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ORIGINAL PAPER

Voltammetric detection of damage to DNA caused by nitro derivatives of fluorene using an electrochemical DNA biosensor

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Abstract An electrochemical DNA biosensor based on the screen printed carbon paste electrode (SPCPE) with an immobilized layer of calf thymus double-stranded DNA has been used for in vitro investigation of the interaction between genotoxic nitro derivatives of fluorene (namely 2-nitrofluorene and 2,7-dinitrofluorene) and DNA. Two types of DNA damage have been detected at the DNA/SPCPE biosensor: first, that caused by direct association of the nitrofluorenes, for which an intercalation association has been found using the known DNA intercalators [Cu $(phen)_2]^{2+}$ and $[Co(phen)_3]^{3+}$ as competing agents, and, second, that caused by short-lived radicals generated by electrochemical reduction of the nitro group (observable under specific conditions only).

Keywords 2-Nitrofluorene · 2,7-Dinitrofluorene · DNA biosensor · Screen printed carbon paste electrode · Electrochemical detection · DNA damage

Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of the 50th Anniversary of receiving the Nobel Prize in Chemistry for the discovery and development of polarographic methods of analysis.

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Introduction

Emissions of gasoline and diesel engines contribute significantly to ever increasing pollution of the environment. A specific part of exhaust gases is composed of nitrated polycyclic aromatic hydrocarbons (NPAHs), which are causally connected with increasing rates of cancer [1]. The presence of these compounds in the environment threatens natural biological functions of ecosystems and healthy organism growth [2]. In the eighties and the nineties of the last century, Möller studied the effect of NPAHs on living organisms and their DNA [3]. Carcinogenic 2-nitrofluorene (2-NF) was chosen as a model marker of NPAHs [4, 5], and lately the genotoxic effect of structurally similar compounds, e.g. of 2,7-dinitrofluorene (2,7-DNF), have also been studied [6]. NPAHs are well known hazardous air and water pollutants [7, 8] and great attention is thus paid to their determination in various matrices [9, 10], modern electrochemical methods being among those frequently tested and used for these purposes [11–14].

Recently, methods and procedures for determination of various NPAHs have been developed in the authors' laboratory, mostly using voltammetric techniques. A hanging mercury drop electrode was utilized as the working electrode for the voltammetric determination of many environmentally important compounds (pollutants) in the past. Nevertheless, it was replaced by other, especially non-toxic sensors (e.g. solid amalgam electrodes [15–17], solid composite electrodes [18–21], solid amalgam composite

electrodes [18, 22], or boron-doped diamond film electrodes [23]). Such electrodes proved to be very suitable for determination of nitro compounds, including NPAHs [24–27].

Electrochemical DNA-based biosensors [28–30], realized by immobilization of oligonucleotides or DNA on a suitable electrode surface used as the signal transducer, are simple to assemble and are widely used to detect the presence of pathogenic microorganisms and genotoxic substances [31, 32], or the protective effects of some substances towards the DNA structure [33, 34]. They can provide reliable results regarding interactions of potential xenobiotics with nucleic acids [35–37]. For example, intercalation into DNA has previously been observed for many planar aromatic molecules [38]. Compounds with positive charge can increase the DNA binding affinity via electrostatic interaction with the negatively charged phosphate backbone [39]. Changes of the intrinsic electrochemical responses of guanine and adenine moieties in calf thymus DNA are often evaluated as a label-free and reagent-less approach to detection [29, 40].

DNA interactions with metal chelates with heterocyclic ligands, for example 1,10-phenanthroline (phen), have been widely studied and utilized as DNA indicators [41–45]. The complex $[Cu(phen)_2]^{2+}$ is also known as a chemical nuclease under specific conditions [44]. These complexes interact, depending on the ionic strength, through hydrophobic interactions with the interior of double-stranded DNA (dsDNA) and through electrostatic interactions with the negatively charged DNA backbone [42, 46] and may compete with DNA association with low-molecular-mass analytes of interest [45, 47]. In the work discussed in this paper, competition between 2-NF or 2,7-DNF and these metal complexes in binding to DNA has been used to characterize the nature of the direct interaction between the studied compounds and DNA.

The first step of electrochemical reduction of 2-NF and 2,7-DNF at mercury electrodes in a wide pH range involves the transfer of four electrons, or of eight electrons in the simultaneous reduction of two nitro groups at 2,7-DNF [48, 49]. The role of nitroreduction in the biological activation of 2-NF and 2,7-DNF has been investigated and the studies indicated that the genotoxicity depends on both nitroreduction and acetylation [4, 6]. Electrochemical DNA biosensors [50–52] have been successfully used to investigate damage to DNA caused by electrochemically generated short-lived radicals [50, 53]. With this in mind, the objective of this work was to confirm binding interactions and damage of the surface attached DNA with health-hazardous nitrofluorene derivatives using a dsDNA modified screen printed carbon paste electrode (DNA/SPCPE) [47, 53]. Electrochemical reduction of 2-NF and 2,7-DNF nitro groups under voltammetric conditions was used for in situ electrochemical generation of reactive intermediates which interact with DNA.

Experimental

Reagents

The stock solutions of 2-nitrofluorene ($c=1\times10^{-3}$ mol L^{-1}; CAS Number 607-57-8; 98%) and 2,7-dinitrofluorene ($c=1\times10^{-4}$ mol L^{-1}; CAS Number 5405-53-8; 97%), both obtained from Sigma–Aldrich, Prague, Czech Republic, in methanol were prepared by dissolving an exact amount of the appropriate substance in 100.0 mL methanol (99%, p.a. purity; Mikrochem, Pezinok, Slovakia). The lower concentration of 2,7-DNF was because of its limited solubility in methanol. More dilute solutions were prepared every day by exact dilution of the stock solutions with methanol. Acetate buffer (AcB; 0.25 mol L^{-1} containing 10 mmol L^{-1} KCl, resulting pH 4.75) or phosphate buffer solutions (PBS; 0.1 mol L^{-1} containing 10 mmol L^{-1} KCl, resulting pH 7.00) were used as main components of supporting electrolytes (KCl was added to buffer solutions because of the use of an Ag/AgCl reference screen printed electrode, to stabilize the electrode potential).

Calf thymus dsDNA was obtained from Sigma–Aldrich, Taufkirchen, Germany (product number D-1501), and used as received. Concentrated stock solution (0.1 mg mL^{-1} dsDNA) was prepared in 10 mmol L^{-1} Tris-HCl and 1 mmol L^{-1} EDTA solution pH 8.0 and stored at −4 °C. The complexes $[Cu(phen)_2](NO_3)_2$ and $[Co(phen)_3](NO_3)_3$ were synthesized at Slovak University of Technology in Bratislava according to the method of Dollimore and Gillard [54]. Other chemicals (all p.a. purity) were supplied by Mikrochem and were used as received. Deionized, double-distilled water was used throughout. Unless stated otherwise, the experiments were carried out at laboratory temperature (23–25 °C). All solutions were kept in glass vessels in the dark at laboratory temperature, except dsDNA concentrated stock solution mentioned above.

Apparatus

Voltammetric measurements were performed with an Autolab PGSTAT-100 potentiostat equipped with an impedance module and driven by GPES 4.9 software (both Eco Chemie, Utrecht, Netherlands). The software worked under the Microsoft Windows XP Professional operating system (Microsoft, USA). A screen printed electrode assembly was purchased from the Food Research Institute, Bratislava, Slovakia. It consists of the carbon paste working electrode (21 mm^2 geometric surface area), an Ag/AgCl reference electrode (with a potential of 284 mV vs a conventional Ag/AgCl saturated KCl electrode), and a silver ink auxiliary electrode and was fabricated using typical screen printing equipment. Silver wires as electric contacts on a plastic support coated with carbon-paste ink

were used as working electrode, silver ink was used as the auxiliary electrode, and silver chloride ink was used as reference electrode. The contacts were covered by an insulating film.

pH was measured using a Metrohm (Herisau, Switzerland) 654 pH meter with a combined glass electrode. An IKAMAG RET basic magnetic stirrer and heater (Fisher, Bratislava, Slovakia) was used for stirring and heating measured solutions. If necessary, oxygen was removed from the measured solutions by bubbling with nitrogen (purity 4.0; Linde, Bratislava, Slovakia) for 5 min.

Preparation of the biosensor (DNA/SPCPE)

The SPCPE surface was pretreated by applying a potential of +1600 mV for 120 s and +1800 mV for 60 s in 10 mL AcB, under stirred conditions; this procedure was necessary to oxidize all contaminants present on the graphite surface and to activate the electrode surface [37, 55] and make it susceptible to the dsDNA immobilization. After the first step, a baseline voltammetric control scan was performed to monitor the state of the working electrode. After washing the electrode surface with deionized water and leaving it to dry, dsDNA stock solution (5 μL; 0.1 mg mL^{-1}) was dropped on to a bare SPCPE and left to evaporate to dryness (overnight) in order to obtain a DNA layer stable during subsequent use of the biosensor in solution [56].

Procedures

DNA/SPCPE and bare SPCPE were used as disposable sensors. Before the measurement, the bare SPCPE was pretreated as described above. The DNA/SPCPE biosensor was used after equilibration for 2 min in the supporting electrolyte without any other treatment. The general procedure to obtain and evaluate voltammograms was as follows. An appropriate volume of stock solution of the studied compound in methanol (or in water in the case of the $[Cu(phen)_2]^{2+}$ and $[Co(phen)_3]^{3+}$ complexes) was placed in a voltammetric cell of total solution volume 10.0 mL at laboratory temperature, or heated to 36 °C by using the water bath and heater (temperature was monitored by means of an immersed mercury-in-glass thermometer). After given incubation time, voltammetric measurement was performed. Unless stated otherwise, scan rate 50 mV s^{-1} and potential step (E_{step}) 5 mV were used in cyclic voltammetry (CV). The pulse amplitude (E_{amp}) +50 mV (anodic scan) or −50 mV (cathodic scan), pulse width 100 ms, scan rate 20 mV s^{-1}, and E_{step} 5 mV were used in differential pulse voltammetry (DPV); E_{amp} 40 mV, frequency 200 Hz, scan rate 3000 mV s^{-1} and E_{step} 15 mV were used in square wave voltammetry (SWV). All voltammetric curves were recorded 3 times and all experiments were repeated 3 times for confirmation. The height of the peaks (I_p) recorded using DPV and SWV was evaluated from the straight line connecting the minima before and after the peak; if necessary, baseline correction of the obtained voltammograms was used. Mathematical and statistical evaluation (for number of measurements n=3 and for significance level α=0.05) was done using the software OriginPro 7.5 (OriginLab, USA).

Results and discussion

DNA binding interaction of the studied compounds and related damage to DNA

Cyclic voltammetric measurements were used to obtain an overall view of the electrochemical behavior of the tested substances at the DNA/SPCPE compared with that at the SCPCE. Figure 1 shows consecutive cathodic/anodic CV scans of 2-NF ($c=1\times10^{-4}$ mol L^{-1}) and 2,7-DNF ($c=5\times10^{-5}$ mol L^{-1}) obtained in the weakly acidic (pH 4.75) medium AcB–methanol (1:1). Usual reduction and follow-up oxidation and electron transfer reactions characteristic of this class of compounds [49] were found using both DNA/SPCPE and SPCPE. The irreversible reduction peaks p_I and p_{IV} (both at −865 mV) correspond to a 4-electron/4-proton transfer reaction, forming hydroxylamine, typical of nitro group reduction in methanolic aqueous acid solution [57]. This is similar to the behavior of these compounds observed at the mercury electrode at the same pH [48]. The cathodic peak p_V represents a hint of reduction of the second nitro group to a hydroxylamino group in the presence of the previously formed hydroxylamino group, well known from nitroreduction at mercury electrodes [49]. The presence of unreduced hydroxylamino group is indicated by the reversible anodic peaks p'_{II} (−65 mV) and p'_{VI} (−45 mV) which can be assigned to 2-electron oxidation of the hydroxylamino group to the nitroso group. Corresponding counterpeaks p_{II} (−275 mV) and p_{VI} (−300 mV) are observed in a second cathodic scan, which is in agreement with the well-known reversibility of the hydroxylamino–nitroso system in the neutral pH region [58]. Condensation reaction between hydroxylamino groups and nitroso groups can take place to give azoxy groups [57]; anodic peaks p'_{III} (+460 mV) and p'_{VII} (+475 mV) probably corresponds to irreversible oxidation of this azoxy group [59] or to expected condensation reactions by oxidation of the reduced intermediates formed [50].

Comparison of the CVs obtained at the DNA/SPCPE and SPCPE indicates the same redox mechanism with some changes in peak height. Enhancement of the analyte response at the biosensor can be taken as confirmation of

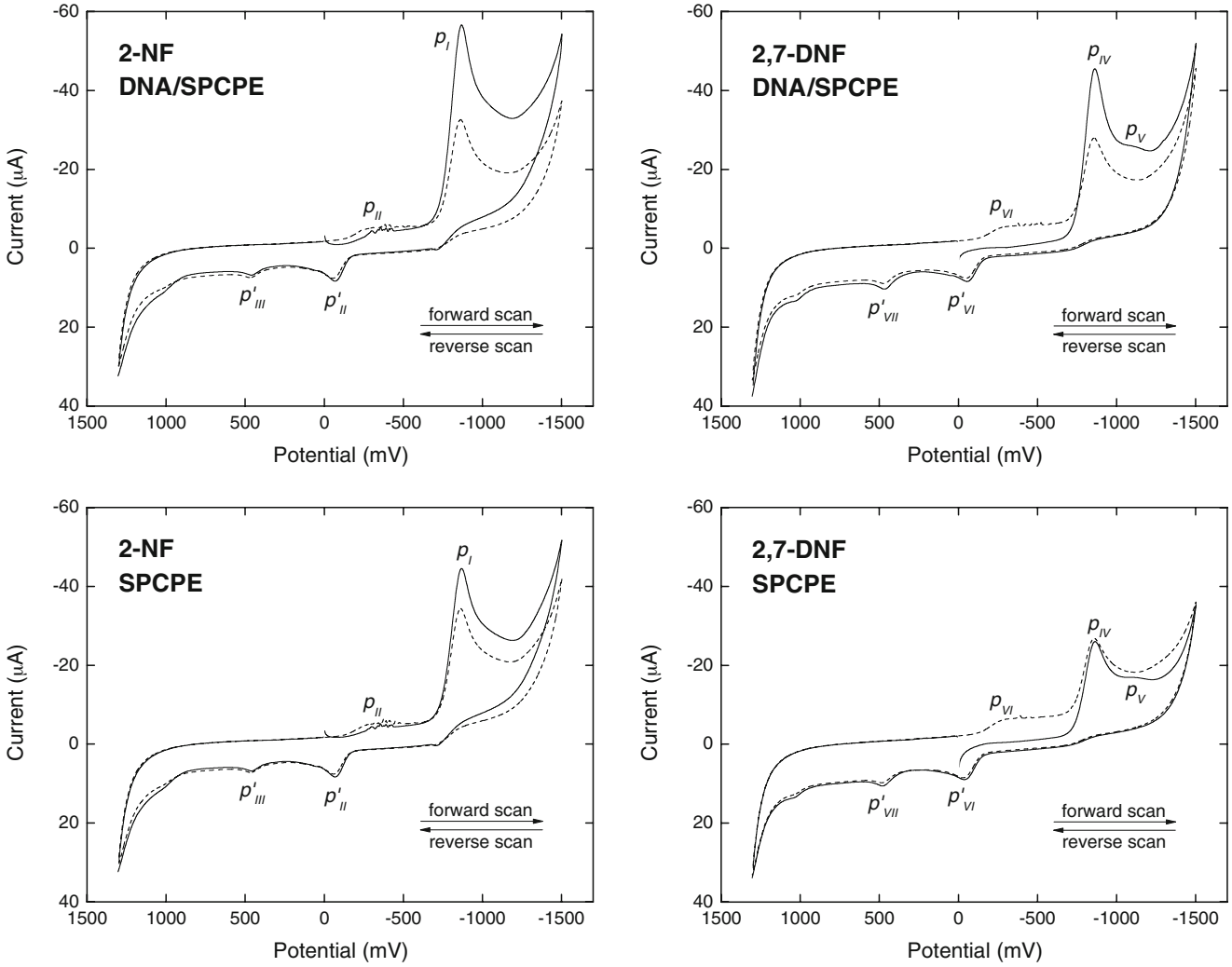

Fig. 1 Cyclic voltammograms of 2-NF ($c=1\times10^{-4}$mol L^{-1}) and 2,7-DNF ($c=5\times10^{-5}$mol L^{-1}) recorded at the DNA/SPCPE and the bare SPCPE in AcB–methanol (1:1) medium; air oxygen was removed from the measured solution by bubbling with nitrogen during electrode incubation for 5 min; scan rate 50 mV s^{-1}, E_{step} 5 mV, 25 °C; the *continuous line* represents the first scan and the *dashed line* the second scan. Peaks description—see text

its accumulation within the DNA layer during electrode incubation for 5 min.

In nature, molecules that bind DNA directly may interact with it in several ways [60]: they can join the nucleic acid and inhibit some of the basic functions of living cells such as replication, transcription, and translation, they can block interaction of DNA with specific, required proteins, or prevent the DNA from adopting conformations required for biological function [37]. Binding of low-molecular-mass compounds to DNA bases is among the DNA damage often investigated by use of DNA-based biosensors using intrinsic DNA bases responses [28]. Figure 2 presents SWV tests of 2-NF at two concentrations including description of the whole experimental procedure. First, the SWV scan of immobilized DNA in a blank solution was performed at several DNA/SPCPEs to evaluate the oxidation of guanine ($I_{p0,G}$) and adenine peak height ($I_{p0,A}$) (at approx. +1000 mV and +1300 mV vs the screen printed reference electrode for guanine and adenine, respectively). Then, a change in the intrinsic DNA responses because of its direct interaction with the tested substances was expressed as normalized peak currents (G (%) and A (%)), using Eqs. 1 and 2:

$$G(\%) = \frac{I_{p,G}}{I_{p0,G}} \times 100 \quad (1)$$

$$A(\%) = \frac{I_{p,A}}{I_{p0,A}} \times 100 \quad (2)$$

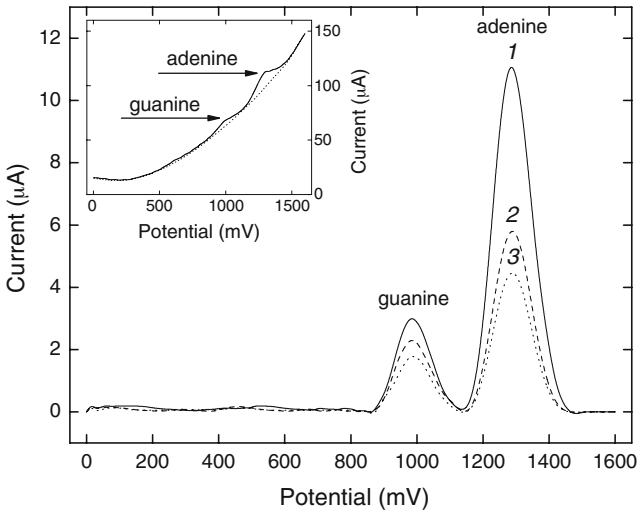

Fig. 2 Baseline-corrected anodic SWV response of DNA bases at the DNA/SPCPE recorded after incubation of the biosensor in 0 mol L^{-1} (*1*), 5×10^{-6}mol L^{-1} (*2*), and 1×10^{-5}mol L^{-1} (*3*) 2-NF stirred AcB–methanol (99:1) solution at 25 °C for 2 min; flushing in blank AcB, and transfer into new AcB medium; E_{amp} 40 mV, frequency 200 Hz, scan rate 3000 mV s^{-1}, E_{step} 15 mV. SW voltammogram of DNA bases with displayed baseline (*dotted line*) is in the *inset*

where $I_{p,G}$ and $I_{p,A}$, $I_{p0,G}$ and $I_{p0,A}$ are, respectively, the guanine or adenine moieties peak currents after and before interaction with the tested compounds.

The studied compounds give no anodic response in the corresponding potential region. The results obtained are summarized in Table 1. It was confirmed that both compounds tested interacted, in a concentration-dependent manner, with DNA immobilized on the SPCPE surface (the $I_{p,G}$ and $I_{p,A}$ values decrease after the interaction). This indicates that the dsDNA structure can be distorted as a result of subtle damage by xenobiotics.

The type of DNA association with the nitrofluorenes was further investigated voltammetrically (using the DPV mode) by competing binding interaction with the copper (II) and cobalt(III) complexes of 1,10-phenanthroline in PBS–methanol (99:1). Because the electrostatic/intercalative binding of $[Cu(phen)_2]^{2+}$ and $[Co(phen)_3]^{3+}$ to dsDNA strongly depends on the ionic strength [45], two concentrations of PBS (5 mmol L^{-1} and 20 mmol L^{-1}) in the supporting electrolyte were used.

The results obtained (depicted in Figs. 3 and 4 for the sake of illustration and summarized in Table 2) confirm:

1. accumulation of the complex indicators because of their association with dsDNA; and
2. the transition in the mode of interaction from dominantly electrostatic to dominantly intercalative with increasing ionic strength of the PBS medium.

The DPV peak current values for the complex indicators obtained at the DNA/SPCPE (compared with SPCPE) in 5 mmol L^{-1} PBS are significantly higher than those measured in 20 mmol L^{-1} PBS, particularly for the $[Co(phen)_3]^{3+}$ complex with higher positive charge. The shift in E_p can also be seen; this depends strongly on the ionic strength. While more negative ΔE_p values were found at the lower ionic strength (dominantly electrostatic analyte–DNA interaction), more positive ΔE_p values correspond to the higher ionic strength (dominantly intercalative analyte–DNA interaction) [45]. When 2-NF or 2,7-DNF are present in the solution, small or no decrease of $[Cu(phen)_2]^{2+}$ or $[Co(phen)_3]^{3+}$ peak current at the DNA/SPCPE in 5 mmol L^{-1} PBS (curves 4) indicates only low or no competition in electrostatic DNA binding. On the other hand, a decrease of the $[Cu(phen)_2]^{2+}$ or $[Co(phen)_3]^{3+}$ peak currents in 20 mmol L^{-1} PBS (curves 6) indicates competition in intercalative DNA binding between 2-NF or 2,7-DNF and the metal complex indicators. This indicator response decrease, i.e. the competing effect, is more intense for $[Cu(phen)_2]^{2+}$ than $[Co(phen)_3]^{3+}$. This is because of the higher intercalation affinity of the $[Cu(phen)_2]^{2+}$ complex towards DNA compared with that of $[Co(phen)_3]^{3+}$, as follows from comparison of voltammetric data obtained at the DNA/SPCPE and SPCPE sensors (Table 2). This difference in the intensity of the decrease in the indicators response can be taken as more evidence of intercalative binding of 2-NF and 2,7-DNF.

Possible blocking of the biosensor surface by the molecules of 2-NF and 2,7-DNF (which could lead to the difference between the curves 6 and 5 in Figs. 3 and 4) was also tested by performing DPV measurements of the indicators in the presence of nitrofluorenes at a bare SPCPE. The responses of $[Cu(phen)_2]^{2+}$ and $[Co(phen)_3]^{3+}$ were not

Table 1 DNA damage during the direct interaction of the studied compounds with the DNA/SPCPE biosensor expressed by normalized values of the SW voltammetric DNA intrinsic responses; $I_{p0,G}$=(3.0±0.1) μA, $I_{p0,A}$=(11.0±0.3) μA; n=3; α=0.05; experimental conditions as for Fig. 2

Compound	Supporting electrolyte	$I_{p,G}$ / $I_{p0,G}$ (%)	$I_{p,A}$ / $I_{p0,A}$ (%)
2-Nitrofluorene			
5 μmol L^{-1}	AcB–methanol (99:1)	77±6	52±5
10 μmol L^{-1}	AcB–methanol (99:1)	60±4	41±4
2,7-Dinitrofluorene			
1 μmol L^{-1}	AcB–methanol (99:1)	88±3	76±5
5 μmol L^{-1}	AcB–methanol (99:1)	80±4	65±6

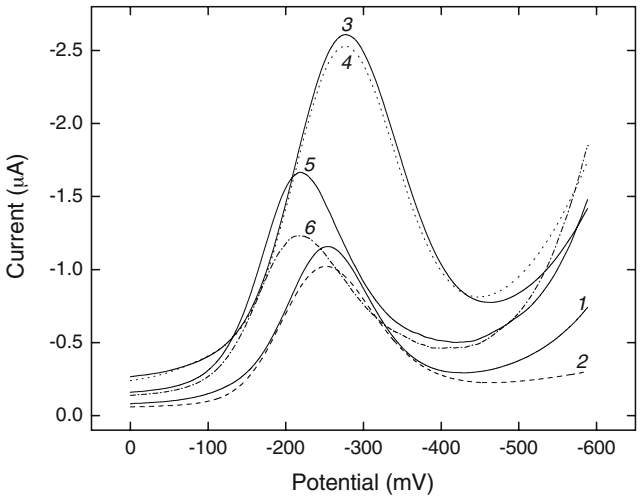

Fig. 3 Cathodic DPV peaks of $[Cu(phen)_2]^{2+}$ ($c=5\times10^{-6}$ mol L^{-1}) recorded in PBS–methanol (99:1); E_{amp} −50 mV, pulse width 100 ms, scan rate 20 mV s^{-1}, E_{step} 5 mV, 25 °C. DP voltammograms recorded in 5 mmol L^{-1} (curves *1*, *3*, *4*) and 20 mmol L^{-1} (curves *2*, *5*, *6*) PBS–methanol (99:1) at the bare SPCPE (curves *1* and *2*), the DNA/SPCPE (curves *3* and *5*), and the DNA/SPCPE in the presence of 2-NF ($c=5\times10^{-6}$ mol L^{-1}) in solution after incubation of the biosensor for 2 min (curves *4* and *6*)

affected by the compounds studied. Thus, under the conditions used, 2-NF and 2,7-DNF derivatives interact with DNA directly as intercalators leading to damage to the DNA.

Damage to DNA caused by electrochemically generated short-lived radicals

It was shown above that the dsDNA layer on the electrode surface enables accumulation of the analyte in the biopolymer matrix. The electrochemical reduction of 2-NF and 2,7-DNF (during the first 4-electron nitro group reduction to the hydroxylamino group) generates short-lived radicals of the $Ar–NO_2H^{\bullet}$ type that may interact with DNA, causing damage [61], as was similarly shown for niclosamide where oxidation of the DNA purine bases was detected voltammetrically [50]. Under these conditions, the DNA damage caused by in situ electrochemical reduction of nitrofluorenes for the solution concentrations of 2-NF and 2,7-DNF of 1×10^{-5} mol L^{-1} was investigated in AcB–methanol (99:1) medium for 2-NF and in AcB–methanol (9:1) medium for 2,7-DNF (the higher methanol content is necessary because of the limited solubility of 2,7-DNF in water). First, the DNA/SPCPE biosensor (and bare SPCPE for comparison) was incubated in a stirred solution of the corresponding compound for 2 min. Then, nitrofluorene reduction was performed to generate $Ar–NO_2H^{\bullet}$ radicals, and, finally, the anodic DPV scan was applied to detect the changes in the intrinsic voltammetric responses of DNA bases (Fig. 5).

Two ways of the reduction of 2-NF and 2,7-DNF to generate their radicals were tested, namely successive CV cathodic/anodic cycling between 0 and −1000 mV (15 scans), and reduction at a fixed potential of −900 mV (for 10 min). Furthermore, effects of solution stirring during the reduction step and temperature (room temperature of 25 °C or human body temperature 36 °C) were also tested. Under conditions of stirred medium heated at 36 °C and successive CV cathodic/anodic cycling between 0 and −1000 mV (15 scans), an increase of guanine ($E_{p,G}$ ~ +800 mV) and adenine ($E_{p,A}$ ~ +1100 mV) DPV peaks was observed (Fig. 5), which demonstrates clearly that damage to the DNA by the reactive radicals took place and caused distortion of the double helix and exposure of the bases to oxidation. Moreover, an additional anodic peak at $E_{p,OG}$ ~ +450 mV was found which can be attributed to electrooxidation of 8-oxoguanine [50], a product of guanine chemical oxidation. All these results are summarized in Table 3. Considering the given error values, a comparable increase in guanine and adenine response was obtained. No changes in the intrinsic DNA responses and no 8-oxoguanine response were found after electroreduction of nitrofluorenes at the fixed potential of −900 mV. To confirm the effect of 2-NF and 2,7-DNF radicals on DNA, similar experiments have been performed with DNA/SPCPE in the absence of nitrofluorenes (i.e. in blank buffer solution) and no change in the intrinsic DNA responses were found.

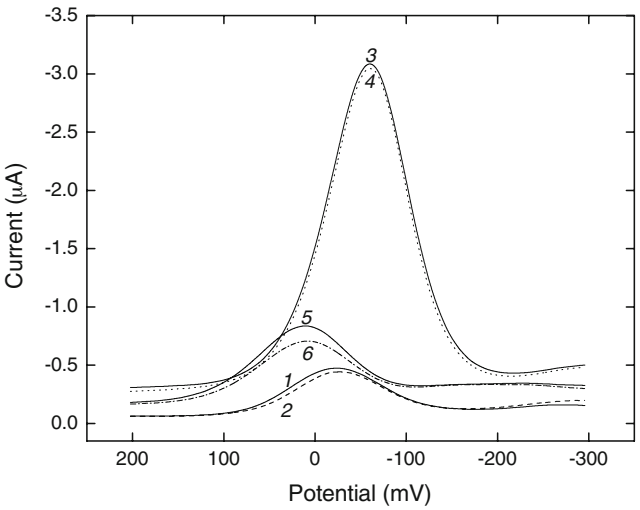

Fig. 4 Cathodic DPV peaks of $[Co(phen)_3]^{3+}$ ($c=5\times10^{-6}$ mol L^{-1}) recorded in PBS–methanol (99:1); E_{amp} −50 mV, pulse width 100 ms, scan rate 20 mV s^{-1}, E_{step} 5 mV, 25 °C. DP voltammograms recorded in 5 mmol L^{-1} (curves *1*, *3*, *4*) and 20 mmol L^{-1} (curves *2*, *5*, *6*) PBS–methanol (99:1) at the bare SPCPE (curves *1* and *2*), the DNA/SPCPE (curves *3* and *5*), and the DNA/SPCPE in the presence of 2,7-NF ($c=5\times10^{-6}$ mol L^{-1}) in solution after incubation of the biosensor for 2 min (curves *4* and *6*)

Table 2 DP voltammetric characteristics of the metal complex indicators used as competing agents in the interaction of 2-NF and/or 2,7-DNF with DNA/SPCPE; $n=3$; $\alpha=0.05$; experimental conditions as for Fig. 3 and 4. E_p represents peak potential of relevant metal complex ($[Cu(phen)_2]^{2+}$ or $[Co(phen)_3]^{3+}$) and ΔE represents the metal complex peak potential shift evaluated at the DNA/SPCPE towards that evaluated at the bare SPCPE

Sensor/indicator	Medium	E_p (mV)	ΔE_p (mV)	I_p (μA)
Bare SPCPE				
5 μmol L^{-1} $[Cu(phen)_2]^{2+}$	5 mmol L^{-1} PBS	−255±3	0±3	−0.95±0.04
	20 mmol L^{-1} PBS	−252±5	0±5	−0.88±0.03
5 μmol L^{-1} $[Co(phen)_3]^{3+}$	5 mmol L^{-1} PBS	−23±3	0±3	−0.37±0.04
	20 mmol L^{-1} PBS	−25±3	0±3	−0.34±0.04
DNA/SPCPE (without 2-NF or 2,7-DNF added)				
5 μmol L^{-1} $[Cu(phen)_2]^{2+}$	5 mmol L^{-1} PBS	−277±3	−22±4	−2.05±0.07
	20 mmol L^{-1} PBS	−216±2	+36±5	−1.34±0.06
5 μmol L^{-1} $[Co(phen)_3]^{3+}$	5 mmol L^{-1} PBS	−60±3	−37±4	−2.70±0.08
	20 mmol L^{-1} PBS	+11±3	+36±4	−0.57±0.06
DNA/SPCPE				
5 μmol L^{-1} $[Cu(phen)_2]^{2+}$	5 mmol L^{-1} PBS–5 μmol L^{-1} 2-NF	−275±3	−20±4	−1.95±0.10
	20 mmol L^{-1} PBS–5 μmol L^{-1} 2-NF	−214±4	+38±6	−0.93±0.10
5 μmol L^{-1} $[Co(phen)_3]^{3+}$	5 mmol L^{-1} PBS–5 μmol L^{-1} 2-NF	−63±2	−40±4	−2.61±0.09
	20 mmol L^{-1} PBS–5 μmol L^{-1} 2-NF	+12±3	+37±4	−0.38±0.08
DNA/SPCPE				
5 μmol L^{-1} $[Cu(phen)_2]^{2+}$	5 mmol L^{-1} PBS–5 μmol L^{-1} 2,7-DNF	−272±2	−17±4	−1.98±0.09
	20 mmol L^{-1} PBS–5 μmol L^{-1} 2,7-DNF	−211±3	+41±6	−0.96±0.09
5 μmol L^{-1} $[Co(phen)_3]^{3+}$	5 mmol L^{-1} PBS–5 μmol L^{-1} 2,7-DNF	−61±4	−38±5	−2.68±0.08
	20 mmol L^{-1} PBS–5 μmol L^{-1} 2,7-DNF	+9±2	+34±4	−0.42±0.06

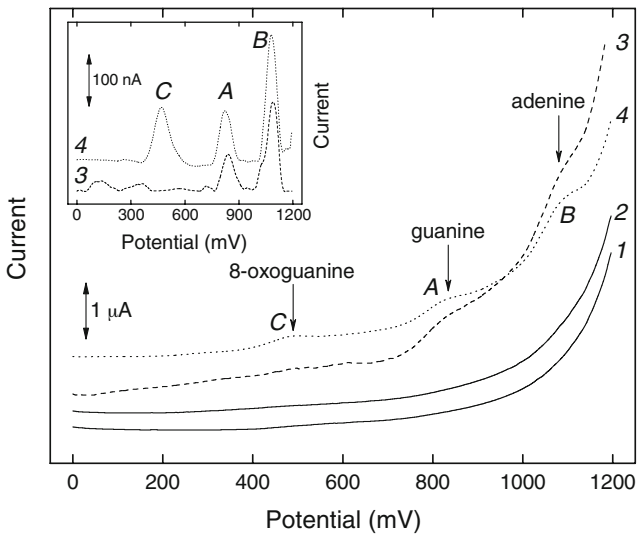

Fig. 5 Anodic DPV response of DNA bases at the DNA/SPCPE recorded in AcB–methanol (99:1) medium; E_{amp} 50 mV, pulse width 100 ms, scan rate 20 mV s^{-1}, E_{step} 5 mV, 36°C. DP voltammograms recorded at the bare SPCPE (curves *1*, *2*) and the DNA/SPCPE (curves *3*, *4*) before (curves *1* and *3*) and after successive CV cathodic/anodic cycling between 0 and −1000 mV (15 scans; scan rate 50 mV s^{-1}) in solution of 2-NF ($c=1\times10^{-5}$mol L^{-1}) in AcB–methanol (99:1) (curves *2* and *4*). Curves *3* and *4* after baseline correction are depicted in the *inset*. Two different DNA/SPCPEs were used to record curves *3* and *4*

Compared with some reports on the induction and control of DNA damage at the electrode surface via electrochemical generation of the damaging (usually radical) species [50, 62], specific conditions were necessary to detect the effect of nitrofluorenes radicals in this work, namely enhanced temperature and potential cycling. The temperature effect could be evidently explained by general rules of chemical kinetics in which increased temperature typically leads to higher reaction yields and efficiency. Potential cycling probably provided the reaction time necessary for the reaction to occur.

Table 3 Detection of DNA damage at the DNA/SPCPE biosensor caused by short-lived radicals generated by electrochemical reduction of 2-NF ($c=1\times10^{-5}$mol L^{-1}; AcB–methanol (99:1) medium) or 2,7-DNF ($c=1\times10^{-5}$mol L^{-1}; AcB–methanol (9:1) medium) during CV cycling between 0 and −1000 mV (15 scans; scan rate 50 mV s^{-1}); $n=3$; $\alpha=0.05$; experimental conditions as for Fig. 5 ($I_{p,G}$, $I_{p,A}$, and $I_{p,OG}$ represent DP anodic peak currents of guanine, adenine, and 8-oxoguanine, respectively)

Compound	$I_{p,G}$ (μA)	$I_{p,A}$ (μA)	$I_{p,OG}$ (μA)
None	+0.07±0.01	+0.17±0.02	–
2-Nitrofluorene	+0.10±0.02	+0.29±0.04	+0.11±0.05
2,7-Dinitrofluorene	+0.12±0.03	+0.34±0.04	+0.15±0.06

Conclusions

Association interactions of calf thymus dsDNA with 2-nitrofluorene and 2,7-dinitrofluorene at the dsDNA-modified screen printed carbon paste electrode were investigated. Subtle DNA damage under conditions of direct DNA–analyte interaction at room temperature, and damage to DNA bases under conditions of electrogeneration of short-lived radicals of nitrofluorenes at human body temperature were found. The type of association of 2-nitrofluorene and 2,7-dinitrofluorene with the surface-attached dsDNA was investigated in detail using competing DNA intercalators and it was found that under in vitro conditions the nitrofluorenes interact with dsDNA by intercalation. The results lead also to the proposal that the toxicity of the compounds under study can be caused by this interaction and their redox activation. The complex study reported here used simple electroanalytical methodology and shows the potential of the disposable DNA/SPCPE biosensor for investigation of genotoxic effects of chemical compounds of environmental and health interest.

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