Langmuir

Article

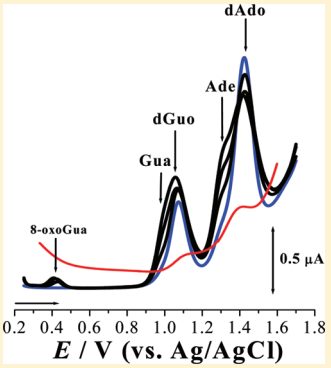
pubs.acs.org/Langmuir

In Situ DNA Oxidative Damage by Electrochemically Generated Hydroxyl Free Radicals on a Boron-Doped Diamond Electrode

S. Carlos B. Oliveira and Ana Maria Oliveira-Brett*

Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal

ABSTRACT: *In situ* DNA oxidative damage by electrochemically generated hydroxyl free radicals has been directly demonstrated on a boron-doped diamond electrode. The DNA-electrochemical biosensor incorporates immobilized double-stranded DNA (dsDNA) as molecular recognition element on the electrode surface, and measures *in situ* specific binding processes with dsDNA, as it is a complementary tool for the study of bimolecular interaction mechanisms of compounds binding to DNA and enabling the screening and evaluation of the effect caused to DNA by radicals and health hazardous compounds. Oxidants, particularly reactive oxygen species (ROS), play an important role in dsDNA oxidative damage which is strongly related to mutagenesis, carcinogenesis, autoimmune inflammatory, and neuro-degenerative diseases. The hydroxyl radical is considered the main contributing ROS to endogenous oxidation of cellular dsDNA causing double-stranded and single-stranded breaks, free bases, and 8-oxoguanine occurrence. The dsDNA-electrochemical biosensor was used to study the interaction between dsDNA immobilized on a boron-doped diamond electrode surface and *in situ* electrochemically generate hydroxyl radicals. Non-denaturing agarose gel-electrophoresis of the dsDNA films on the electrode surface after interaction with the electrochemically generated hydroxyl radicals clearly showed the occurrence of *in situ* dsDNA oxidative damage. The importance of the dsDNA-electrochemical biosensor in the evaluation of the dsDNA–hydroxyl radical interactions is clearly demonstrated.



INTRODUCTION

Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), peroxyl ($ROO^{\bullet}$), and hydroxyl ($OH^{\bullet}$) radicals are generated inside cells as products of metabolism, by leakage from mitochondrial respiration, and also under the influence of exogenous agents—such as ionizing radiation, quinones, and peroxides. The biological function of ROS in the organism so far is ambiguous. On the one hand, ROS can assist the immune system, mediate cell signaling, and be essential in apoptosis. On the other hand, excess ROS are responsible for causing DNA oxidative modifications and mutations, which can initiate carcinogenesis and may play a role in the development of several age-correlated degenerative diseases.[1−12]

Among ROS, hydroxyl radicals are the most reactive and are frequently assumed to be directly responsible for the steady-state level of DNA oxidative modifications observed under natural growth conditions as well as for the damage induced by exposure to peroxides, quinones, and many other oxidants.[13−16] Moreover, based on the results with radical scavengers, 70% of the DNA cellular damage induced by ionizing radiation has been attributed to hydroxyl radicals.[1,16,17] Consequently, the interaction mechanism of $OH^{\bullet}$ radicals with dsDNA has been investigated by several authors[1,8−13] and the chemical damage to dsDNA has been separated into DNA single-stranded and double-stranded breaks and base oxidation.

DNA strand breakage is due to direct action of $OH^{\bullet}$ radicals on the dsDNA backbone or indirectly as a consequence of oxidation of the sugar moiety[1,13] and has been confirmed by the decrease in viscosity[13] and molecular weight determined by sedimentation and diffusion measurements.[13]

The effect of $OH^{\bullet}$ radicals on DNA base oxidation has been extensively studied.[4,6,18] DNA samples exposed to $OH^{\bullet}$ radicals showed increased levels of 8-oxo-7,8-dihydroguanine (8-oxoGua)[19,20] and 8-oxo-7,8-dihydroadenine (8-oxoAde)[21,22] important biomarkers of DNA oxidative damage.

Various methods with great sensitivity and specificity have helped to characterize the nature of $OH^{\bullet}$ interaction with DNA, such as UV−vis, single cell gel electrophoresis, HPLC-ECD, GC/MS, post labeling, and electrochemical methods.[6,12,17] However, for different reasons, conflicting results have been reported[12,17] such as (I) the high reactivity and the short life of the $OH^{\bullet}$ radicals; $OH^{\bullet}$ reacts only with molecules that are very close to it, that is, only a few nanometers distance; (II) the nature of the $OH^{\bullet}$ interaction with DNA has been investigated in different conditions so that results cannot be compared; and (III) different techniques with comparable sensitivity for detection DNA base oxidation give different results. Consequently, consensus and clarification of the interaction of $OH^{\bullet}$ with dsDNA is still needed.

Electrochemical techniques, such as pulse techniques, are suitable for studies of biological systems, since they are fast and have high sensitivity. One advantage of the use of pulse techniques is that they lead to a great improvement in the signal-to-noise ratio compared to steady-state techniques and in many cases greater selectivity.[23]

Received: January 5, 2012
Revised: February 13, 2012
Published: February 15, 2012

A dsDNA-electrochemical biosensor is an integrated receptor−transducer device that uses DNA as a biomolecular recognition element to measure specific binding processes with DNA, through electrochemical transduction. The most important factor for the construction of efficient dsDNA−electrochemical biosensors is the immobilization of the DNA probe on the electrode surface. Different adsorption immobilization procedures, electrostatic adsorption or evaporation, of a monolayer or multilayer DNA films have been used. The multilayer dsDNA-electrochemical biosensor consists of an electrode with dsDNA deposited on the transducer surface by successive monolayer coverage.[24]

The dsDNA-electrochemical biosensor, using differential pulse voltammetry, has been successfully utilized to investigate the interaction of small molecules, heavy metals, and other hazardous compounds with dsDNA[24−27] and compared with other methods shows great sensitivity toward detecting small perturbations of the double-helical structure and the detection of DNA oxidative damage, allowing the unraveling of detailed mechanistic interactions. Another important advantage of using dsDNA-electrochemical biosensors is the possibility of the generation of highly reactive intermediates *in situ*, on the electrode surface, and the electrochemical detection of their direct interaction with dsDNA.[24]

Among the electrochemical transducers, the boron-doped diamond electrode (BDDE) presents unique properties.[28−30] The BDDE has an inert character, weak adsorption properties, with an excellent extensive positive potential window. The BDDE behavior is strongly related to the controlled *in situ* electrochemical generation (by water discharge) of hydroxyl radicals ($OH^{\bullet}$) and their subsequent reactions. The $OH^{\bullet}$ electrochemically generated on a BDDE has been investigated in different electrolyte media.[28−30]

The oxidation of organic compounds at the BDDE surface can follow two different mechanisms depending on the applied potential. In the region of water stability before oxygen evolution, only reactions involving simple electron transfer occur. At high positive applied potentials, in the potential region of oxygen evolution (water discharge)[30] a sequence of reactions occur which are initiated by the $OH^{\bullet}$ formed at the BDDE surface with the organic compound in solution.

The extremely reactive hydroxyl radicals cause dsDNA oxidative damage, and it is very important to explain and characterize the electrogenerated $OH^{\bullet}$-dsDNA interaction mechanisms, which can be electrochemically evaluated on the BDDE surface.

The aim of this work was to investigate the *in situ* interaction and oxidative damage caused by hydroxyl radicals to dsDNA. A thick multilayer dsDNA-electrochemical biosensor was prepared on the BDDE surface. The BDDE surface is the source of the electrochemically generated $OH^{\bullet}$ and the transducer to detect dsDNA oxidative damage, caused by $OH^{\bullet}$ to the dsDNA immobilized on the BDDE surface. Differential pulse voltammetry was used to monitor the occurrence and changes to the oxidation peaks of dsDNA and its oxidation products.[29]

EXPERIMENTAL SECTION

Materials and Reagents. The sodium salt of highly polymerized calf thymus dsDNA (length, 10 000−15 000 bp) was obtained from Sigma-Aldrich and used without further purification. A stock solution of 30 mg mL^{-1} dsDNA was prepared in deionized water and diluted to the desired concentration in the supporting electrolyte. The supporting electrolyte solution used was 0.1 M acetate buffer pH = 4.5. All solutions were prepared using analytical-grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤0.1 μS cm^{-1}).

The concentration of nucleic acids in aqueous solution was determined by measuring their absorbance at 260 nm and considering the relation between optical units and micrograms given by the Sigma−Aldrich certificate of analysis. A Specord S 100 UV−vis spectrophotometer (Carl Zeiss Technology, Analytic Jena, Jena, Germany) was used to measure the absorbance of the nucleic acid solutions. The purity level of calf thymus dsDNA samples was checked spectrophotometrically by determining their UV 260/280 nm absorbance ratio and was always higher than 1.8.

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pipettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out using a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C).

Voltammetric Parameters and Electrochemical Cells. Voltammetric experiments were performed using a μAutolab running with *GPES 4.9* software, Metrohm, Autolab, Utrecht, The Netherlands. Differential pulse (DP) voltammetry conditions were as follows: pulse amplitude, 50 mV; pulse width, 70 ms; and scan rate, ν = 5 mV s^{-1}. Measurements were carried out using a boron-doped diamond working electrode, a Pt wire counter electrode, and a Ag/AgCl (3 M KCl) as reference, in a 2 mL one-compartment electrochemical cell.

The boron-doped diamond films were prepared at the Centre Suisse de Electronique et de Microtechnique SA (CSEM), Neuchatel, Switzerland, on silicon wafers using the hot filament chemical vapor deposition (HF-CVD) technique with a filament temperature in the range 2440−2560 °C and a gaseous mixture containing CH_4, H_2, and trimethylboron (TMB). The boron dopant is introduced in the diamond film by *in situ* doping during the CVD process through a TMB gas source. This HF-CVD process gives a columnar, randomly textured polycrystalline boron-doped diamond film with the surface dominated by facets. The final boron content was of the order of 8000 ppm, 5.7 × 6.1 mm^2 surface area, ∼ 1 μm thickness.

Acquisition and Presentation of Voltammetric Data. Some voltammograms presented were baseline-corrected using the moving average application with a step window of 5 mV included in *GPES v* 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artifact, although the peak intensity is in some cases reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

BDDE Surface Conditioning. An anodic electrochemical pretreatment of the BDDE surface was carried out[28,29] applying a potential of E_{ap} = +3.0 V for 15 min, in pH = 4.5 0.1 M acetate buffer. The BDDE surface was oxidized, Ox-BDDE, together with a long and extensive oxygen evolution. After the Ox-BDDE was placed in 0.1 M acetate buffer pH = 4.5, electrolyte and various DP voltammograms were recorded until a steady-state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

Thick Multilayer DNA-Electrochemical Biosensor Preparation and Incubation Procedure. The thick multilayer dsDNA-electrochemical biosensors were prepared by covering the Ox-BDDE surface successively with six drops each of 40 μL 10 mg mL^{-1} dsDNA solution. After placing each drop on the electrode surface, the biosensor was dried under a constant flux of N_2. The prepared thick multilayer dsDNA-BDDE biosensors were transferred to the 0.1 M acetate buffer pH = 4.5, where the dried film was hydrated for 30 min and afterward +0.30 V applied during 30 min, in order to have a stronger adsorption of dsDNA to the BDDE surface. This potential was chosen because it is not positive enough to irreversibly oxidize the DNA bases inside the molecule[27] and was considered to be sufficient to stabilize the negatively charged DNA film on the Ox-BDDE surface.

dx.doi.org/10.1021/la300070x | *Langmuir* 2012, 28, 4896−4901

Electrogeneration of Hydroxyl Radicals. The $OH^{\bullet}$ were *in situ* electrogenerated on the thick multilayer dsDNA-BDDE biosensor surface by applying different positive potentials, + 2.0 V, + 2.5 V, and +3.0 V, for different periods of time, 15 min, 30 min, 1 h, 2 h, and 3 h.

DNA Gel Electrophoresis. Nondenaturing agarose (0.5%, ultrapure DNA grade from Sigma) gel was prepared in TA buffer (10 mM Tris base and 4.4 mM acetic acid). 40 μL dsDNA control solution and hydroxyl radical-dsDNA sample aliquots (with 0.25% bromophenol blue in water) were loaded into wells, and electrophoresis was carried out in TAE buffer (10 mM Tris base, 4.4 mM acetic acid, and 0.5 mM EDTA, pH = 8) for 1 h at 100 V. After 2% ethidium bromide (EtBr) staining, DNA was visualized and photographed under UV (312 nm) transillumination to visualize DNA mobility. The dsDNA films oxidatively damaged by the electrochemically generated $OH^{\bullet}$, on the BDDE surface, in different experimental conditions, were removed from the BDDE surface, transferred to Eppendorf tubes and dissolved in 100 μL of pH = 4.5 0.1 M acetate buffer. The procedure for the dsDNA control solution was the same. The control dsDNA film was prepared on the BDDE, hydrated for 30 min, and afterward a potential of +0.30 V was applied for 30 min.

RESULTS AND DISCUSSION

Electrogeneration of Hydroxyl Radicals *in Situ* at the BDDE Surface. The BDDE surface was pretreated anodically in acetate buffer, as described in the Experimental Section. The *in situ* electrochemical generation of $OH^{\bullet}$ at the Ox-BDDE surface was studied by DP voltammetry, between the potential limits E_1 = 0.0 V and E_2 = +2.10 V, in pH = 4.5 0.1 M acetate buffer, Figure 1.

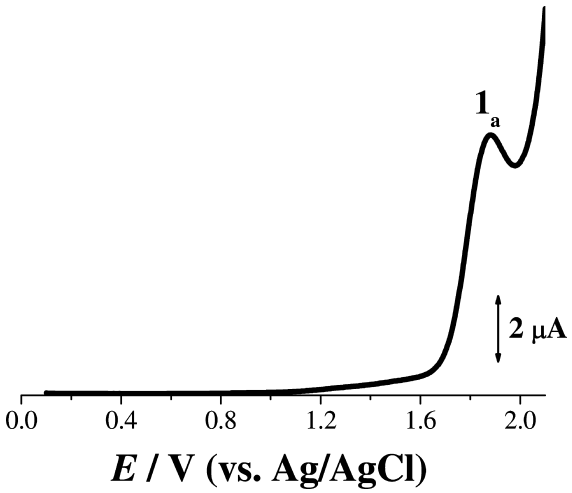


Figure 1. DP voltammograms in pH = 4.5 0.1 M acetate buffer at Ox-BDDE surface.

The DP voltammogram presented a very high oxidation peak 1a, at E_{p1a} ~ +1.9 V, corresponding to the *in situ* electrochemical generation of $OH^{\bullet}$ at the Ox-BDDE surface, by water discharge, reaction 1.

$$BDDE + H_2O \rightarrow BDDE(^{\bullet}OH) + H^{+} + e^{-} \quad (1)$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \quad (2)$$

The Ox-BDDE surface undergoes little interaction with the electrolyte, the overpotential for oxygen evolution is high, reaction 2 occurs in the solution due to the $OH^{\bullet}$ high reactivity, but the oxygen evolution reaction, via H_2O_2, does not occur.[30−33]

The kinetics of the $H_2O/OH^{\bullet}$ redox couple at the Ox-BDDE surface is pH-dependent and the applied potential also influences the rate of water discharge.[28−30]

Considering that at potentials higher than +1.8 V water discharge occurs, the potentials of +2.0, +2.5, and +3.0 V were chosen to investigate the interaction with dsDNA of *in situ* electrogenerated $OH^{\bullet}$ on the thick multilayer dsDNA-BDDE biosensors at different potentials applied during different periods of time.

The concentration profile of hydroxyl radicals during oxygen evolution as a function of the distance from the electrode surface can be calculated.[30] For the current density used $j \sim 300$ A m^{-2}, the reaction layer thickness was ~1 μm whereas the maximum (surface) concentration of hydroxyl radicals reached several tenths of μM.

Electrochemical Behavior of dsDNA at Ox-BDDE Surface. The electrochemical behavior of dsDNA, all DNA bases, nucleosides, nucleotides, and biomarker 8-oxoGua was investigated at BDDE surfaces.[25] All DNA bases and 8-oxoGua are electroactive on the Ox-BDDE surface and it is possible to evaluate the integrity of dsDNA by a DP voltammogram. The peak potentials obtained for the oxidation of each of the four free bases in solution, Figure 2A, occurs at less positive potentials compared with the peak potentials found for their corresponding nucleotides[29] Figure 2B, enabling the evaluation of dsDNA integrity by the detection of the oxidation peaks due to the presence of free bases and oxidized free bases. Peak overlapping occurs when the free bases and nucleotides are present. However, the detection of Gua and 8-oxoGua[29] oxidation peaks is a clear indication of the occurrence of DNA oxidative damage, Figure 2A.

As shown in Figure 2A and B, voltammetric methods are an excellent analytical tool, giving important information in assessing the occurrence of any physical, chemical, enzymatic, or oxidative damage to DNA that can cause the release of free bases or strand breaks disrupting the polymer morphology, or generate the products of purine base oxidation.

***In Situ* Evaluation of Hydroxyl Radical−dsDNA Interaction.** The dsDNA-BDDE biosensor was used to evaluate the interaction of the immobilized dsDNA on the BDDE surface with the *in situ* electrogenerated $OH^{\bullet}$ at different potentials, +2.0 V, +2.5 V, and +3.0 V, applied during different periods of time, 15 min up to 3 h.

The thick multilayer dsDNA-BDDE biosensor was always prepared as described in the Experimental Section, in which the hydration of the dsDNA film and the application of +0.3 V to the BDDE surface are very important. All experiments were performed in pH = 4.5 0.1 M acetate buffer since the dsDNA oxidation peak currents are much higher at pH = 4.5 than at physiological pH ~ 7[27] thus allowing lower detection limits for the identification of DNA oxidative damage.

The results obtained with the dsDNA-BDDE biosensor to evaluate the interaction of the immobilized dsDNA with the *in situ* electrogenerated $OH^{\bullet}$ on the BDDE surface at different positive potentials applied during different times were always compared with those obtained with the control dsDNA-BDDE biosensor, which was not subjected to applied high positive potentials.

The control dsDNA-BDDE biosensor was characterized by DP voltammetry in pH = 4.5 0.1 M acetate buffer, showing well-defined small oxidation peaks due to the oxidation of desoxyguanosine (dGuo), at E_{pa} = +1.10 V, and desoxyadenosine (dAdo), at E_{pa} = +1.38 V, Figure 3. The dGuo and dAdo

dx.doi.org/10.1021/la300070x | *Langmuir* 2012, 28, 4896−4901

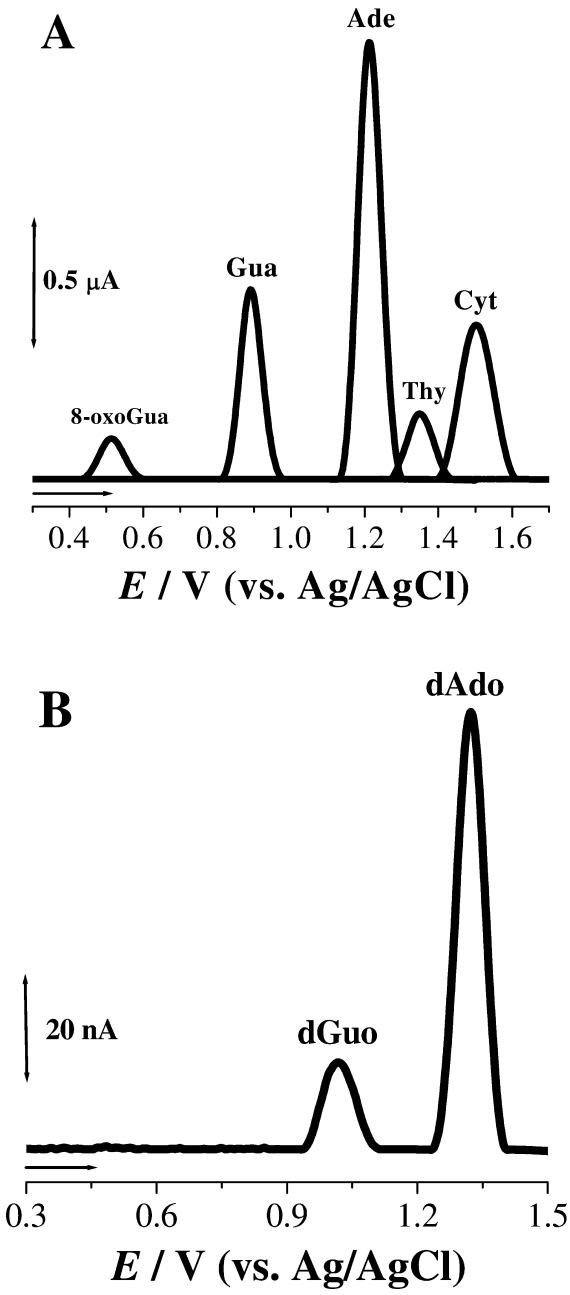


Figure 2. DP voltammograms baseline corrected in pH = 4.5 0.1 M acetate buffer at Ox-BDDE surface: (A) filtered supernatant from a saturated solution of 1 mM of 8-oxoguanine (8-oxoGua), 5 μM guanine (Gua), 10 μM adenine (Ade), 20 μM of thymine (Thy), and 20 μM of cytosine (Cyt) and (B) 200 μg mL^{-1} of dsDNA.

oxidation peaks are small due to the difficulty of electron transfer from the inside of the rigid dsDNA structure to the BDDE surface, similar to the results obtained using a glassy carbon electrode.[17,31]

A potential of +2.0 V was applied for 15 min and 3 h to newly prepared dsDNA-BDDE biosensors, and the interaction of the immobilized dsDNA with the OH$^{\bullet}$ electrogenerated on the BDDE surface was detected. DP voltammograms after 15 min showed an increase of the oxidation peak currents of dGuo and dAdo compared with those obtained with the control dsDNA-BDDE biosensor. The DP voltammograms after 3 h showed a larger increase of the oxidation peak currents of dAdo, that occurred at a more negative potential, and a new

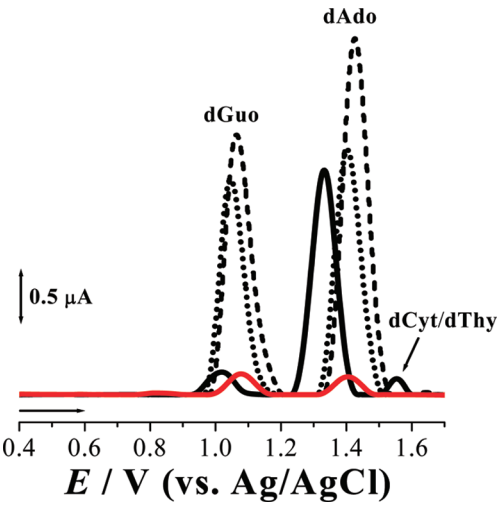


Figure 3. DP voltammograms baseline corrected in pH = 4.5 0.1 M acetate buffer with a thick multilayer dsDNA-BDDE biosensor: (red line) control; and after applying +2.5 V for (●●●) 15 min, (---) 30 min, and (—) 2 h to the BDDE surface causing electrogeneration of hydroxyl radicals.

oxidation peak corresponding to oxidation of the pyrimidine, cytosine and thymine, nucleotides (dCyt/dThy), at E_{pa} ~ +1.55 V. However, the interaction of dsDNA with the electrogenerated OH$^{\bullet}$ did not lead to the occurrence of base oxidation products.

A potential of +2.5 V was applied for 15 min, 30 min, and 2 h to new dsDNA-BDDE biosensors, Figure 3, and as expected, the DP voltammograms after 15 and 30 min showed a large increase of the oxidation peak currents of dGuo and dAdo compared with the results obtained with the control dsDNA-biosensor. The DP voltammograms obtained for the dsDNA-BDDE biosensor after 2 h showed the same behavior as those for an applied potential of +2.0 V for 3 h, a large increase of the dAdo oxidation peak currents, that also occurred at a more negative potential, and a new oxidation peak corresponding to dCyt/dThy, at E_{pa} ~ +1.55 V, but the interaction did not lead to the occurrence of base oxidation products.

The results in Figure 3 are due to hydrogen bond breaking, unfolding and strand breaking of the DNA double helix structure, causing the appearance of short DNA single strands and enabling oxidation of the DNA bases morphologically more accessible to the Ox-BDDE surface. For the applied potentials of +2.0 V and +2.5 V, the new peak dCyt/dThy only occurs after a long time, because the *in situ* concentration of OH$^{\bullet}$ electrogenerated on the BDDE surface is low at these potentials and the damage to the dsDNA on the BDDE surface is not electrochemically detectable. Voltammetric analysis is not easy because overlap of the oxidation peaks of the free bases and nucleosides or nucleotides occurs, all present in the DNA damaged film on the BDDE surface.

After applying a potential of +3.0 V during 30 min and 1 h to newly prepared dsDNA-BDDE biosensors, the interaction of the immobilized dsDNA with the OH$^{\bullet}$ electrogenerated on the BDDE surface was detected. The DP voltammograms showed a large increase of the oxidation peak currents of dGuo and dAdo, due to the (OH$^{\bullet}$)−DNA interaction, compared with the results obtained with the control dsDNA-BDDE biosensor, Figure 4, explained by the DNA double helix unfolding, causing the DNA bases to be more exposed and in contact with the electrode surface, thus facilitating their oxidation.

dx.doi.org/10.1021/la300070x | *Langmuir* 2012, 28, 4896−4901

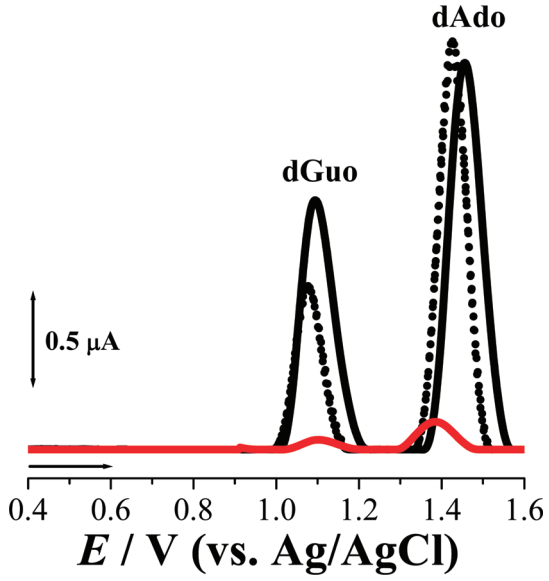


Figure 4. DP voltammograms baseline corrected in pH = 4.5 0.1 M acetate buffer with a thick multilayer dsDNA-BDDE biosensor: (red line) control and after applying +3.0 V for (●●●) 30 min and (—) 1 h to the BDDE surface causing electrogeneration of hydroxyl radicals.

Applying +3.0 V for 2 h to a new dsDNA-BDDE biosensor, compared with the results obtained with the control dsDNA-BDDE biosensor, showed a large increase in the oxidation peak currents of dGuo and dAdo, and the occurrence of three new oxidation peaks corresponding to the oxidation of 8-oxoGua, at E_{pa} = +0.42 V, and of the free purine bases Gua, at E_{pa} ~ +1.0 V, and Ade, at E_{pa} ~ +1.3 V. These are released from the DNA double helix after a longer incubation time exposure, i.e., to a higher concentration of electrogenerated OH•, Figure 5.

A decrease of the oxidation peak currents of dAdo and dGuo and an increase of the oxidation peak currents of 8-oxoGua, Gua, and Ade were observed in subsequent scans, Figure 5, confirming dsDNA oxidative damage caused by a higher concentration of electrogenerated OH• under the conditions of +3.0 V for 2 h.

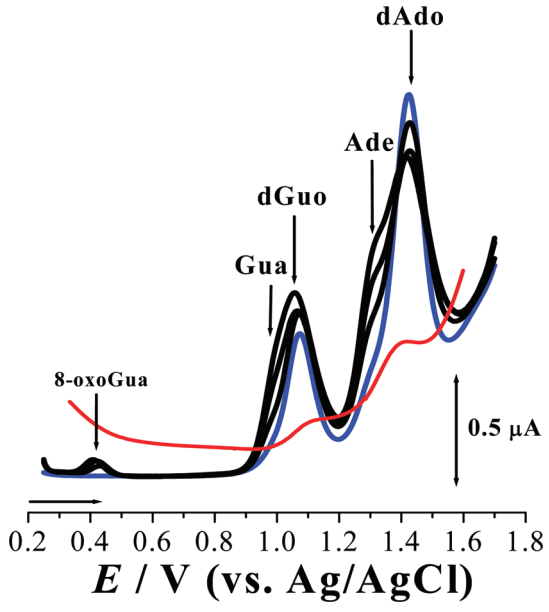


Figure 5. DP voltammograms in pH = 4.5 0.1 M acetate buffer with a thick multilayer dsDNA-BDDE biosensor: (red) control and (blue) first scan and (black) subsequent scans after applying +3.0 V for 2 h to the BDDE surface causing electrogeneration of hydroxyl radicals.

The results showed direct evidence of the (OH•)−DNA interaction in acidic media at pH = 4.5, where the dsDNA predominating structure is the B-form, and it can be concluded that the (OH•)−DNA interaction can easily occur at physiological pH.[26,27]

Electrophoresis Evaluation of Hydroxyl Radical-dsDNA Interaction. Gel electrophoresis was performed as described in the Experimental Section, to detect conformation changes in dsDNA after interaction with *in situ* electrogenerated OH•. The main objective of the electrophoresis experiments was to compare the relative migration profile of dsDNA after interaction with *in situ* electrogenerated OH• with that of control native dsDNA. Therefore, the use of markers for molecular mass determination was not considered necessary.

To visualize the dsDNA mobility and damage, the EtBr binding assay for dsDNA damage was used.[34,35] In general, the smaller the DNA fragment length, the greater the electrophoretic mobility.[27,28] Moreover, the migration distances for dsDNA and ssDNA fragments of the same length are expected to be different due to the accelerated mobility of ssDNA relative to dsDNA throughout the agarose gel matrix, although for the same length, the double negatively charged dsDNA would sense a stronger electrophoretic mobility force.[34,35]

The electrophoretic migration profile of the dsDNA after interaction with *in situ* OH• electrogenerated on the BDDE surface after applying +2.0 V for 2 h (line 2) and +2.0 V for 30 min (line 4), + 2.5 V for 3 h (line 5), and +3.0 V for 2 h (line 3) and +3.0 V for 3 h (line 6) is shown in Figure 6. The control dsDNA (line 1) was analyzed and the band observed in the absence of OH• interaction corresponded to different-sized length fragments present in the loaded samples.[34,35]

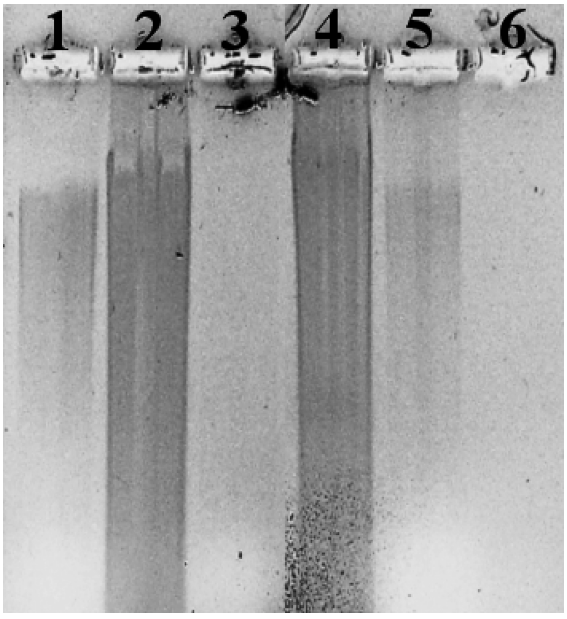


Figure 6. Nondenaturing agarose (0.5%) gel electrophoresis: dsDNA control (line 1) and dsDNA from a thick multilayer dsDNA-BDDE biosensor after applying: +2.0 V for 2 h (line 2) and 30 min (line 4); +2.5 V for 3 h (line 5) and +3.0 V for 2 h (line 3) and 3 h (line 6) to the BDDE surface causing electrogeneration of hydroxyl radicals.

dx.doi.org/10.1021/la300070x | *Langmuir* 2012, 28, 4896−4901

The electrophoresis obtained for dsDNA after interaction with *in situ* $OH^{\bullet}$ electrogenerated on the BDDE surface for a lower concentration of electrogenerated $OH^{\bullet}$ (lines 2 and 4) showed that the electrophoretic migration mobility increased indicating the presence of a population of different-length small ssDNA fragments. The large decrease in total fluorescence, observed in lines 3, 5, and 6, corresponds to accumulation of damage in the DNA exposed for a longer period of time to a higher concentration of electrogenerated $OH^{\bullet}$, and is in agreement with the $OH^{\bullet}$-induced loss of DNA-EtBr fluorescence, explained by the occurrence of free bases and breakage of the sugar phosphate backbone.

These electrophoretic experiments confirm the results for ($OH^{\bullet}$)−DNA interaction detected using voltammetric methods, which is of crucial importance to interpret and explain the occurrence of dsDNA oxidative damage.

■ CONCLUSIONS

An electrochemical dsDNA-BDDE biosensor prepared by immobilization of dsDNA on Ox-BDDE surface was successfully used to identify different kinds of dsDNA damage caused by *in situ* electrogenerated $OH^{\bullet}$. The dsDNA-BDDE biosensor enabled preconcentration of the $OH^{\bullet}$ electrogenerated on the dsDNA-BDDE biosensor surface. By controlling the applied potential, different concentrations of $OH^{\bullet}$ were electrochemical generated *in situ*. By monitoring the modification of oxidation peak currents of the purine and pyrimidine deoxynucleoside residues, it was concluded that $OH^{\bullet}$ oxidatively damage the immobilized dsDNA, leading to modifications in the dsDNA structure, exposing more purinic and pyrimidinic residues to the electrode surface and facilitating their oxidation. DNA oxidative damage was confirmed by electrophoresis and demonstated the occurrence of the 8-oxoGua peak, a biomarker of DNA oxidative damage. The dsDNA-BDDE biosensors enable an inexpensive direct and fast detection procedure that can contribute to elucidation of the mechanism by which DNA is oxidatively damaged by radicals and hazard compounds.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: brett@ci.uc.pt. Tel/FAX: +351-239-835295.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from Fundação para a Ciência e Tecnologia (FCT), Post-Doctoral Grant SFRH/BPD/71965/2010 (SCB Oliveira), project, PTDC/QUI-QUI/098562/2008, POFC-QREN (cofinanced by the European Community Fund FEDER/COMPETE), and CEMUC-R (Research Unit 285), is gratefully acknowledged.

■ REFERENCES

(1) Abolfath, R. M.; Van Duin, A. C. T.; Brabec, T. *J. Phys. Chem. A* **2011**, *115*, 11045−11049, http://qmsimulator.wordpress.com.
(2) Dizdaroglu, M. *Free Radical Biol. Med.* **1991**, *10*, 225−242.
(3) Jeong, J. B.; De Lumen, B. O.; Jeong, H. J. *Cancer Lett.* **2010**, *293*, 58−64.
(4) Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. *FASEB J.* **2003**, *17*, 1195−1214.
(5) Nishikawa, M. *Cancer Lett.* **2008**, *266*, 53−59.
(6) Dizdaroglu, M.; Jaruga, P.; Birincioglu, M.; Rodriguez, H. *Free Radical Biol. Med.* **2002**, *32*, 1102−1115.
(7) Halliwell, B.; Gutteridge, J. M. *Arch. Biochem. Biophys.* **1986**, *246*, 501−514.
(8) Sies, H. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1058−1071.
(9) Sies, H. *Oxidative Stress: Oxidants and Antioxidants*; Academic Press: London, 1991.
(10) Joenje, H. *Mutat. Res.* **1989**, *219*, 193−208.
(11) Clayson, D. B.; Mehta, R.; Iverson, F. *Mutat. Res.* **1984**, *317*, 25−42.
(12) Jing-Jing, Z.; Bin, W.; Yan-Fen, L.; Wen-Li, J.; Hui, C.; Huai-Sheng, W. *Electroanalysis* **2008**, *20*, 1684−1689.
(13) Rhaese, H. J.; Freese, E. *Biochim. Biophys. Acta* **1968**, *155*, 476−490.
(14) Prutz, W. A. *Radiat. Environ. Biophys.* **1984**, *23*, 1−6.
(15) Prutz, W. A. *Radiat. Environ. Biophys.* **1984**, *23*, 7−18.
(16) Bernd, E.; Daniel, B.; Waldemar, A.; Günther, N. G.; Chantu, R. S. M. *Nucleic Acids Res.* **1996**, *24*, 1625−1631.
(17) Piedade, J. A. P.; Oliveira, P. S. C.; Lopes, M. C.; Oliveira-Brett, A. M. *Anal. Biochem.* **2006**, *355*, 39−49.
(18) Jean, C.; Thierry, D.; Thierry, D.; Didier, G.; Jean-Pierre, P.; Jean-Luc, R.; Sylvie, S. *Mut. Res.* **1999**, *424*, 9−21.
(19) Mei, N.; Tamae, K.; Kunigita, N.; Hirano, T.; Kasai, H. *Environ. Mol. Mutagen.* **2003**, *41*, 332−338.
(20) Helbock, H. J.; Beckman, K. B.; Ames, B. N. *Method. Enzymol.* **1990**, *300*, 156−165.
(21) Cadet, J.; Douki, T.; Gasparutto, D. *Radiat. Phys. Chem.* **2005**, *72*, 293−299.
(22) Kasai, H.; Lida, A.; Yamaizumi, Z.; Nishimura, S.; Tanooka, H. *Mutat. Res.* **1990**, *243*, 249−253.
(23) Brett, C. M. A.; Oliveira-Brett, A. M. In *The Encyclopedia of Electrochemistry, Instrumentation and Electroanalytical Chemistry*, Bard, A. J., Stratmann, M., Eds.; Wiley-VCH Verlag: Weinheim, Germany, 2003; Vol. 3, Chapter 2.2, pp 105−124.
(24) Oliveira, S. C. B.; Oliveira-Brett, A. M. *Comb. Chem. High Throughput Screening* **2010**, *13*, 628−640.
(25) Chiorcea-Paquim, A. M.; Corduneanu, O.; Oliveira, S. C. B.; Diculescu, V. C.; Oliveira-Brett, A. M. *Electrochim. Acta* **2009**, *54*, 1978−1985.
(26) Oliveira, S. C. B.; Oliveira-Brett, A. M. *Anal. Bioanal. Chem.* **2010**, *398*, 1633−1641.
(27) Oliveira, S. C. B.; Corduneanu, O.; Oliveira-Brett, A. M. *Bioelectrochemistry* **2008**, *72*, 53−58.
(28) Oliveira, S. C. B.; Oliveira-Brett, A. M. *Electrochim. Acta* **2010**, *55*, 4599−4605.
(29) Oliveira, S. C. B.; Oliveira-Brett, A. M. *J. Electroanal. Chem.* **2010**, *648*, 60−66.
(30) Kapalka, A.; Fóti, G.; Comninellis, C. *Electrochim. Acta* **2009**, *54*, 2018−2023.
(31) Marselli, B.; Gomez, J. G.; Michaud, P. A.; Rodrigo, M. A.; Comninellis, C. *J. Electrochem. Soc.* **2003**, *150*, D79−D83.
(32) Kapalka, A.; Lanova, B.; Baltruschat, H.; Fóti, G.; Comninellis, C. *Electrochem. Commun.* **2008**, *10*, 1215−1218.
(33) Brillas, E.; et al. *Electrochim. Acta* **2004**, *49*, 4487−4496.
(34) Brody, J. R.; Kern, S. E. *Anal. Biochem.* **2004**, *333*, 1−13.
(35) Martin, R. *Gel Electrophoresis Nucleic Acids*; Bios Scientific Publishers Limited: Oxford, 1996.