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Study of interactions between actinomycin D and DNA on carbon paste electrode (CPE) and on the hanging mercury drop (HMDE) surface

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Abstract

The interaction of actinomycin (ACTD) with double stranded (ds) calf thymus DNA and single stranded (ss) DNA was studied at the carbon paste electrode surface by means of transfer voltammetry in 0.2 M phosphate buffer solution (pH 7.4). Accordingly the interaction of actinomycin (ACTD) with ds calf thymus DNA, ss DNA and supercoiled (sc) DNA was studied using hanging mercury drop electrode in 0.3 M NaCl, and 50 mM sodium phosphate buffer (pH 8.5). The different electrochemical behaviours are presented and compared in the article.

Keywords: Actinomycin-D; DNA intercalators; CPE; HMDE

1. Introduction

Actinomycin D (ACTD) is an antitumor antibiotic that contains a 2-aminophenoxazin-3-one chromophore and two cyclic pentapeptide lactones. This drug has been used clinically for the treatment of highly malignant tumors and also in combination with other antitumor agents to treat high-risk tumors [1,2].

There is general agreement that its antitumor activity is directly attributable to its ability to bind within duplex DNA via intercalation of the planar chromophore, preferably at the GpC sequence with the two pentapeptide rings resting on the

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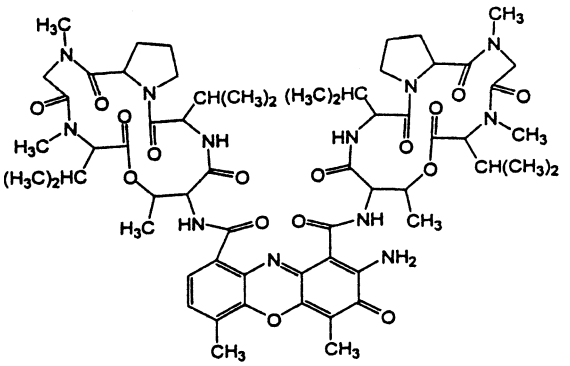
minor groove. A model of DNA–ACTD complex has been accepted according to which the phenoxazone ring is intercalated between the G·C and C·G basepairs (where 7guanine residues are on opposite strands), forming strong hydrogen bonds in the minor groove between the guanine 2-amino groups and the carbonyl oxygen atoms of the L-threonine residues of the cyclic pentapeptides. There have also been recent reports indicating that ACTD may also bind strongly via some non-classic modes, such as to single-stranded DNA and to some DNA sequences containing no GpC site. Calorimetric studies, optical methods and capillary electrophoresis have been used so far in order to investigate the nature of the binding to DNA and DNA sequences [3–7].

Among a wide range of established techniques, for the study of ligand-DNA binding, from the simple measurement of UV adsorption and melting temperature to demanding techniques like NMR or X-ray crystallography, electrochemical methods have been developed and reinforced DNA research. Voltammetric signals obtained with mercury electrodes are strongly affected by the DNA structure and present high sensitivity to minor damage especially to strand breaking and degradation [8,18]. On the other hand, carbon paste electrodes in combination with stripping techniques lead to quantitative results regarding the determination of low-molecular weight compounds with affinity for DNA [8–18].

Our study is the first one focusing on the interaction between DNA and ACTD based on the development of an electrochemical biosensor either on a carbon paste (CPE) or a hanging mercury drop electrode (HMDE). The different electrochemical behaviour of ACTD is presented and compared with the behaviour of other model intercalators, such as ethidium bromide or acridine orange which were studied in the same way. Our aim is to prove that electrochemical methods are competitive enough and can be adequately applied to the characterization of such interactions and expand them on the detection of selective ligand binding.



Actinomycin (D) (ACTD)

2. Experimental

2.1. Reagents

Double stranded (ds) calf thymus DNA (D-1501, highly polymerized) was purchased from Sigma. ACTD was purchased from Sigma (16,053-9) p.a. and was of 95% purity. The supporting electrolyte of differential pulse voltammetric experiments was phosphate buffer solution 0.2 M (pH 7.4), while the one of alternating current voltammetric experiments was 0.3 M NaCl, 50 mM sodium phosphate buffer (pH 8.5).

Single stranded (ss) DNA was prepared by boiling a solution of ds DNA for 15 min and lefting it to stand at 4 °C for 10 min. Plasmid DNA (pUC119) was isolated from *Escherichia coli* cells using the method of alkaline lysis. For the study of the electrochemical behaviour of dsDNA and ssDNA on the CPE surface, the stock solutions (1 g/l for dsDNA and 0.5 g/l for ssDNA) were prepared in 10 mM Tris–HCl and 1 mM EDTA at pH 8.0, while for the electrochemical behaviour on the HMDE the stock solutions of dsDNA (80 mg/l), ssDNA (40 mg/l) and scDNA (150 mg/l) were prepared in 10 mM Tris–HCl at pH 7.5. Stock solutions of ACTD were prepared with water, while dilute solutions were prepared just before use. The water used was doubly distilled and sterilized.

All water and pipette tips were sterilized by autoclaving for 20 min. The electrochemical cells were cleaned with diluted nitric acid, rinsed with water and sterilized for 20 min. Ultrapure nitrogen was used to bubble the solutions of dissolved oxygen for 1 min before each experiment.

2.2. Apparatus

Differential pulse and alternating current voltammetric measurements were performed with a Metrohm 647 VA-Stand controlled by a 646 VA-Processor. The working electrode for the differential pulse voltammetric measurements was a CPE of 6 mm diameter, the reference electrode was a saturated Ag/AgCl and the counter electrode was a platinum wire electrode, while the working electrode for the alternating current voltammetric measurements was a HMDE.

2.3. Preparation of working electrodes

2.3.1. Carbon paste electrode

The carbon paste was prepared in the usual way by hand-mixing graphite powder and nujol oil. The ratio of graphite powder to nujol oil was 75:25. The resulting paste was packed tightly into a Teflon sleeve. Electrical contact was established with stainless steel screw. The surface was polished to a smooth finish before use. The electrode was pretreated by applying a potential at +1.7 V for 1 min without stirring prior to the accumulation step. According to other researchers [19] the electrochemical pretreatment produces a more hydrophilic surface state and a concomitant removal of organic layers.

DNA modified electrode was prepared by applying a potential of + 0.5 V for 5 min after the electrode's pretreatment. Different DNA concentrations were used and immobilised on the CPE surface in order to detect the optimal concentration for full coverage of the CPE surface. Fig. 1 presents the different peak heights obtained by varying DNA concentration. A concentration of 0.1 g/l (in the case of dsDNA) and 0.05 g/l (in the case of ssDNA) were selected as the most suitable, since at these concentrations is observed full electrode surface coverage.

2.3.2. Hanging mercury drop electrode

DNA was absorbed at the electrode surface from 10 μl of stock solutions after dipping the mercury drop for 120 s. The DNA modified electrode was washed twice by distilled water and by background 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 the voltage scan.

3. Procedures

3.1. Interaction of solution-phase DNA with ACTD on the CPE surface

The analysis of solution-phase DNA with ACTD consisted of mixing the two components, followed by accumulation and transduction by transfer voltammetry using differential pulse as stripping mode. The electrode was rinsed for 5 s prior to each medium exchange. Stock DNA (0.1 g/l for dsDNA and 0.05 g/l for ssDNA) and ACTD solutions were added to 0.2 M phosphate buffer to produce the required concentrations and the mixture was left to stand for 10 min. A freshly polished CPE was immersed into the mixture solution. Subsequently to the electrode's pretreatment, the accumulation of the mixture was performed by applying a potential of +0.5 V for 5 min. The transduction was carried out in the blank phosphate buffer solution, with an initial potential of +0.1 V and a scan rate of 50 mV/s.

3.2. Interaction of surface-confined DNA with ACTD on the CPE surface

The procedure consists of DNA immobilization, interaction of ACTD with immobilized DNA and transduction by transfer voltammetry using differential pulse as stripping mode. Prior to each medium exchange, the electrode was rinsed carefully with water for 5 s. After the pretreatment of the CPE, as previously described, the nucleic acid was subsequently immobilized onto the activated electrode surface by adsorptive accumulation for 5 min at +0.5 V. The dsDNA-coated electrode was

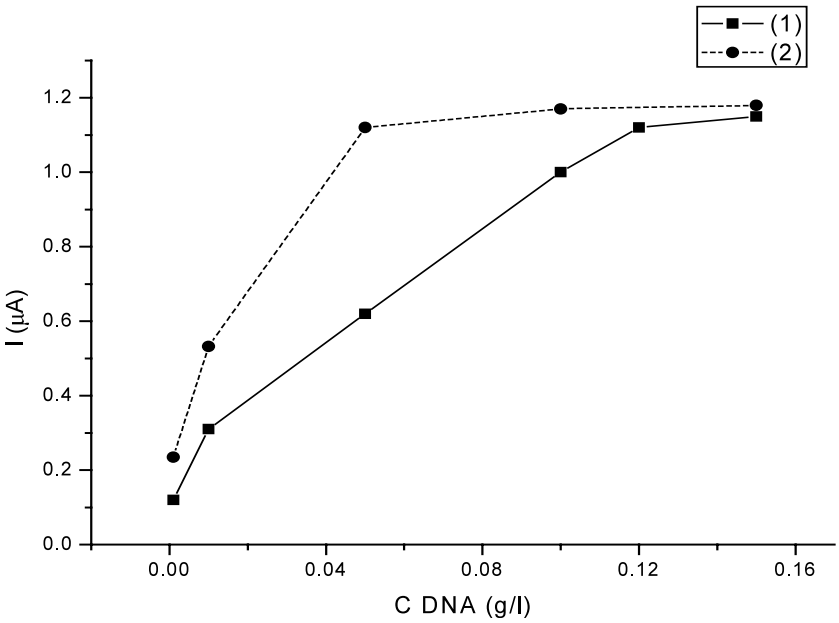


Fig. 1. (1) Dependence of peak current of the characteristic guanine peak of dsDNA in relation with increasing concentrations of dsDNA. (2) Dependence of peak current of the characteristic guanine peak of ssDNA in relation with increasing concentrations of ssDNA. *The experiment was performed under the experimental conditions described in the text.*

transferred to the stirred sample solution (analyte plus 0.2 M phosphate buffer solution pH 7.4) 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 the immobilization of ssDNA and the study of the ssDNA-sensor interaction with ACTD.

3.3. Interaction of surface-confined analyte with DNA on the CPE surface

After the pretreatment of the CPE surface, ACTD was first accumulated at the surface of the CPE and then the resulting ACTD-coated electrode was rinsed with water for 5 s and subsequently immersed into the stirred sample solution containing DNA plus 0.2 M phosphate buffer solution pH 7.4. The surface of the electrode was not renewed prior to each assay. All the experiments were conducted at room temperature (20 °C).

3.4. Treatment of DNA with ACTD in solution and immobilization on the HMDE surface

DNA was incubated with ACTD at different concentrations for 45 min prior to adsorption at the electrode surface. Ten microliters 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 background electrolyte. The electrode was then immersed into the blank background electrolyte which was previously deareated with argon.

The mercury electrode should be fully covered with DNA and the optimal concentration for the full coverage of the HMDE surface is 80 mg/l for the dsDNA, 40 mg/l for the ssDNA and 150 mg/l for the scDNA. The interaction between the different forms of DNA and increasing concentrations of ACTD was studied. The transduction was carried out in blank background electrolyte with an initial potential of −0.1 V, a scan rate of 20

mV/s, a frequency of 230 Hz and a peak to peak amplitude of 10 mV.

4. Results

4.1. Transfer voltammetry using differential pulse mode of ACTD and DNA at CPEs

For our studies we used 0.2 M sodium phosphate (pH 7.4) as a background electrolyte which was more suitable compared with the 0.001 M phosphate buffer at pH 7.4 that was also examined. Native ds DNA yielded two positive peaks at +0.92 and at +1.22 V, which were more intense in the first supporting electrolyte. The same happened with thermally denatured (single-stranded) DNA, which yielded two higher peaks at +0.93 and at +1.22 V. In both of these two cases, the first peak corresponds to the oxidation of the guanine residues and the second one corresponds to the oxidation of adenine residues.

The accumulation potential and the accumulation time have a profound effect upon the DPV response. As it has been shown in our previous studies [20], 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 influence of ionic strength was also studied as a factor affecting the electrochemical behaviour of dsDNA and ssDNA using a 0.001 M phosphate buffer pH 7.4. Under these conditions the electrochemical behaviour of DNA has changed. The dsDNA yielded two less intense peaks, while ssDNA yielded three peaks, first one at +0.67 V, a second one at +0.92 V and another at +1.20 V.

ACTD produces a peak at +1.12 V with a preconcentration step at +0.2 V for 120 s. The DPV response decays at other preconcentration potentials, such as +0.4, +0.6 and +0.8 V. The calibration curve at Fig. 2 shows the current response in relation with increasing concentrations of ACTD at the above optimal conditions.

4.1.1. Interactions of surface-confined DNA with ACTD in solution

The CPE electrode was first pretreated by applying a potential at +1.7 V for 1 min. The dsDNA-modified electrode was prepared by immersing the CPE in a solution of dsDNA at a concentration of 0.1 g/l in 0.2 M phosphate buffer (pH 7.4) for 5 min at +0.5 V. The electrode was washed and immersed in ACTD solutions of different concentrations ranging between 0 and 6.05×10^{-11} M (in 0.2 M sodium phosphate buffer, pH 7.4, for 120 s at +0.2 V). The selection of the interaction time was done according to the potential changes in the characteristic oxidation peak of DNA and a potential appearance of any new peak declaring the formation of a complex or the excess of the drug into the solution.

By increasing the ACTD's concentration, both peaks of dsDNA were gradually decreased. A decrease was also observed in the case of the use of thermally denatured (ss) DNA. In this case the ssDNA-modified electrode was prepared as already mentioned and was immersed in ACTD solutions of different concentrations ranging from 0 to 2.587×10^{-11} M (in 0.2 M sodium phosphate buffer, pH 7.4, for 120 s at +0.2 V). It has to be mentioned that when ACTD's concentration reaches the value of 2.59×10^{-11} M, the two characteristic peaks of ssDNA disappeared. Figs. 3 and 4 present the decrease of the peak currents of dsDNA and ssDNA in relation to increasing concentrations of the drug into the solution.

The same decrease of the DNA characteristic peaks was observed during the interaction of dsDNA or ssDNA and the drug in the 0.001 M phosphate buffer pH 7.4.

4.1.2. Interactions of ACTD and DNA in solution

The incubation time of the two components is a very important factor affecting the DPV response. Fig. 5 shows the effect of the incubation time of ACTD and DNA in solution. It is shown that the current response is increased as the incubation time is increased. The time selected was 10 min, since no dramatic change at the peak current occurs after this point.

Double stranded DNA solution of 0.1 g/l (final concentration) was left to react for 10 min with different concentrations of ACTD ranging from 0 to 8.716×10^{-9} M. By increasing concentrations of ACTD into the solution, the peak current of dsDNA due to the oxidation of guanine residues

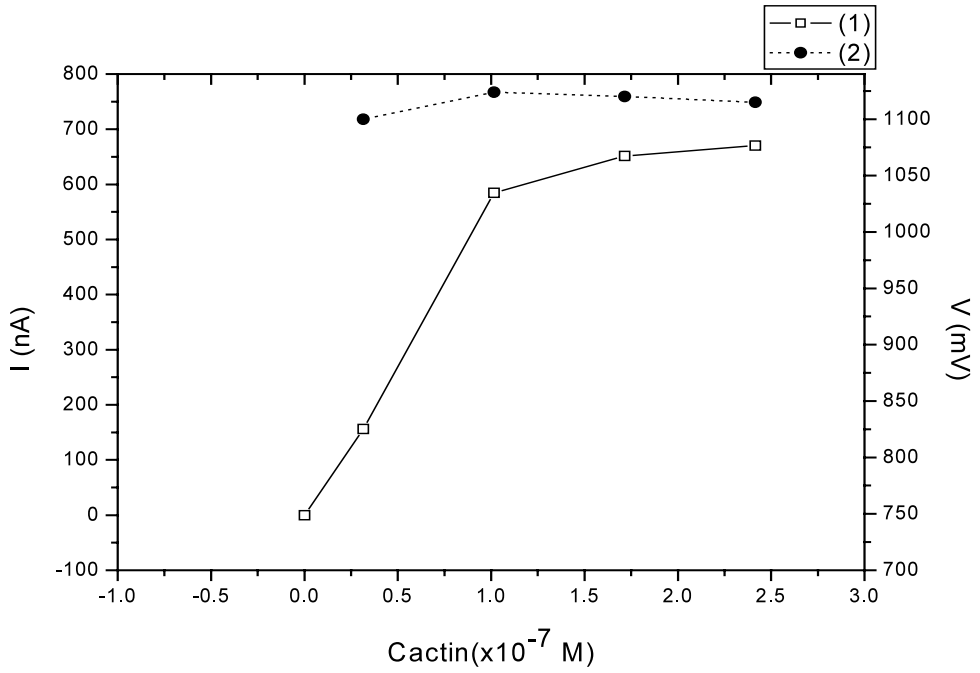


Fig. 2. Calibration curve of ACTD in 0.2 M phosphate, pH 7.4. (1) Dependence of peak current of ACTD oxidation on different concentrations. (2) Dependence of the oxidation potential on the increasing concentrations of ACTD. *The experiment was performed under the experimental conditions described in the text.*

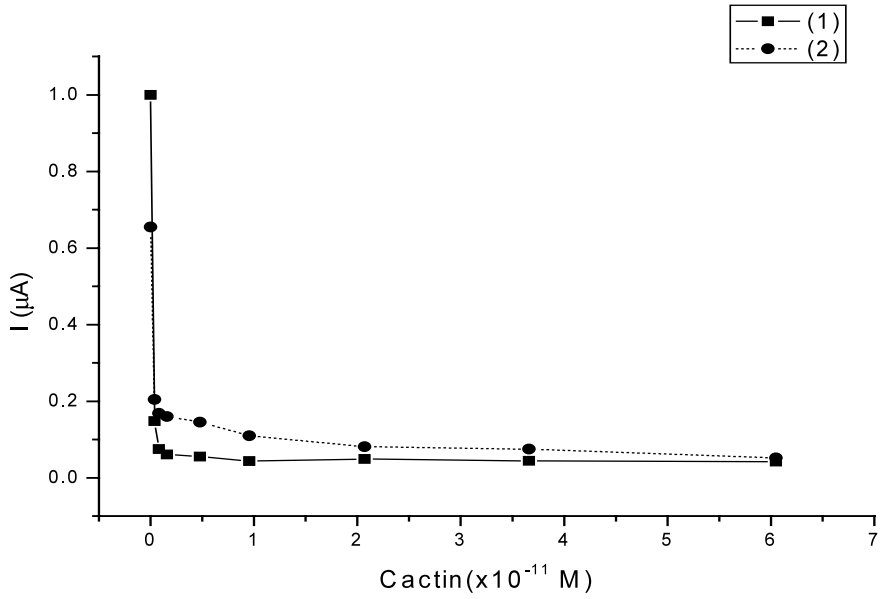


Fig. 3. (1) Dependence of peak current of the characteristic peak of dsDNA at +0.925 V on increasing concentrations of ACTD. (2) Dependence of peak current of the characteristic peak of dsDNA at +1.217 V on increasing concentrations of ACTD into the solution. Experimental conditions are as described above. *The experiment was performed under the experimental conditions described in the text.*

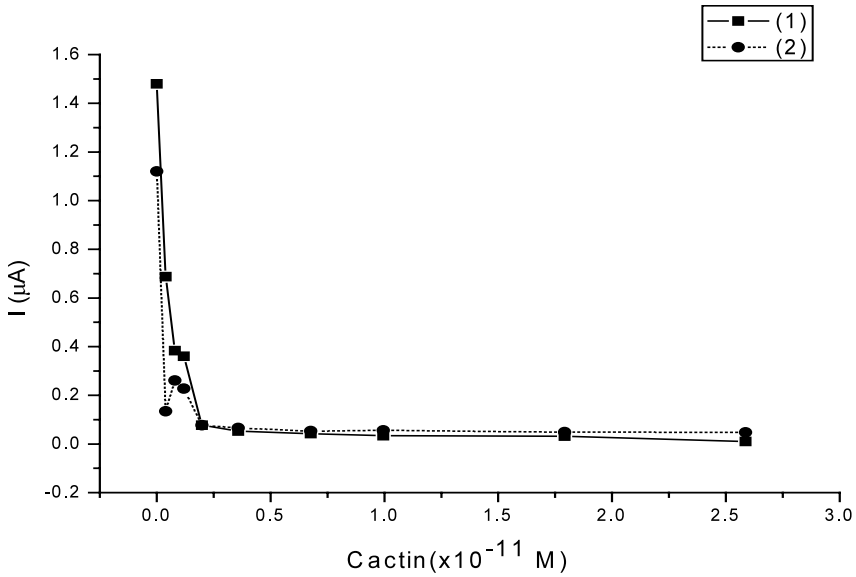


Fig. 4. (1) Dependence of peak current of the characteristic peak of ssDNA at +0.932 V on increasing concentrations of ACTD. (2) Dependence of peak current of the characteristic peak of ssDNA at +1.218 V on increasing concentrations of ACTD into the solution under the above conditions. *The experiment was performed under the experimental conditions described in the text.*

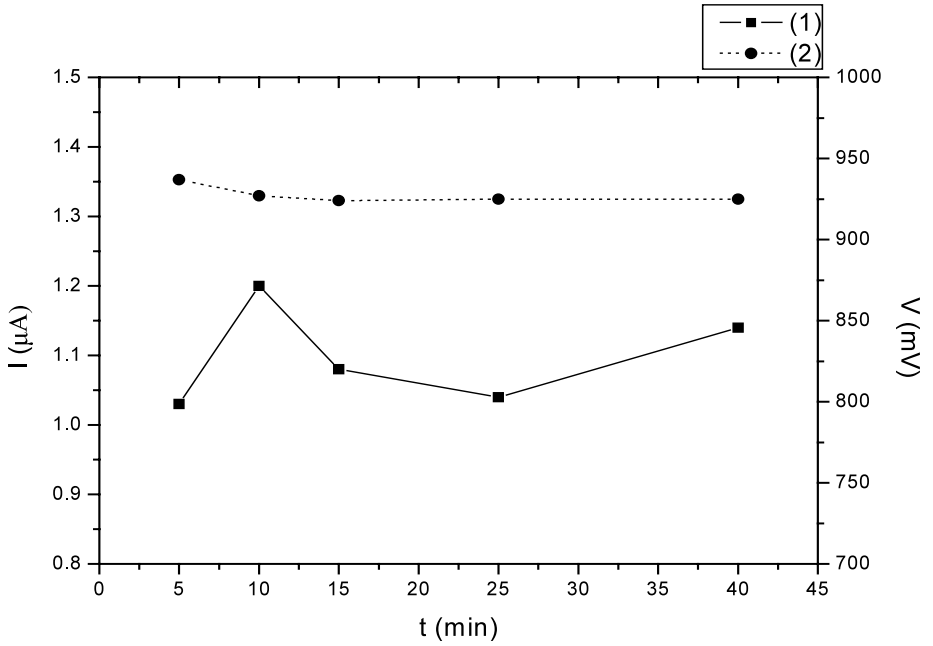


Fig. 5. (1) Dependence of peak current of the characteristic peak of dsDNA at +0.925 V being in solution with a constant concentration of ACTD (0.3984×10^{-10} M) on the incubation time in relation to the potential. (2) Dependence of the oxidation potential on the incubation time under the above conditions. *The experiment was performed under the experimental conditions described in the text.*

remains at about the same values and its characteristic potential is shifted to negative values, while the other peak at +1.215 V decreases gradually. This behavior could be attributed to the conformational changes in the DNA structure, where the adenine residues seem to be more accessible to oxidation. In Fig. 6 are presented the differentiations at the peak current of both peaks of dsDNA in relation to increasing concentrations of the drug.

The same experimental procedure was performed using ss DNA. The final concentration of ssDNA into the solution was constant and equal to 5×10^{-2} mg/ml, while the drug's concentrations ranged from 0 to 6.3×10^{-9} M. In this case the first oxidation peak of ssDNA at +0.93 V remained stable, while the one at +1.22 V was increased with increasing concentrations of the drug into the solution. Fig. 6 shows the differentiations at the peak current of DNA with increasing amounts of the drug (Fig. 7).

4.1.3. ACTD is bound to the electrode surface

It is already proven that DNA, RNA and other biomacromolecules are strongly adsorbed to the mercury and CPEs. So, it is possible to prepare a DNA-modified electrode by dipping the electrode into the DNA solution for a short period of time followed by washing. Low-molecular mass substances do not have the tendency to adhere to the surface of an electrode or they are removed by washing. In some cases strong binding of low-molecular mass compounds, such as mitomycin or daunomycin, to the electrode surface was observed due to chemisorption, polymerization, condensation, etc. of the given substance. ACTD gives a well-developed peak at +1.12 V obtained with CPE, which was first pretreated and then immersed into a solution of 1.4×10^{-7} M ACTD for 2 min at +0.5 V, washed and transferred into a blank background electrolyte. This was the way to prepare an ACTD-modified electrode and to use it for the study of the surface-confined ACTD with increasing concentrations of dsDNA in solution.

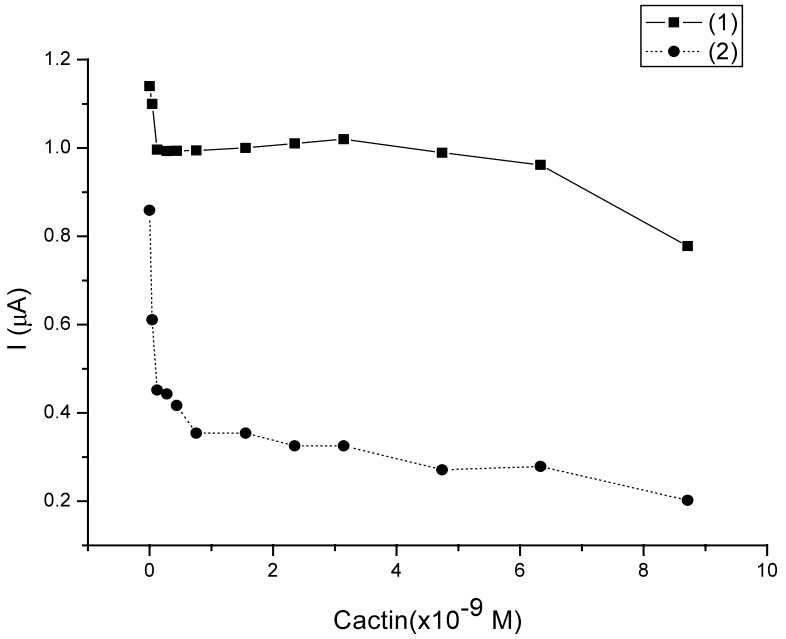


Fig. 6. (1) Dependence of peak current of the characteristic peak of dsDNA at +0.925 V on increasing concentrations of ACTD after incubation of stock dsDNA with ACTD in 0.2 M phosphate buffer for 10 min. (2) Dependence of peak current of the characteristic peak of dsDNA at +1.217 V on increasing concentrations of ACTD after incubation of stock dsDNA with ACTD in 0.2 M phosphate buffer for 10 min. *The experiment was performed under the experimental conditions described in the text.*

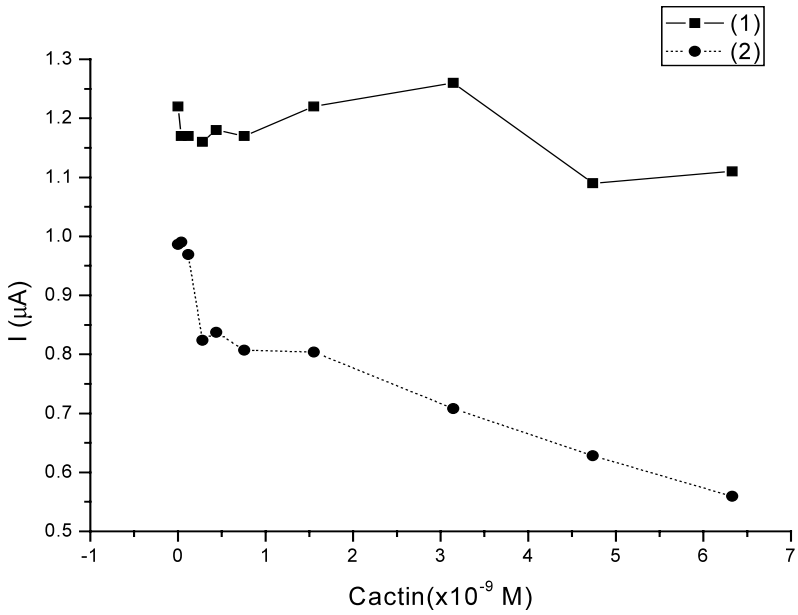


Fig. 7. (1) Dependence of peak current of the characteristic peak of ssDNA at +0.932 V on increasing concentrations of ACTD after incubation of stock ssDNA solution with ACTD in 0.2 M phosphate buffer pH 7.4 for 10 min. (2) Dependence of peak current of the characteristic peak of ssDNA at +1.218 V on increasing concentrations of ACTD after incubation of stock ssDNA solution with ACTD in 0.2 M phosphate buffer pH 7.4 for 10 min. *The experiment was performed under the experimental conditions described in the text.*

4.1.4. Interaction of the surface-confined ACTD with DNA in solution

The ACTD-modified electrode was immersed into a solution of dsDNA at a final concentration ranging from 0 to 4.8×10^{-3} mg/l. The incubation time was 120 s at a potential of +0.2 V. As a result of the immersion, a well defined peak at +1.12 V appeared. By increasing the amounts of dsDNA into the solution, the characteristic peak of ACTD was decreased, while the potential shifted at +1.10 V. Furthermore, another peak at +0.92 V appeared and increased by increasing concentrations of dsDNA into the solution. As a conclusion the immobilization of ACTD at the electrode surface did not prevent the interaction of ACTD with dsDNA.

4.2. Alternating current voltammetric responses of ds, ss and scDNA at full electrode coverage

The mercury electrode was immersed into a 10 μl drop of the DNA solution for an accumulation time of $t_A = 120$ s. The DNA concentration was adjusted to secure full coverage of the electrode surface under the given conditions. The concentration of dsDNA was 80 mg/l, the one of ssDNA was 40 mg/l and the one of scDNA was 150 mg/l [11]. All the solutions were prepared in 10 mM Tris–HCl pH 7.5. The electrode was washed and transferred into the electrolytic cell containing a blank background electrolyte and the alternating current voltammetric measurement was performed.

4.2.1. Alternating current voltammetric response of ACTD

ACTD produces a well-developed peak at −0.40 V with a preconcentration step at −0.1 V for 120 s and subsequent transfer at blank background electrolyte. The calibration curve at Fig. 8 shows the current response in relation to increasing concentrations of ACTD at the above conditions.

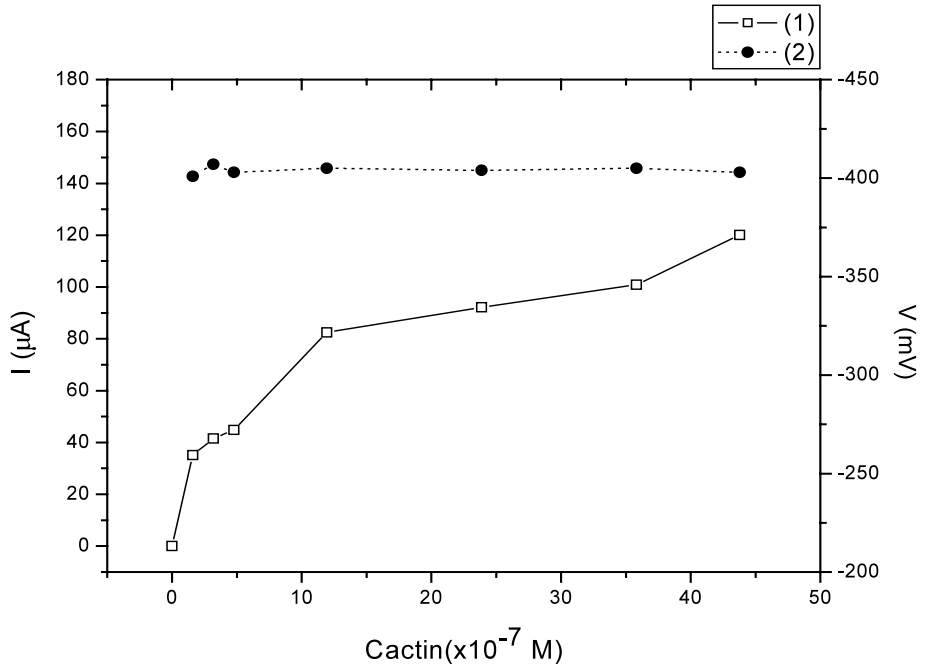


Fig. 8. Calibration curve of ACTD on the HMDE surface into the buffer solution (0.3 M NaCl, 50 mM phosphate, pH 8.5), containing increasing concentrations of ACTD. The transduction was performed into blank background electrolyte. (1) Dependence of peak current of ACTD reduction on different concentrations. (2) Dependence of the reduction potential on increasing concentrations of ACTD. *The experiment was performed under the experimental conditions described in the text.*

4.2.2. Interaction between ACTD and DNA on the HMDE surface

The mercury electrode was immersed into a 10 μl drop of a DNA solution for an accumulation time of 120 s. The electrode was washed and transferred into the electrolytic cell containing blank background electrolyte to perform the alternating current voltammogram. Supercoiled DNA yielded a peak at −1.25 V, dsDNA yielded peak I at −1.17 V, peak III at −1.42 V and peak II due to unwinding at −1.30 V, and thermally denatured (ssDNA) yielded a more intense peak III at −1.42 V and peak I at −1.18 V [11].

DsDNA and ACTD were incubated for 45 min. Ten microliters of this solution were immobilised on the HMDE surface and alternating current voltammetry was performed in blank supporting electrolyte. By increasing the concentration of ACTD, peak II increases as dsDNA is subjected to a more intense unwinding due to the intercalated drug and peak III is decreased. When the drug's concentration reached 3×10^{-6} M, two new peaks appear, the one at −1.55 V probably due to the intercalated ACTD and the other at −0.42 V obviously due to the amount of the drug in excess. As the concentration of the drug increases, these two peaks are also increased. Fig. 9 presents the differentiations into the configuration of dsDNA after interaction with increasing concentrations of ACTD.

The same procedure was followed with ssDNA and its interaction with ACTD. When the concentration of the drug reaches the value of 7×10^{-6} M, two new peaks appear as previously described. Peak III increased when the drug's concentration equals 7.96×10^{-8} M and then decreases gradually. The interaction between scDNA and the drug after incubation and immobilisation on the HMDE was also studied with the same way. At a drug concentration of 3×10^{-5} M, the peak at −1.55 V appears and increases with increasing concentrations of the drug which interacts with scDNA. The concentration of the drug provoking the differentiations in the scDNA

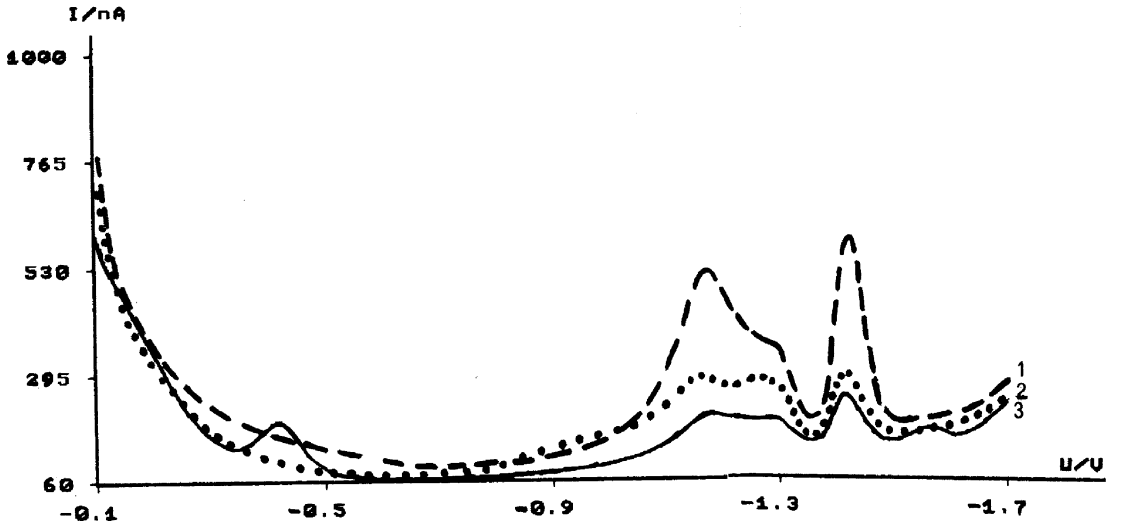


Fig. 9. (1) Alternating current voltammogram of dsDNA (80 mg/l) immobilised on the HMDE surface. (2) Alternating current voltammogram of the mixture dsDNA (80 mg/l)+3.98×10^{-8} M ACTD incubated in solution prior to immobilisation on the HMDE surface. (3) Alternating current voltammogram of the mixture dsDNA (80 mg/l)+3.98×10^{-6} M ACTD incubated in solution prior to immobilisation on the HMDE surface. *The experiment was performed under the experimental conditions described in the text.*

form is higher, since the sc-form is very strong and rigid compared to the other forms of DNA. Figs. 10 and 11 present the differentiations in ssDNA form and scDNA form after incubation with increasing concentrations of ACTD.

Peak III which appears due to the desorption of the DNA segments firmly adsorbed via bases, is a very important indicator of DNA cleavage at the electrode surface [8]. In this case peak III is decreased and does not appear in the case of the

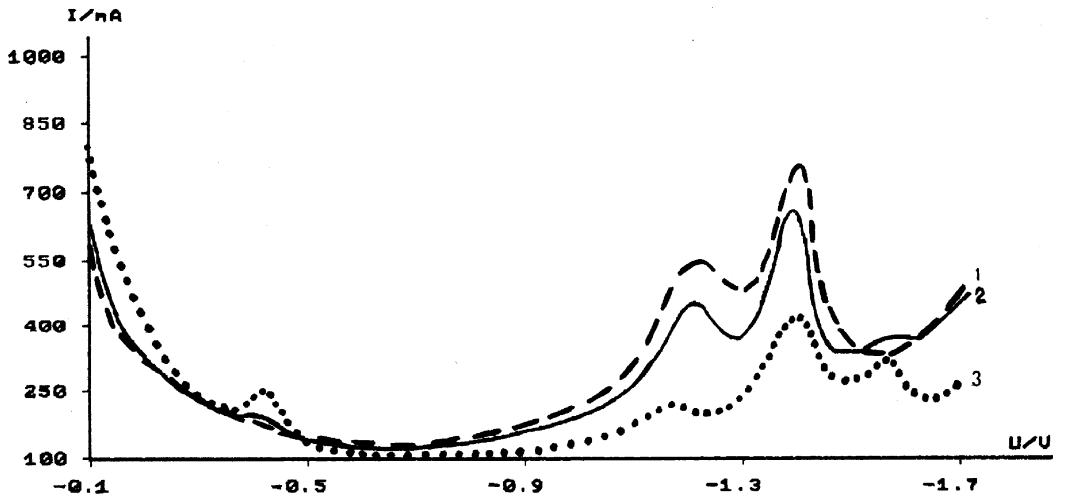


Fig. 10. (1) Alternating current voltammogram of ssDNA (40 mg/l) immobilised on the HMDE surface. (2) Alternating current voltammogram of the mixture ssDNA (40 mg/l)+1.5×10^{-6} M ACTD incubated in solution prior to immobilisation on the HMDE surface. (3) Alternating current voltammogram of the mixture ssDNA (40 mg/l)+3.98×10^{-5} M ACTD incubated in solution prior to immobilisation on the HMDE surface. *The experiment was performed under the experimental conditions described in the text.*

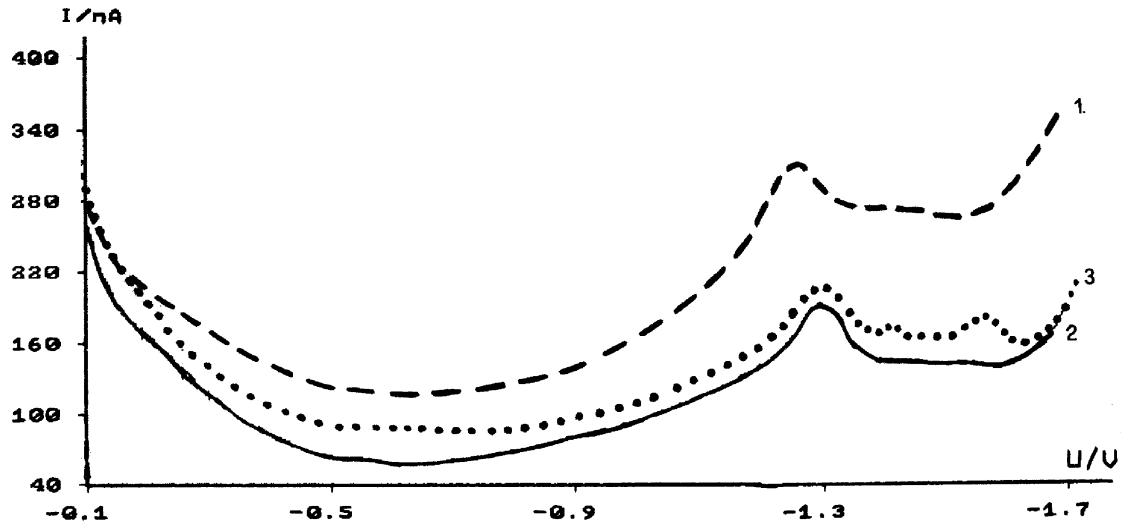


Fig. 11. (1) Alternating current voltammogram of scDNA (150 mg/l) immobilised on the HMDE surface. (2) Alternating current voltammogram of the mixture scDNA (150 mg/l)+1.5×10^{-5} M ACTD incubated in solution prior to immobilisation on the HMDE surface. (3) Alternating current voltammogram of the mixture scDNA (150 mg/l)+3.98×10^{-5} M ACTD incubated in solution prior to immobilisation on the HMDE surface. *The experiment was performed under the experimental conditions described in the text.*

interaction between scDNA and ACTD. The new peak appearing at −1.55 V is a clear evidence of the differentiation to DNA configuration due to the interaction with the drug.

5. Conclusions

In this paper we have shown that intercalation of ACTD within DNA being in solution or being immobilized on the CPE surface can be monitored by adsorptive transfer stripping voltammetry with differential pulse mode. Furthermore, the interaction at the HMDE surface with alternating current voltammetry can play a complementary role in order to study the results of the interaction.

5.1. Interactions on CPE surface

The result of the binding of ACTD to the immobilised dsDNA was the decrease of both characteristic oxidation peaks of dsDNA. The same decrease was observed after the interaction of the drug with ssDNA. The behaviour of dsDNA and ACTD incubated in solution was completely different. Increasing concentrations of the drug in the bulk of the solution incubated with the same concentration of dsDNA led to a stabilisation of the peak at +0.92 V, while the peak at +1.22 V decreased gradually probably due to conformational changes. It has to be mentioned that the oxidation potential of the peak at +0.93 V was shifted to +0.91 V leading to the conclusion that electrostatic interactions take place in the DNA double helix. The same was observed in the case of incubation of ssDNA with increasing concentrations of the drug, but in this case we cannot speak about intercalation phenomena. The ionic strength of the supporting electrolyte is a factor affecting the conformation of DNA into the solution and on the electrode surface. In this case lower ionic strength acts as an inhibiting factor to the optimal signal of DNA.

We have also shown that ACTD adheres to the CPE surface resisting washing of the electrode surface more strongly than acridine orange. The concentration of ACTD equals the optimum value to secure the full electrode surface coverage, which is according to calibration curve (Fig. 2) equal to 1.4×10^{-7} M. By immersing this electrode in dsDNA solution, we observed a decrease at the oxidation peak of the immobilised ACTD, while a

peak at +0.92 V started to appear by increasing concentrations of dsDNA. This observation can lead us to the conclusion that ACTD is capable of interacting with dsDNA molecules diffusing to the surface from the bulk solution. We could also assume that the positioning of the DNA molecule has changed, since the electrode has undergone a different modification, but the modification is different than those of acridine orange immobilisation, where a new peak at +0.87 V appeared with increasing concentrations of dsDNA. Further studies could be done on the different positioning of the DNA molecule at the electrode surface and the mobility of the DNA molecules from the bulk of the solution to the electrode surface along with the potential use of ACTD as an indicator.

5.2. Interactions on HMDE surface

At the mercury surface, dsDNA can be slowly unwound in the potential region around −1.2 V. During the slow scaning of potential to the negative values in AC voltammetry DNA unwinding cannot be avoided. DNA molecules containing free ends, i.e. open circular (oc) DNA, linearized (lin) as well as oc and linDNAs containing additional single strand breaks (ssb) yield well developed peak III. On the contrary, DNA molecules lacking free ends (including supercoiled covalently closed circular DNA, scDNA) cannot be extensively unwound at the electrode surface and do not produce peak III.

These strong differences were exploited in this paper in the development of a dsDNA, a ssDNA and a scDNA-sensor for the detection of DNA damage. DsDNA yields a less intense peak I compared to the one yielded by ssDNA, since the latter is a relaxed and more accessible form. By increasing the concentration of ACTD into the incubated solution, we observed that peak II in dsDNA form due to the unwinding of the dsDNA becomes wider, while peak I and peak III decreased. At higher drug concentrations a new peak appears at −1.56 V probably due to the intercalated drug, while a peak at −0.4 V appeared declaring the non-intercalated drug. Concerning the interaction with scDNA, a very small peak at −1.42 V appeared due to the presence of a small amount of oc-DNA, not detectable with gel electrophoresis, which remained stable after incubation with different concentrations of the drug. No increase of peak III occurred due to the presence of ACTD into the incubated solution leading to the conclusion that ACTD does not induce cleavage of DNA.

In conclusion, we applied a dsDNA and a ssDNA-modified electrode on a carbon paste surface, which can be used in the detection of ACTD. The changes of the characteristic peak of guanine and adenine residues can be useful for the determination of very low concentrations of ACTD. The study of these changes and the different way of action with dsDNA or ssDNA could highlight the mechanism of the interaction between different chemical compounds. We have also shown that the HMDE surface (in combination with alternating current voltammetry) separates the responses of different DNA forms and presents high sensitivity to minor damage to the DNA double helix. In this paper the application of the HMDE sensors for the detection of ACTD in solution showed higher sensitivity compared to the one attempted on the CPE surface. These results could be combined in order to study the mechanism of interaction between DNA and various intercalative agents, like ethidium bromide or acridine orange which were electrochemically studied. Finally, we have shown that adsorptive transfer stripping voltammetry with differential pulse mode in combination with alternating current voltammetry can be used in order to study the effects of intercalation causing locally prestretching of DNA resulting in the formation of a complex.

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