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Study of interactions between DNA-ethidium bromide (EB) and DNA-acridine orange (AO), in solution, using hanging mercury drop electrode (HMDE)

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Abstract

The interaction of ethidium bromide (EB) and acridine orange (AO) with double stranded (ds), thermally denatured (ss) and supercoiled (sc) DNA, in solution, was studied by alternating current voltammetry (AC voltammetry) at the hanging mercury drop electrode (HMDE) in 0.3 M NaCl+50 mM sodium phosphate buffer (pH 8.5). Their interaction with DNA is shown to be time dependent and completely different. The changes at peak 2 (peak at −1.20 V) of dsDNA form and the appearance of peak 3 (peak at −1.42 V) in scDNA form are presented as criteria declaring the different mechanism of interaction of EB and AO with DNA. Additionally, the appearance of a new peak at around −0.44 V as a result of DNA and AO interaction, differentiates the studied behaviors. The comparison of the electrochemical behaviors of these compounds highlights the differences in the mechanism of interaction.

Keywords: Acridine orange; Ethidium bromide; Alternating current voltammetry; DNA intercalators

1. Introduction

Acridine orange (AO) and ethidium bromide (EB) are dyes and well-known DNA intercalators [1]. The importance of the interaction phenomena in nucleic acid research has raised the concern of researchers in the development of faster, more sensitive and less laborious techniques, such as electrochemical techniques that appear as a very promising alternative.

Important research work was devoted to the study of interactions of compounds, with affinity for DNA, with different DNA forms using electrochemical techniques [2–11]. Such an electrochemical technique is alternating current (AC) voltammetry, combined with the HMDE, which

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is very sensitive to minor damage to the DNA double helix [2,12,13]

The adsorption of nucleic acids on the HMDE electrode, results in the production of three different reduction peaks. More specifically peak at −1.2 V is produced both by native (ds) and thermally denatured (ss) DNA and was due to "segmental desorption or reorientation of the helical regions adsorbed mainly via the sugar-phosphate backbone". Peak at −1.14 V was assigned to "the open ds regions" while the most negative peak at −1.38 V was assigned to "the desorption of the DNA segments firmly adsorbed via bases" [2].

Among the numerous binding studies of small molecules to DNA, EB was one of the most frequently studied intercalators. The binding constant for the intercalation of EB to DNA was determined using amperometry [14]. We have also reported preliminary results on the behavior of AO as a DNA intercalator [15]. In this work we are focusing on the electrochemical study of the interaction between EB and AO with DNA, at the (HMDE) by AC voltammetry. The interaction of the two compounds with DNA gives different electrochemical behaviors, thus giving proofs of the specific interaction mechanism.

2. Experimental

2.1. Materials

Calf thymus DNA (D-1501, highly polymerized), was purchased from Sigma. EB p.a was of 95% purity and AO hydrochloride was of 99% purity (Aldrich). All aqueous solutions were prepared with sterilized doubly distilled water.

The supporting electrolyte was 0.3 M NaCl and 50 mM sodium phosphate buffer (pH 8.5). We have determined DNA concentration by measuring the absorbance at 260 nm [16]. DsDNA, ssDNA and scDNA solutions were prepared in 0.2 M NaCl and 10 mM Tris–HCl (pH 7.5). Plasmid DNA (pUC119) was isolated from *Escherichia coli* cells using the method of alkaline lysis. Single stranded (ss) DNA was prepared by boiling a solution of ds DNA for 15 min and left at 4 °C for 10 min.

2.2. Apparatus

AC voltammetric measurements were performed using a Metrohm 647 VA-Stand controlled by a 646 VA-Processor. The working electrode was a hanging mercury electrode, the reference electrode was a saturated Ag/AgCl/3 M KCl and the counter electrode was a platinum wire electrode. Ultrapure argon was used to bubble the solutions of dissolved oxygen for 15 min before each experiment. The following parameters were chosen: frequency 230 Hz, amplitude 10 mV, scan rate 20 mV s^{-1}. All measurements were performed in 0.3 M NaCl and 50 mM sodium phosphate (pH 8.5) buffer solution.

2.3. Preparation of nucleic acid-modified electrodes

The procedure was established by other researchers [5] and is the following: DNA was accumulated at the electrode surface from 10 μl of a solution containing 0.2 M NaCl and 10 mM Tris–HCl, pH 7.5 for 120 s. The DNA modified electrode was washed twice by distilled water and subsequently by supporting electrolyte solution. It was then transferred to deareated 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 each voltage scan.

2.4. Treatment of DNA with EB or AO in solution

20 μl dsDNA of (80 mg l^{-1}) or ssDNA (40 mg l^{-1}) or scDNA (150 mg l^{-1}) were incubated with, varying concentrations of, EB or AO for 30 min prior to immobilization at the electrode surface. 10 μl of the solution were absorbed at the surface of the electrode for 120 s and the modified electrode was washed twice with doubly-distilled water and then with supporting electrolyte. The electrode was then immersed into the blank supporting electrolyte which was previously deareated with argon.

3. Results and discussion

3.1. Alternating current voltammetric responses of double stranded (ds), single stranded (ss) and supercoiled (sc) DNA at full electrode coverage

The mercury electrode was immersed into a 10 μl drop of the DNA solution for an accumulation time of 120 s. The DNA concentration was adjusted to secure full coverage of the electrode surface under the given conditions. Figs. 1–3 present the relation between the characteristic peak current values (depending on each DNA form) and increasing DNA concentrations. The concentrations were selected as the optimal values and were: 80 mg l^{-1} for dsDNA, 40 mg l^{-1} for ssDNA and 150 mg l^{-1} for scDNA.

All the solutions were prepared in 0.2 M NaCl and 10 mM Tris–HCl pH 7.5. The electrode was washed and transferred into the electrolytic cell containing a blank supporting electrolyte and the AC voltammetric measurement was performed.

Supercoiled DNA yielded a peak at −1.25 V, dsDNA yielded peak 1 at −1.17 V, peak 2 due to unwinding at −1.30 V and peak 3 at −1.42 V, and thermally denatured (ss DNA) yielded a more intense peak 3 at −1.417 V and peak 1 at −1.177 V in very good agreement with the results obtained from other researchers [5].

3.2. Alternating current voltammetric responses of EB and AO

EB produces a well-developed peak at −1.33 V with a preconcentration step at −0.1 V for 120 s and a subsequent transfer at blank supporting electrolyte. An AC voltammogram is shown in Fig. 4. Linearity is observed in the range (2–135) $\times 10^{-9}$ M (sensitivity 1.32 nA nM^{-1}, r = 0.990) while saturation of the electrode surface is observed at higher concentrations.

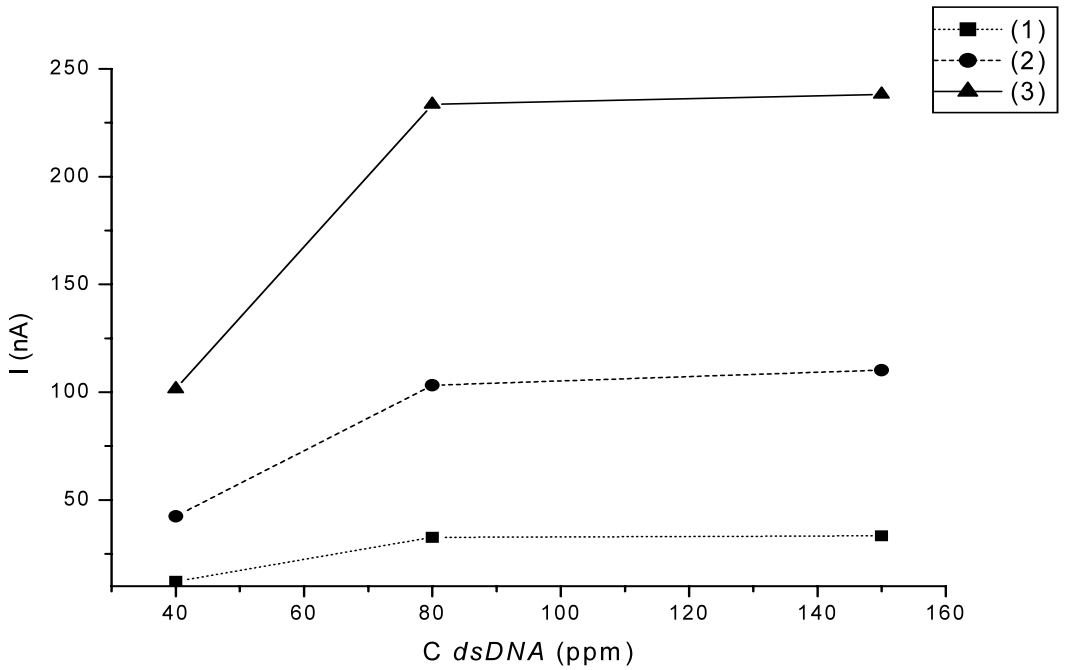

Fig. 1. Dependence of peak current of the characteristic peaks of dsDNA in relation with increasing concentrations of dsDNA. (1) Peak 2 (−1.28 V), (2) peak 1 (−1.18 V), (3) peak 3 (−1.43 V).

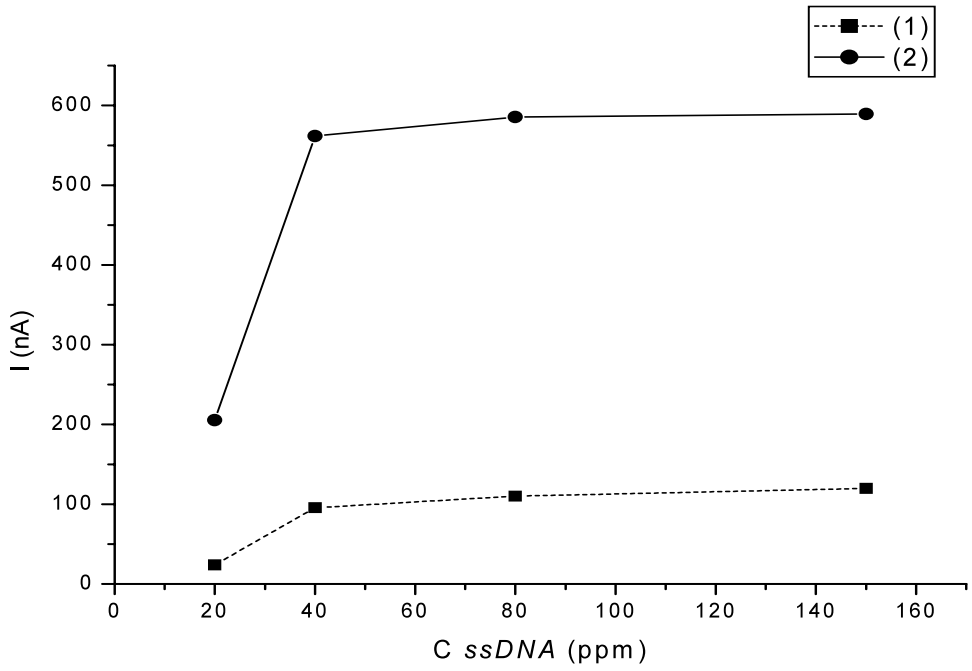

Fig. 2. Dependence of peak current of the characteristic peak of ssDNA in relation with increasing concentrations of ssDNA. (1) Peak 1 (−1.18 V), (2) peak 3 (−1.4 V).

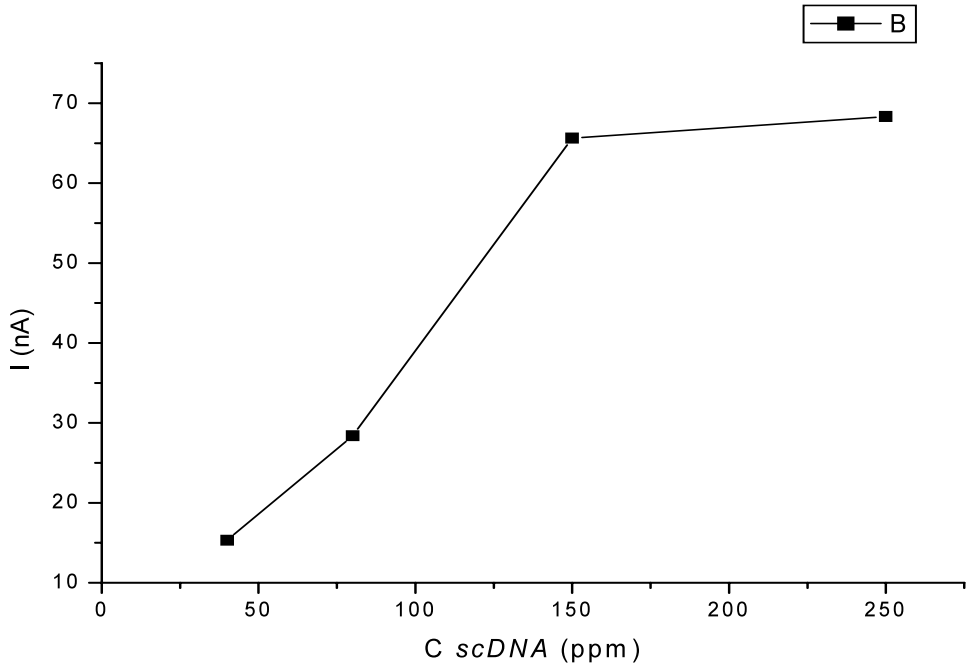

Fig. 3. Dependence of peak current, of the characteristic peak of sc DNA at −1.25 V, in relation with increasing concentrations of scDNA.

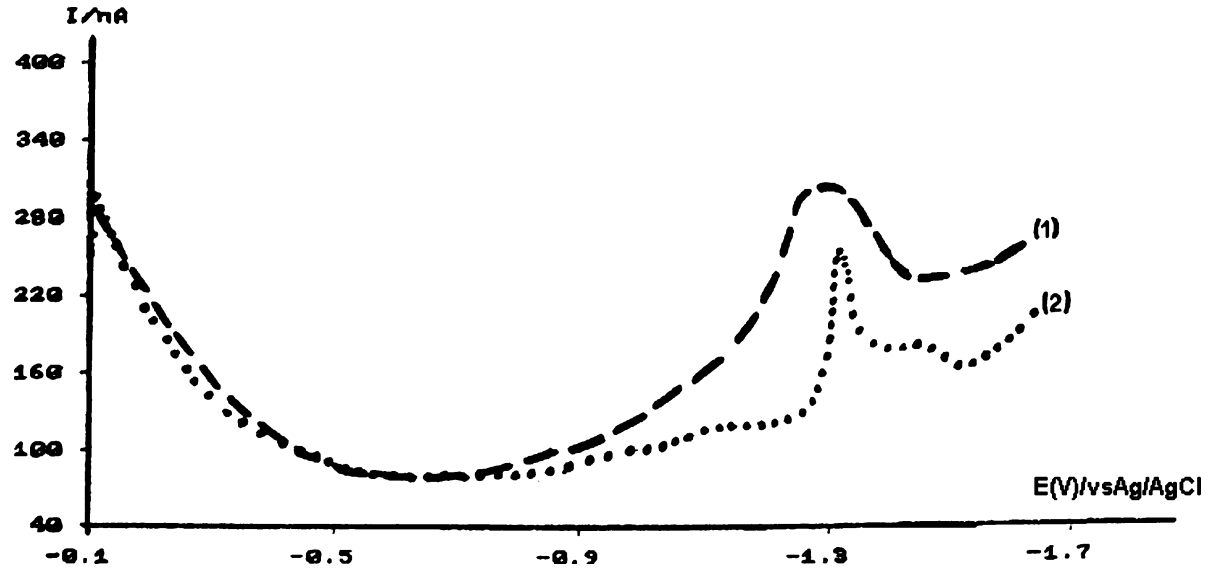

Fig. 4. AC voltammograms of (1) EB (9×10^{-8} M), (2) AO (3.5×10^{-7} M) into the buffer solution 0.3 M NaCl and 50 mM phosphate, pH 8.5.

AO produces a peak at −1.33 V. The AC voltammogram was performed in blank supporting electrolyte after a preconcentration step at −0.1 V for 120 s. An AC voltammogram is shown in Fig. 4. Linearity is observed in the range $(0.02–0.54) \times 10^{-6}$ M (sensitivity 684.8 nA μM^{-1}, r = 0.998) while saturation of the electrode surface is observed at higher concentrations.

3.3. Interaction between DNA and EB in solution

Different concentrations of EB between 10^{-8} and 10^{-4} M were studied in relation with the three different forms of DNA.

By increasing concentrations of EB during the incubation with dsDNA, peak 2 becomes wider and its characteristic potential is shifted to more negative potentials, while peak 3 decreases. The presence of EB causes local distortions of DNA double-helix and/or overall untwisting (unwinding) of dsDNA molecules thus leading to the increase of peak 2. In addition, the adsorption of the underwound DNA segments prevent surface DNA denaturation and formation of DNA regions with freely accessible bases resulting in a decreased intensity of peak 3 [18]. Fig. 5 presents the effect of the EB at a concentration of 5×10^{-9} M on the dsDNA configuration.

In the case of thermally denatured DNA (ssDNA), after its incubation with EB, peak 3 increases slightly, while peak 1 remains stable. In this case, the behavior is different, since no intercalation occurs. Fig. 6 depicts the difference at ssDNA configuration after incubation with 5×10^{-9} M EB. A new peak appears at −0.99 V probably due to different reorientation of some phosphodiesteric bonds not fully adsorbed [18].

The interaction of scDNA and EB resulted in the appearance of peak 3 as a sign of introduction of "free ends" in the DNA structure, where the bases of DNA are more accessible to reduction [17,18]. As is shown in Fig. 7, increasing concentrations of EB result in the appearance of peak 3 which increases gradually.

The incubation time between DNA and EB was selected according to the appearance of peak 3 in the scDNA-form. Peak 3 increases with time during the incubation of scDNA and EB up to 30 min and it levels off after this point. This is the reason that the optimal incubation time selected

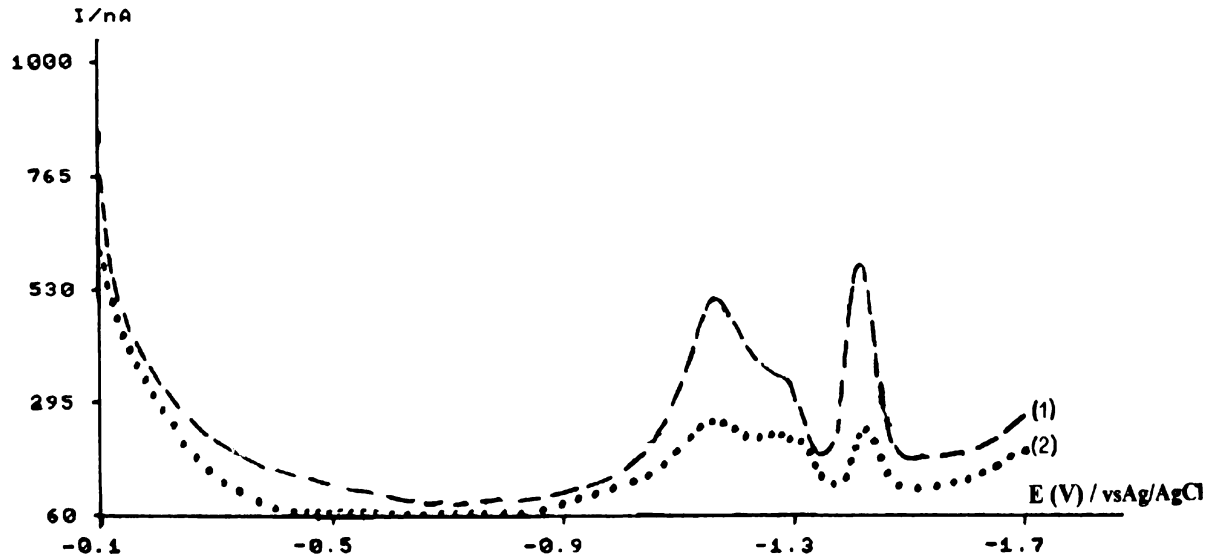

Fig. 5. (1) AC voltammogram of dsDNA (80 mg l^{-1}) immobilized on the HMDE surface. (2) As in (1) + 5×10^{-9} M EB immobilized on the electrode surface.

was the one of 30 min for EB and in order to compare the different behaviors it was decided to incubate AO and the different forms of DNA also for 30 min. Fig. 8 presents the effect of time on peak 3, while the concentration of EB was constant and equal to 5×10^{-6} M.

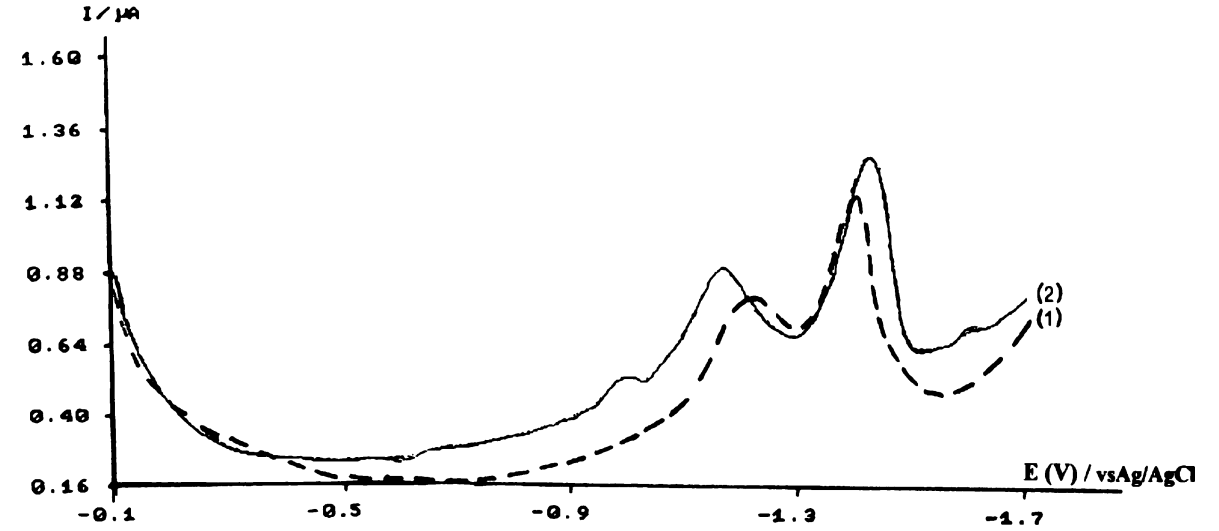

Fig. 6. (1) AC voltammogram of ssDNA (40 mg l^{-1}) immobilized on the HMDE surface. (2) As in (1) + 5×10^{-9} M EB immobilized at the electrode surface.

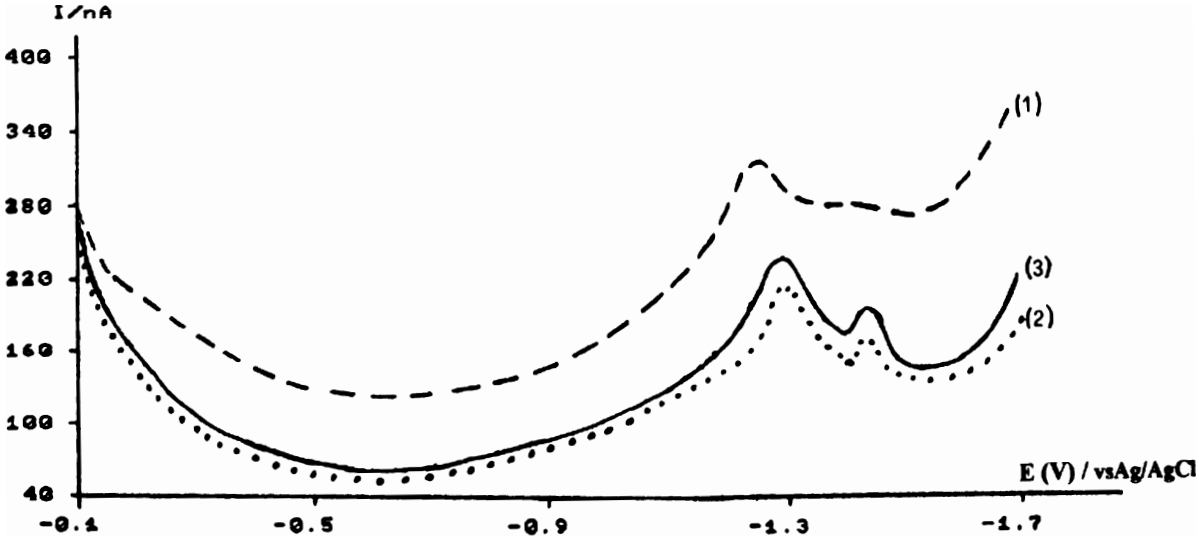

Fig. 7. (1) AC voltammogram of scDNA (150 mg l^{-1}) immobilized on the HMDE surface. (2) As in (1)+5×10^{-8} M EB immobilized on the HMDE surface. (3) As in (1)+2.5×10^{-8} M EB immobilized on the HMDE surface.

3.4. Interaction between AO and DNA in solution

Different concentrations of AO between 10^{-8} and 10^{-4} M were studied in relation with the three different forms of DNA.

By raising the concentration of AO during the incubation with dsDNA a new peak appears at −0.44 V probably due to conformational changes caused by the intercalated AO. Such a behavior was not observed after the incubation of dsDNA

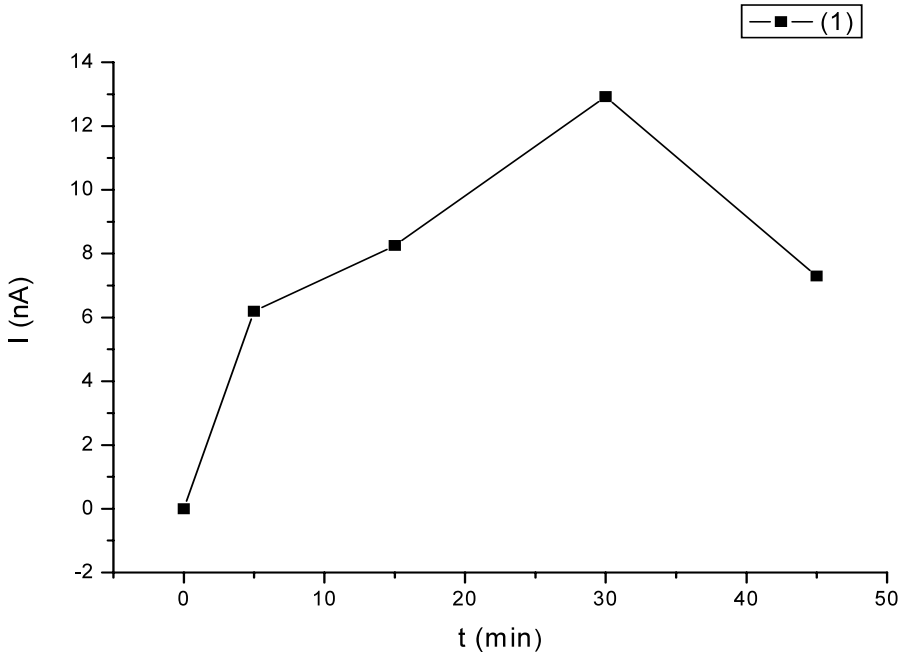

Fig. 8. The effect of incubation time on peak 3 (−1.43 V) between scDNA (150 mg l^{-1}) and EB (5×10^{-6} M).

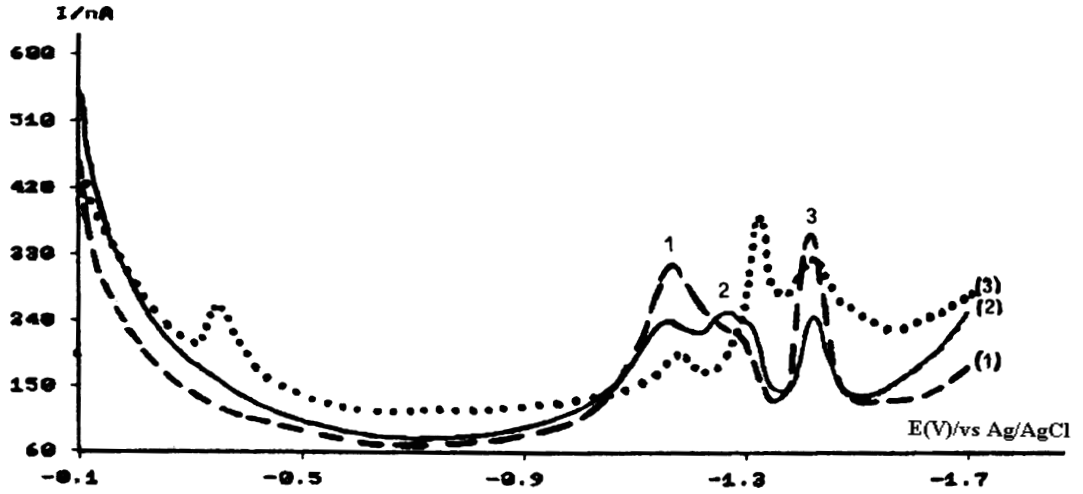

Fig. 9. (1) AC voltammogram of dsDNA (80 mg l^{-1}) immobilized on the HMDE surface. (2) As in (1)+2.5×10^{-7} M AO immobilized on the HMDE surface. (3) As in (1)+4×10^{-5} M AO immobilized on the HMDE surface.

and EB leading to the conclusion that the mechanism of interaction, in these two cases, is different. Fig. 9 presents the effect of two representative AO's concentrations on the dsDNA configuration, where the new peak reaches its optimal characteristics at a concentration 4×10^{-5} M of AO.

In the case of interaction between ssDNA and AO the characteristic peak of ssDNA at −1.18 V decreases probably due to non-adsorbed phosphodiesteric bonds in the presence of AO [18]. As is shown in Fig. 10 the characteristic peak of ssDNA at −1.18 V decreases and slightly shifts to −1.22

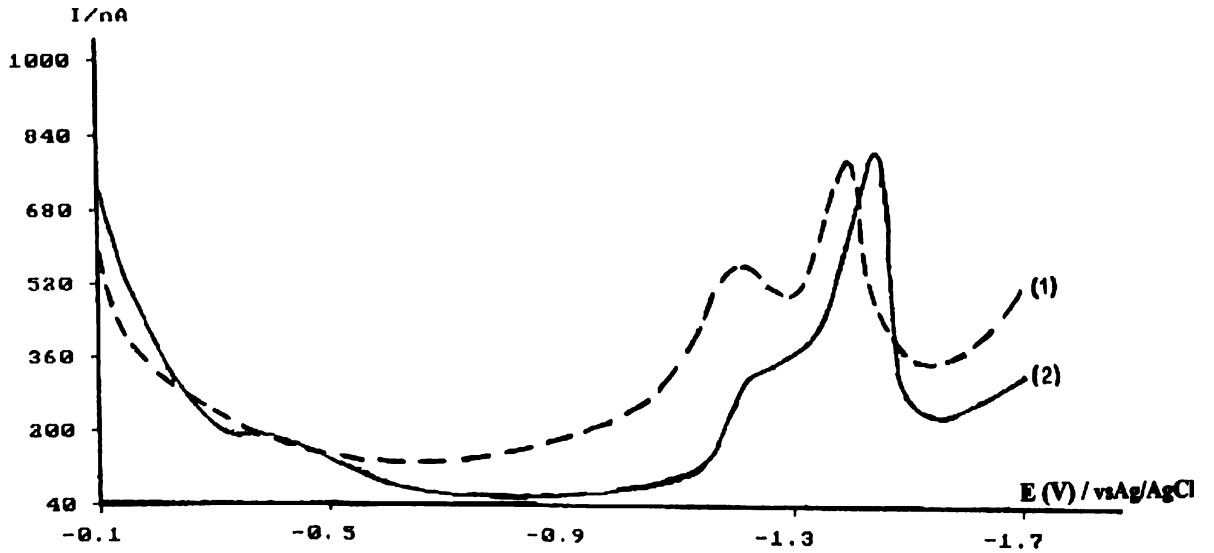

Fig. 10. (1) AC voltammogram of ssDNA (40 mg l^{-1}) immobilized on the HMDE surface. (2) As in (1)+2.5×10^{-5} M AO immobilized on the HMDE surface.

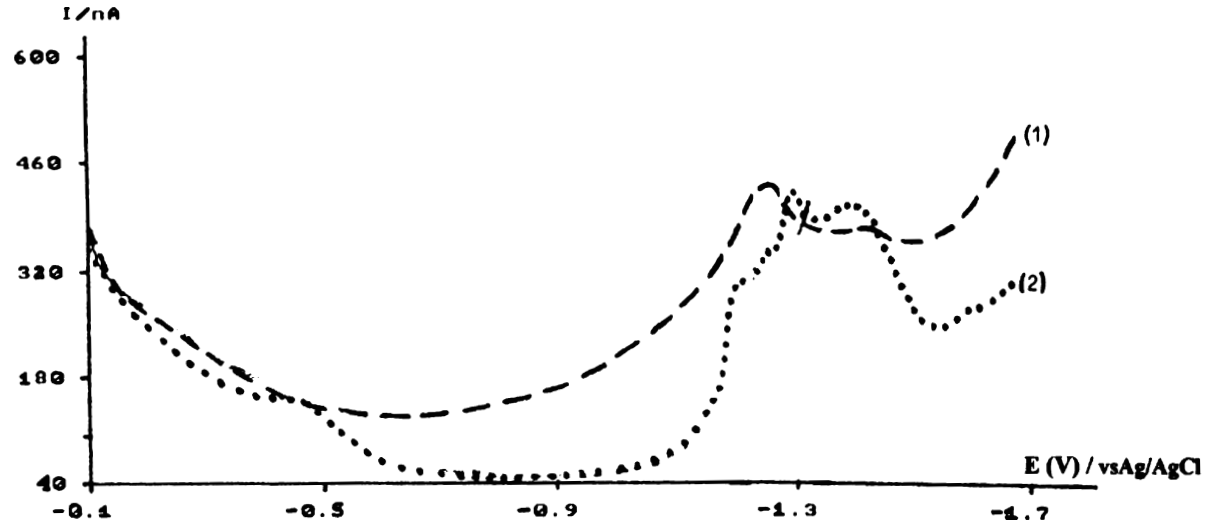

Fig. 11. (1) AC voltammograms of scDNA (150 mg l^{-1}) immobilized on the HMDE surface. (2) As in (1)+4×10^{-5} M AO immobilized on the electrode surface.

V. The other characteristic peak at −1.43 V slightly increases, when the concentration of AO equals the value of 2.5×10^{-5} M. The new peak appears also at −0.39 V, but even at a concentration of 4×10^{-5} M of AO, this peak is less intense compared with the one shown in Fig. 9.

After the interaction of scDNA and AO, peak 3 does not appear. By increasing concentrations of AO the characteristic peak of scDNA decreases, while the characteristic peak of AO becomes more and more intense. The new peak appears at −0.44 V as the concentration of AO equals 4×10^{-5} M and could be attributed to the intercalated AO. This behavior is shown in Fig. 11.

4. Conclusions

The nucleic acid-modified mercury electrode is a useful tool in DNA research, capable of competing with a number of currently used methods including gel electrophoresis because of its sensitivity, simplicity and versatility.

In this paper we have shown that interaction of EB with DNA in solution can be monitored by AC voltammetry on the HMDE surface along with the study of AO and DNA in solution at the same system. Their interaction with DNA is shown to be time dependent and the optimal reaction time was investigated. Furthermore, the comparison of the electrochemical behavior of these two intercalators highlights the differences into their mechanism of action.

4.1. Interaction of EB with DNA

At the mercury surface, dsDNA is slowly unwound in the potential region around −1.2 V (peak 2). The increase of peak 2 after the interaction with EB leads to the conclusion that EB causes local prestretching of DNA. According to Palecek [2,19] peak at −1.2 V is due to opening of the DNA double helix at the electrode surface, resulting in an increased accessibility of DNA bases. A more intense peak 3 appears in ssDNA compared with the dsDNA form, where peak 3 decreases gradually, which becomes higher after interaction with EB. This slight increase can be attributed to electrostatic forces resulting in the relaxation of DNA. These differences concerning peak 3 can be used as criteria for the characterization of a compound as intercalator. The appearance of peak 3 after interaction of EB with scDNA, which is a molecule lacking free ends

and cannot be extensively unwound at the electrode surface, is the most important evidence leading to the conclusion that EB is an intercalative introducing "free ends" in the DNA structure [19].

4.2. Interaction of AO with DNA

The interaction of AO with dsDNA resulted in a decrease of peak 3 at low concentrations of AO, while at higher concentrations a new peak at −0.44 V appeared probably due to the intercalated AO. Peak 3 appearing after the interaction with ssDNA has a tendency to increase, while the nature of the new peak at −0. 39 V is different, since there does not occur any intercalation phenomenon between ssDNA and AO. It is obvious that there is an interaction between the two of them probably due to electrostatic forces. Peak 3 does not appear after the interaction of AO with scDNA, but there is a peak which appears at −0.44 V and could be attributed to the intercalated AO. The latter peak is less intense compared with the one appearing after the interaction of dsDNA and AO maybe because the sc form is more stable and rigid compared with the ds form.

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References

[1] M.J. Waring, Annu. Rev. Biochem. 50 (1981) 159.
[2] E. Palecek, Electroanalysis 8 (1996) 7.
[3] A.M.O. Brett, S.H.P. Serrano, T.A. Macedo, D. Raimundo, M.H. Marques, M.A. La-Scalea, Electroanalysis 8 (1996) 992.
[4] J. Wang, G. Rivas, X. Cai, H. Shiraishi, P.A.M. Farias, N. Dontha, D. Luo, Anal. Chim. Acta 332 (1996) 139.
[5] M. Fojta, E. Palecek, Anal. Chim. Acta 342 (1997) 1.
[6] A.M.O. Brett, S.H.P. Serrano, I.G.R. Gutz, M.A. La-Scalea, Electroanalysis 9 (1997) 110.
[7] X. Chu, G.-L. Shen, J.-H. Jiang, T.-F. Kang, B. Xiong, R.-Q. Yu, Anal. Chim. Acta 373 (1998) 29.
[8] G. Marazza, I. Chianella, M. Mascini, Anal. Chim. Acta 387 (1999) 297.
[9] V. Brabec, Electrochimica Acta 45 (2000) 2929.
[10] C. Teijeiro, P. Perez, D. Marin, E. Palecek, Bioelectrochem. Bioenerg. 38 (1995) 77.
[11] J. Wang, M. Ozsoz, X. Cai, G. Rivas, H. Shiraishi, D. Grant, M. Chicharro, J. Fernández, E. Palecek, Bioelectrochem. Bioenerg. 45 (1998) 33.
[12] F. Jelen, M. Fojta, E. Palecek, J. Electroanal. Chem. 427 (1997) 49.
[13] E. Palecek, Talanta 56 (2002) 809.
[14] T.-C. Tang, H.-J. Huang, Electroanalysis 11 (1999) 1185.
[15] I. Gherghi, S. Girousi, A. Pantazaki, R. Tzimou-Tsitouridou A. Voulgaropoulos, Int. J. Env. Anal. Chem., in press.
[16] G.D. Fasman, Handbook of Biochemistry and Molecular Biology, third ed., CRC Press, Cleveland, 1975, p. 589.
[17] E. Palecek, M. Fojta, Q. Anal. Chem. 73 (2001) 74A.
[18] M. Fojta, L. Havran, J. Fulneckova, T. Kubicarova, Electroanalysis 12 (2000) 926.
[19] E. Palecek, Bioelectrochem. Bioenerg. 28 (1992) 71.