a]; Stella Th. Girousi[b]; Anastasia A. Pantazaki[c]; Anastasios N. Voulgaropoulos[b]; Roxani Tzimou-Tsitouridou[a]

[a] Analytical Chemistry Laboratory Department of Chemical Engineering Aristotle University of Thessaloniki 54124 Thessaloniki Greece, [b] Analytical Chemistry Laboratory Department of Chemistry Aristotle University of Thessaloniki 54124 Thessaloniki Greece, [c] Biochemistry Laboratory Department of Chemistry Aristotle University of Thessaloniki 54124 Thessaloniki Greece,

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ELECTROCHEMICAL DNA BIOSENSORS APPLICABLE TO THE STUDY OF INTERACTIONS BETWEEN DNA AND DNA INTERCALATORS

IOANNA CH. GHERGHI[a,*], STELLA TH. GIROUSI[b,†], ANASTASIA A. PANTAZAKI[c], ANASTASIOS N. VOULGAROPOULOS[b] and ROXANI TZIMOU-TSITOURIDOU[a]

[a]*Analytical Chemistry Laboratory, Department of Chemical Engineering;* [b]*Analytical Chemistry Laboratory, Department of Chemistry;* [c]*Biochemistry Laboratory, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece*

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Electrochemical DNA biosensors, based either on carbon paste electrode (CPE) or hanging mercury drop electrode (HMDE) were prepared. These biosensors were used in the study of interaction between double stranded DNA (dsDNA) and single stranded DNA (ssDNA) and acridine orange, a well known DNA intercalator. The different electrochemical behaviors were compared in the article.

Keywords: Electrochemical DNA biosensor; DNA; Acridine orange; Intercalation; Carbon paste electrode; Hanging mercury drop electrode

INTRODUCTION

In an electrochemical DNA biosensor, DNA is being immobilized on the electrode surface and its electrochemical behavior is being studied in relation with compounds which potentially interact with DNA and possess toxic or therapeutic properties. As electrode materials mainly mercury and carbon electrodes (glassy and carbon paste) have been used [1–5].

Voltammetric signals obtained with mercury electrodes are strongly affected by the DNA structure and sensitively respond to minor DNA damage especially to strand breaking and degradation [6]. A reduction peak at −1.4 V appears due to the disruption of DNA sugar–phosphate backbone [7].

On the other hand, voltammetric signals obtained with carbon paste are less sensitive to changes in the DNA structure compared to mercury electrodes. This problem

*Scholarship holder from the Greek State Scholarship Foundation
†Corresponding author. Fax: +-30-31-997719. E-mail: girousi@chem.auth.gr

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could be overcome by employing chronopotentiometric analysis thus making it possible to obtain quantitative results of this interaction in the determination of low-molecular weight compounds with affinity for DNA by their effect on the oxidation signal of the guanine peak of DNA immobilized on the electrode sensor [8].

Alternatively, qualitative results could be obtained on the basis of different electrochemical behaviors of the nucleic-acid-binding molecules in the absence, and in the presence, of DNA. The shifts of the formal potentials of the redox couple caused by the intercalation of nucleic-acid-binding molecules into DNA double helix- and the reduction of peak current resulting from the dramatic decrease in the apparent diffusion coefficient of the nucleic-acid-binding molecule after association with double-stranded DNA can be exploited [9,10].

The interaction between chemical compounds and DNA occurs in 4 different mechanisms [11,12]: (i) intercalation within DNA (i.e. actinomycin D, daunomycin), (ii) nonintercalative DNA binding (i.e. triostin, netropsin), (iii) covalent binding to DNA (i.e. mitomycin), (iv) strand-breaking interactions (i.e. bleomycin, streptonigrin).

The concern of the biochemists is aroused by the fact that these drugs are inhibitors of nucleic acid synthesis. At present biochemists mainly use agarose gel electrophoresis in order to study the DNA damage due to physical and chemical environmental agents. Gel electrophoresis is a time consuming and laborious technique moreover it is not able to detect small damages to DNA induced by ionizing radiation, enzymatic digestion or treatment with chemicals, damages which can be detected by adsorptive transfer alternating current (AdTS AC) voltammetry [13]. Electrochemical DNA biosensors appear as an interesting alternative in the research field.

Acridine orange is a dye [14] which interacts with DNA by intercalation. In this article, an electrochemical DNA biosensor, based either on carbon paste electrode (CPE) or hanging mercury drop electrode (HMDE), was prepared in order to study the interaction between DNA and acridine orange. The different electrochemical behaviors were studied in the article.

EXPERIMENTAL

Materials

Calf thymus DNA (Catalog No. D-1501, highly polymerized), was purchased from Sigma Single stranded DNA (ssDNA) was prepared by boiling a solution of double stranded DNA (dsDNA) for 15 min and left at 4°C for 10 min. Acridine orange hydrochloride p.a. was purchased from Aldrich (31, 833-7). All reagents were of analytical-reagent grade. Graphite powder was purchased from Fluka. All aqueous solutions were prepared with sterilized doubly-distilled water. In the case of CPE phosphate buffer (pH 7.0) was used as supporting electrolyte and with HMDE 0.3 M NaCl, 50 mM sodium phosphate buffer (pH 8.5). Acridine orange was dissolved in doubly-distilled water, a stock solution of 10^{-3} mol/L was prepared. For the study of the electrochemical behavior of dsDNA and ssDNA on the CPE surface, stock solutions of 1 g/L were prepared in 10 mM Tris–HCl and 1 mM EDTA at pH 8.0, while with the HMDE stock solutions of dsDNA (80 mg/L) and ssDNA (40 mg/L) were prepared in 10 mM Tris–HCl at pH 7.5.

Apparatus

Differential pulse and alternating current (ac) voltammetric measurements were performed with a Metrohm 647 VA-Stand controlled by a 646 VA-Processor. The working electrode was either a CPE or a HMDE, the reference electrode was a saturated Ag/AgCl and the counter electrode was a platinum wire electrode. Ultrapure argon was used to deaerate the solutions for 15 min before each experiment.

Preparation of Working Electrodes

Carbon Paste Electrode

The CPE was prepared by thoroughly mixing in a mortar, 0.75 g of graphite powder and 0.5 mL nujol oil. A portion of the resulting paste was packed into the well of the METROHM electrode body (5 mm diameter, 3 mm deep). Electrical contact was established with stainless steel screw. The surface was polished to a smooth finish before use.

Hanging Mercury Drop Electrode

DNA was absorbed at the electrode surface from 10 μL of solution containing 10 mM Tris–HCl pH 7.5 for 60 s. The DNA modified electrode was washed twice by distilled water and by background electrolyte solution. It was then transferred to deaerated blank background solution, which was initially bubbled with argon for 100 s. The initial potential (E_I) was applied at the electrode for 15 s prior to the voltage scan.

Procedures

Interaction of Surface-confined DNA with Acridine Orange on the CPE Surface

The procedure consists of DNA immobilization, interaction of acridine orange with immobilized DNA and transduction by transfer voltammetry using differential pulse mode. Prior to each medium exchange, the electrode was rinsed carefully with water for 5 s. A freshly polished CPE was first pretreated by applying a potential of +1.7 V for 1 min without stirring in a phosphate buffer solution (pH 7). The nucleic acid was subsequently immobilized onto the activated electrode surface by adsorptive accumulation. For the immobilization of dsDNA the adsorptive accumulation lasted 5 min at +0.5 V, while the adsorptive accumulation for the immobilization of ssDNA lasted 2 min at +0.5 V. The DNA-coated electrode was transferred to the stirred sample solution (analyte plus phosphate buffer pH 7) for 120 s, while holding a potential of +0.2 V. The transduction was performed in the blank phosphate buffer solution. The same procedure was followed for both the dsDNA-sensor and the ssDNA-sensor for the study of the interaction with acridine orange.

Interaction of DNA with Acridine Orange on the HMDE Surface

The mercury electrode fully covered with DNA was immersed into the solution of acridine orange. The optimal concentration for the full coverage of the HMDE was 80 mg/L for dsDNA and 40 mg/L for ssDNA [15]. The interaction between the

two different forms of DNA and increasing concentrations of acridine orange was studied.

RESULTS AND DISCUSSION

Interactions of Surface Confined DNA on CPE with Acridine Orange in Solution

For our studied we used 50 mM sodium phosphate (pH 7) as a background electrolyte. Native dsDNA yielded two oxidation peaks, one at +1.21 V and another at +0.92 V. Thermally denatured ssDNA yielded three intense peaks at +1.20, +0.92, and at +0.66 V.

The accumulation potential and time have a profound effect upon the DPV response. According to our recent studies the peak current is slightly affected by increasing the potential between 0.0 and +0.6 V, while it decreases rapidly at higher potentials. The optimal accumulation time, which has to do with the affinity of the nucleotides to the carbon surface, was found to be 5 min for dsDNA and 2 min for ssDNA.

Acridine orange produces a well developed oxidation peak at +0.78 V with a preconcentration step at +0.2 V for 2 min. Concerning the dsDNA-modified electrode, it was prepared by immersing the CPE into a solution of dsDNA at a concentration of 0.1 g/L in 50 mM phosphate buffer (pH 7) for 5 min at +0.5 V. The electrode was washed and immersed into acridine orange solutions of concentrations varying within 0 and 11×10^{-7} M (in 50 mM phosphate buffer, pH 7, for 2 min at +0.2 V). By increasing acridine's concentration, the two characteristic peaks of dsDNA at +1.21 and at +0.92 V were gradually lowered. This behavior is shown in Fig. 1. When acridine orange reached a concentration value of 5×10^{-7} M a new peak appeared at +0.83 V, probably due to the formation of a complex between DNA and acridine orange.

The ssDNA-modified electrode was prepared with the same way and was immersed in acridine orange solutions of the same range. The three characteristic peaks of ssDNA oxidation were lowered while a new peak at +0.80 V appeared when the concentrations of acridine orange reached the value of 9×10^{-7} M. This peak could be attributed to the excess of acridine orange. The behavior is demonstrated in Fig. 2.

Interaction Between Acridine Orange and DNA at the HMDE

The behavior of acridine orange with DNA was also studied using HMDE as working electrode into a 50 mM sodium phosphate buffer pH 8.5 containing 0.3 M NaCl. Under these conditions acridine orange is being reduced at −1.30 V. The mercury electrode was immersed into a 10 μL drop of ssDNA (40 mg/L) and was washed twice with doubly-distilled water and then with background electrolyte. Then it was transferred into the electrolytic cell and the alternating current voltammogram was performed. An ac voltammogram of ssDNA on the HMDE, is shown in Fig. 3 (1).

Gradually increasing concentrations of acridine orange (Peak A), in the buffer solution containing 40 mg/L acridine orange, were added till the concentration reached a value of 8×10^{-8} M. After a preconcentration step of 15 s at −0.1 V,

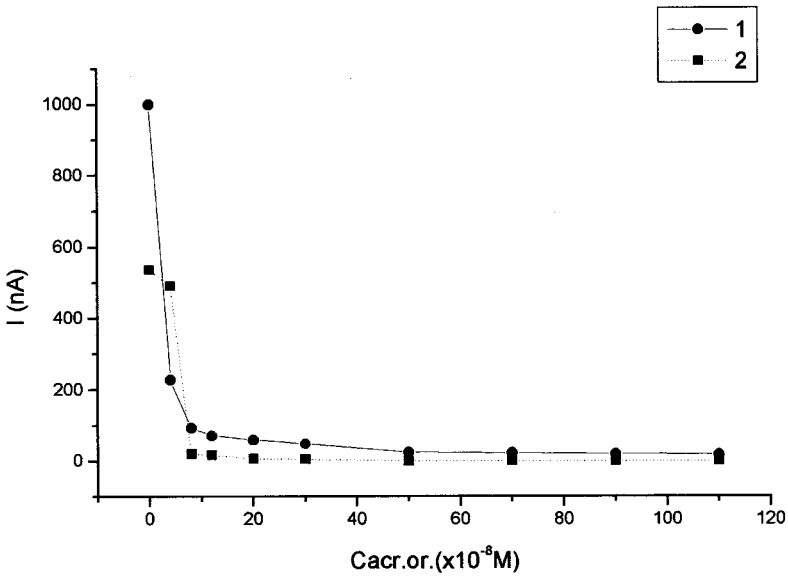

FIGURE 1 Dependence of peak current of dsDNA at: (1) + 1.01 V; (2) + 0.92 V on increasing concentrations of acridine orange. The CPE was pretreated at + 1.7 V for 1 min followed by adsorptive accumulation of dsDNA at + 0.5 V for 5 min and was immersed in acridine orange solutions of different concentrations. Experimental conditions: working electrode: CPE, supporting electrolyte: phosphate buffer pH 7.0, stripping mode: Differential pulse voltammetry.

the electrochemical profile of ssDNA was affected. An ac voltammogram is shown in Fig. 3 (2).

The same procedure was followed in order to study the interaction between dsDNA (80 mg/L) and acridine orange. DsDNA was immobilized on the HMDE surface and then transferred into the buffer solution, an ac voltammogram is shown in Fig. 4 (1).

The dsDNA-modified electrode was immersed into the buffer solution containing increasing concentrations of acridine orange (Peak A), until the acridine's orange concentration became equal to 8×10^{-8} M. The electrochemical configuration of dsDNA was influenced due to the appearance of the new peak (Peak A). An ac voltammogram is shown in Fig. 4 (2).

In both experiments, the electrochemical profiles of ssDNA and dsDNA were affected seriously after the addition of acridine orange, showing that there is evidence of interaction between DNA and acridine orange.

CONCLUSIONS

The different behaviors of acridine orange with DNA on CPE and HMDE could be attributed to a particular way of interaction between DNA and acridine orange. A reasonable conclusion could be that in the case of acridine orange no strand breaking occurs but only intercalative binding, as shown by the results of the study with the dsDNA-modified CPE.

It is well known that voltammetry in combination with mercury electrodes is very sensitive to minor damage to the DNA double helix due to strand breaking or

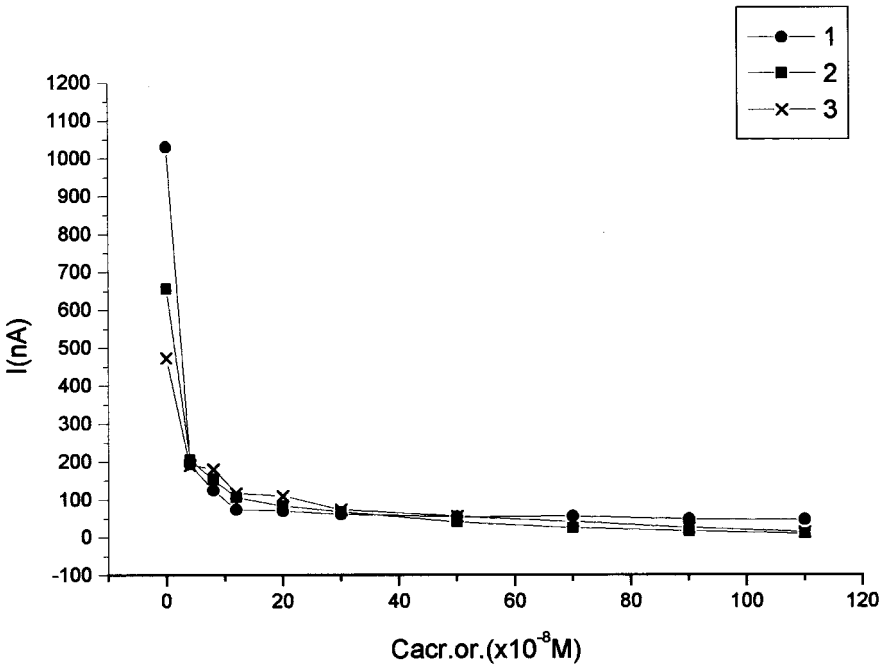

FIGURE 2 Dependence of peak current of ssDNA oxidation at: (1) +1.20 V; (2) +0.92 V; (3) +0.66 V immobilized on the electrode surface on increasing concentrations of acridine orange. The CPE was pretreated at +1.7 V for 1 min followed by adsorptive accumulation of ssDNA at +0.2 V for 5 min and was immersed in acridine orange solutions of different concentrations. The incubation prior to each scan was 2 min. Experimental condition: working electrode: CPE supporting electrolyte: phosphate buffer pH 7.0, stripping mode: Differential pulse voltammetry.

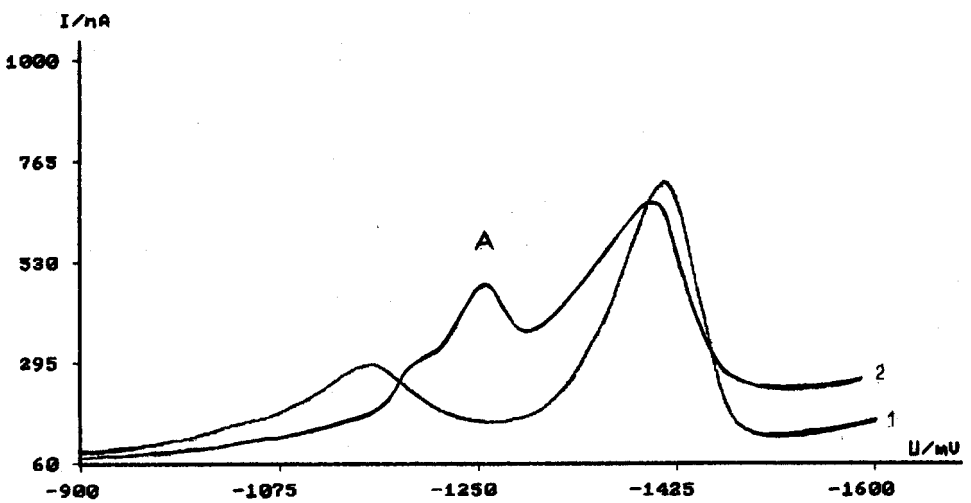

FIGURE 3 (1) Alternating current voltammogram of ssDNA (40 mg/L); (2) ac voltammogram of ssDNA (40 mg/L) + 8×10^{-8} M acridine orange (Peak A, acridine orange). Experimental conditions: working electrode: HMDE, supporting electrolyte: phosphate buffer pH 8.5 + 0.3 M NaCl, stripping mode: Alternating current voltammetry.

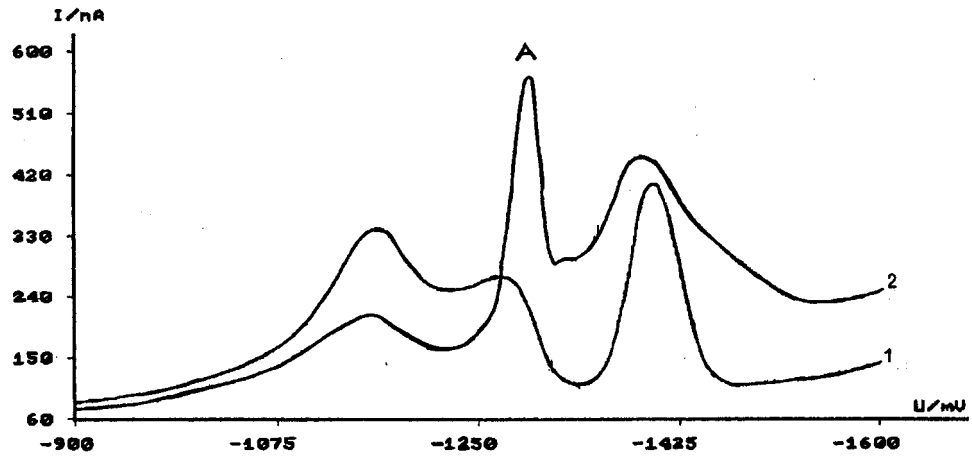

FIGURE 4 (1) ac voltammogram of dsDNA (80 mg/L) and (2) ac voltammogram of dsDNA (80 mg/L) + 8×10^{-8} M acridine orange) (peak A, acridine orange). Experimental conditions: working electrode: HMDE, supporting electrolyte: phosphate buffer pH 8.5 + 0.3 M NaCl, stripping mode: Alternating current voltammetry.

degradation. Voltammetric analysis of nucleic acids with carbon electrodes has shown much lower sensitivities, but recently it has been shown that application of the constant current chronopotentiometry with carbon electrodes in the analysis of DNA [16,17] may provide sensitivities comparable with those yielded by mercury electrodes in combination with voltammetric methods.

Moreover, solid electrodes are more convenient and easier to handle for the construction of various kinds of biosensors involving nucleic acids and proteins. Immobilization of the peptide nucleic acid at the carbon electrode recently produced a highly specific DNA hybridization sensor capable of detecting single base mismatch [18] (point mutation) in DNA. Proper use of the mercury and solid electrodes in nucleic acid research may soon bring electrochemical analysis closer to the needs of contemporary biochemical and molecular-biological research.

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