Chapter 20

DNA-electrochemical biosensors for investigating DNA damage

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20.1 INTRODUCTION

Electrochemical research on DNA is of great relevance to explain many biological mechanisms. The interaction of many chemical compounds, including water, some metal ions and their complexes, small organic molecules and proteins, with DNA is reversible and stabilizes DNA conformations. However, hazard compounds such as drugs and carcinogens interact with DNA causing irreversible damage and these interactions have to be carefully studied. DNA-electrochemical biosensors are a very good model for simulating nucleic acid interactions with cell membranes, potential environmental carcinogenic compounds and clarifying the mechanisms of interaction with drugs and chemotherapeutic agents.

The electrochemical behaviour and the adsorption of nucleic acid molecules and DNA constituents have been extensively studied over recent decades [1–6]. Electrochemical studies demonstrated that all DNA bases can be electrochemically oxidized on carbon electrodes [7–13], following a pH-dependent mechanism. The purines, guanine (G) and adenine (A), are oxidized at much lower positive potentials than the pyrimidines, cytosine (C) and thymine (T), the oxidation of which occurs only at very high positive potentials near the potential corresponding to oxygen evolution, and consequently are more difficult to detect. Also, for the same concentrations, the oxidation currents observed for pyrimidine bases are much smaller than those observed for the purine bases. Consequently, the electrochemical detection of oxidative changes occurring in DNA has been based on the detection of purine base oxidation peaks or of the major

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oxidation product, 8-oxoguanine (8-oxoGua) [14,15], which is a biomarker of oxidative stress.

Electrochemical oxidation of natural and synthetic DNA performed at pyrolytic graphite [16] and glassy carbon [3–6,17,18] electrodes showed that at pH 4.5 only the oxidation of the purine residues in polynucleotide chains is observed. Using differential pulse voltammetry, the less positive peak corresponds to the oxidation of guanine residues and the peak at more positive potentials is due to the oxidation of adenine residues.

Large differences in the currents obtained at carbon electrodes for dsDNA and ssDNA oxidation were observed. The greater difficulty for the transition of electrons from the inside of the rigid helix form of dsDNA to the electrode surface than from the flexible ssDNA, where guanine and adenine residues can reach the surface, leads to much higher peak currents for ssDNA. Thus, the oxidation currents of guanine and adenine residues in DNA can be used to probe individual adenine–thymine (A–T) and guanine–cytosine (G–C) pairs in dsDNA. In this way, the irreversible DNA damages caused by health hazardous compounds and oxidizing substances can be monitored electrochemically either by using the changes in the oxidation peaks of the purinic DNA bases, guanine and adenine or by the appearance of 8-oxoGua characteristic peaks due to DNA oxidative stress.

When natural or synthetic DNA molecules interact with electrode surfaces adsorption occurs. The knowledge about the adsorption of nucleic acids onto the electrode surface leads to the development of DNA-modified electrodes, also called electrochemical DNA biosensors [3–6,19–24]. An electrochemical DNA biosensor is an integrated receptor–transducer device that uses DNA as the biomolecular recognition element to measure specific binding processes with DNA, using electrochemical transduction.

This chapter describes different methodologies used in the design of DNA-electrochemical biosensors, their surface morphological characterization as well as their application to DNA–drug interaction studies.

20.2 AFM IMAGES OF DNA-ELECTROCHEMICAL BIOSENSORS

A DNA-electrochemical biosensor is formed by a DNA film, which constitutes the molecular recognition element (the probe), directly immobilized on the electrochemical transducer. The performance, sensitivity and reliability of the DNA biosensor and the electrochemical response are dictated by the DNA immobilization procedure. The DNA biophysical properties, such as flexibility, and DNA–drug interactions, are influenced

by the adsorbed DNA structure (ssDNA or dsDNA), concentration, pH and supporting electrolyte [25–28]. Therefore, a full understanding of the surface morphology of the DNA-electrochemical biosensor is necessary to guarantee the correct interpretation of the experimental results.

Magnetic AC atomic force microscopy (MAC Mode AFM) has proved to be a powerful surface analysis technique to investigate the interfacial and conformational properties of biological samples softly bound to the electrode surface and can be used as an important tool to characterize DNA-electrochemical biosensor surfaces [25,27].

Carbon electrodes such as glassy carbon, carbon fibres, graphite or carbon black have wide electrochemical applications but they do not represent a good substrate to obtain AFM images. Highly oriented pyrolytic graphite (HOPG), which is easy to clean, inert in air and has extremely smooth terraces on its basal plane (Fig. 20.1A) can be used with success for imaging biological molecules. Thus the MAC Mode AFM surface characterization of the nanoscale DNA adsorbed films was performed using HOPG electrodes.

Depending on the required application, three different methods of preparation of DNA films were developed. The optimized experimental conditions used to obtain AFM images are described in Procedure 28 (see in CD accompanying this book).

The AFM image of the HOPG substrate modified by a thin layer of dsDNA, when small DNA concentrations are used, is shown in Fig. 20.1B. The dsDNA molecules adsorbed on the HOPG surface formed two-dimensional lattices with uniform coverage of the electrode.

DNA films grown in acid buffer solutions presented greater DNA surface coverage, due to overlaying and superposition of DNA molecules, in relation to films formed in neutral buffer solutions [26]. The DNA network patterns define nanoelectrode systems with different active surface areas on the graphite substrate and form a biomaterial matrix to attach and study interactions with molecules such as drugs [27].

The thin dsDNA films do not completely cover the HOPG electrode surface and the network structure has holes exposing the electrode underneath. The drug molecules from the bulk solution will also diffuse and adsorb non-specifically on the electrode's uncovered regions. If the drugs are electroactive, this leads to two different contributions to the electrochemical signal, one from the simple adsorbed drug and the other due to the damage caused to immobilized DNA, being difficult to distinguish between them.

A complete coverage of the electrode surface is obtained using the multi-layer and thick dsDNA films described in Procedure 28 (see in CD

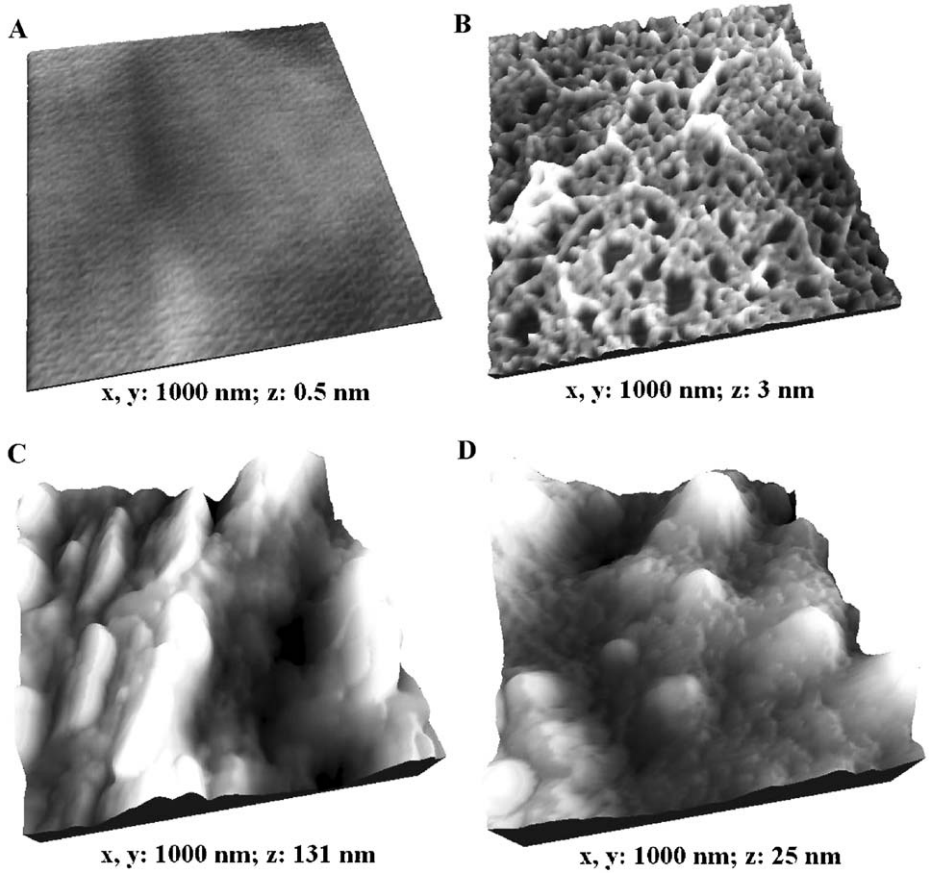


Fig. 20.1. MAC Mode AFM three-dimensional images in air of (A) clean HOPG electrode; (B) thin-film dsDNA-biosensor surface, prepared onto HOPG by 3 min free adsorption from 60 μg/mL dsDNA in pH 4.5 0.1 M acetate buffer; (C) multi-layer film dsDNA biosensor, prepared onto HOPG by evaporation of three consecutive drops each containing 5 μL of 50 μg/mL dsDNA in pH 4.5 0.1 M acetate buffer; (D) thick-film dsDNA biosensor, prepared onto HOPG by evaporation from 37.5 mg/mL dsDNA in pH 4.5 0.1 M acetate buffer. With permission from Refs. [28,29].

accompanying this book). The AFM images of both multi-layer and thick-layer films show uniform and complete coverage of the electrode, with regularly dispersed peaks and valleys (Figs. 20.1(C) and (D)). The DNA–electrode surface interactions are stronger and these DNA films are more stable on the HOPG surface. The big advantage of the dsDNA thick film is that the HOPG surface is completely covered by dsDNA and consequently the undesired binding of molecules to the electrode

surface is not possible. As a result, the multi-layer and thick-film DNA biosensors [27,29] are useful tools to study, *in situ*, the interactions between DNA and health hazardous compounds and drugs with therapeutic activity.

20.3 DNA-ELECTROCHEMICAL BIOSENSORS FOR DETECTION OF DNA DAMAGE

The aim of developing DNA-modified electrodes is to study the interaction of DNA immobilized on the electrode surface with analytes in solution and to use the DNA biosensor to evaluate and to predict DNA interactions and damage by health hazardous compounds based on their ability to bind to nucleic acids. In this way, DNA acts as a promoter between the electrode and the biological molecule under study.

The DNA-biosensing design usually employs electrochemical, optical and mechanical transduction techniques [5]. Electrochemical methods have the advantage of being rapid, sensitive and cost effective. Nevertheless, the most important advantage in using electrochemical DNA biosensors is the possibility of *in situ* generation of reactive intermediates and the detection of their interaction with DNA. Comprehensive descriptions of research on DNA and DNA sensing [1–6,19–22,30–34] show the great possibilities of using electrochemical transduction in DNA diagnostics.

The electrochemical sensor for DNA damage consists of an electrode with DNA immobilized on the surface. The interactions of the surface-immobilized DNA (either by electrostatic adsorption or by evaporation) with the damaging agent are converted, via changes in electrochemical properties of the DNA recognition layer, into measurable electrical signals corresponding to the oxidation of DNA purine bases [35]. The double-stranded DNA structure makes access of the bases to the electrode surface difficult, hindering their oxidation. The occurrence of DNA damage causes the unwinding of the double helix. As the double helix unwinds, closer access of the bases to the surface is possible, leading to the possibility of voltammetric detection of DNA damage. This biosensor has been successfully applied to study the interaction of several substances with dsDNA and the interpretation of the results has contributed to the elucidation of the mechanisms by which DNA is damaged by health hazardous compounds [5,6,35–37].

Although several different DNA adsorption methods have been used on different types of electrodes [19,25], the immobilization of dsDNA to

electrode surfaces can be attained very easily by evaporation or adsorption and no reagents or DNA modifications will occur since the immobilization does not involve formation of covalent bonds with the surface. Therefore, DNA-electrochemical biosensors have been prepared using a glassy carbon electrode (GCE) instead of HOPG as the conducting transducer substrate for DNA adsorption (see Procedure 29 in CD accompanying this book).

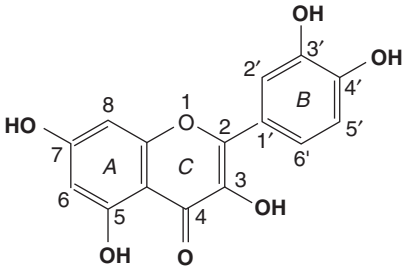
Differential pulse (DP) voltammetry, a voltammetric technique with high sensitivity, is normally performed and the equipment as well as the electrochemical procedures used for the voltammetric studies of DNA–drug interaction are described (see Procedure 29 in CD accompanying this book).

20.4 DNA DAMAGE PRODUCED BY REACTIVE OXYGEN SPECIES (ROS)

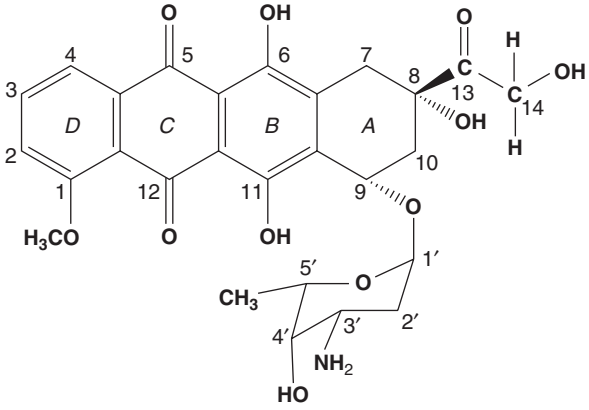
Free radicals are produced in living cells by normal metabolism and by exogenic sources such as carcinogenic compounds and ionizing radiation. Several drugs, such as nitroimidazoles, show biological activity after *in vivo* reduction to produce free radicals, hydroxylamine or nitroso derivatives [38], which react with biological molecules in the cell, including DNA. The result of these interactions usually involves DNA damage giving rise to the so-called oxidative stress, which is the main cause of mutagenesis, carcinogenesis and ageing [39].

By analogy, the DNA immobilization on a conducting solid support provides an interface that models the processes occurring in the living cell where DNA interacts with charged surfaces. Therefore, by controlling (conditioning) the electrode/biosensor potential, oxidation or reduction of different compounds previously linked to DNA can occur. These redox reactions cause the *in situ* formation of reactive intermediates, such as free radicals, and their action on DNA is detected by electrical measurable signals in a voltammogram using the DNA-electrochemical biosensor.

Next, some typical examples will be presented of how a DNA-electrochemical biosensor is appropriate to investigate the DNA damage caused by different types of substances, such as the antioxidant agent quercetin (Scheme 20.1), an anticancer drug adriamycin (Scheme 20.2) and nitric oxide. In all cases, the dsDNA damage is detected by changes in the electrochemical behaviour of the immobilized dsDNA, specifically through modifications of the purinic base oxidation peak current [3,5,40].



Scheme 20.1. Chemical structure of quercetin.



Scheme 20.2. Chemical structure of adriamycin.

20.4.1 Quercetin

Flavonoids, compounds found in rich abundance in all land plants, owing to their polyphenolic nature often exhibit strong antioxidant properties [41]. They are considered as potential chemopreventive agents against certain carcinogens since it was demonstrated that the intake of a large quantity of flavonoid inhibited the incidence of ROS producing damage to DNA. However, in contrast with this commonly accepted role, there is also evidence that flavonoids themselves are mutagenic and have DNA damaging ability [42,43].

Quercetin (Scheme 20.1) a major flavonoid in human diet, acts as a pro-oxidant [42–44] under certain circumstances. Although the mechanism of interaction of quercetin with DNA is not yet fully understood, there is considerable evidence that quercetin-induced DNA damage occurs via reaction with Cu(II). Quercetin can directly reduce transition metals, thus

providing all the elements necessary to generate the highly oxidizing hydroxyl radical (•OH). On the other hand, there is experimental support that the formation of quercetin radicals via auto-oxidation of the catechol ring leads to the generation of superoxide radicals. Therefore, it was proposed that quercetin can promote oxidative damage to DNA through the generation of ROS.

DNA–Cu(II)–quercetin interactions can be followed electrochemically using a DNA-electrochemical biosensor [29,35]. This knowledge about the electrochemical behaviour of the dsDNA incubated with quercetin–Cu(II) complexes at GC electrode [45] is an important feature to understand quercetin–DNA interactions at a DNA-electrochemical biosensor. The preparation of the solutions and the quercetin–Cu(II) complex used during the characterization of *in situ* electrochemical DNA damage promoted by the quercetin–Cu(II) complex using a DNA biosensor is described (see Procedure 29 in CD accompanying this book).

20.4.1.1 Quercetin–dsDNA interaction at thick DNA biosensors

In Fig. 20.2 are shown DP voltammograms obtained with a thick-layer DNA biosensor previously immersed into a quercetin–$CuSO_4$ solution for 30 min and for 6 h. During incubation of the DNA biosensor, the solution containing quercetin and Cu(II) was continuously stirred. For comparison, a DP voltammogram obtained with the dsDNA-modified GCE in acetate buffer is also presented in Fig. 20.2.

After 30 min of incubation of the thick-layer dsDNA biosensor in a solution of quercetin–Cu(II), a typical quercetin oxidation peak 1 is observed followed by a small peak at about +0.45 V. Increasing the incubation time to 6 h led to the total disappearance of quercetin peak 1 and the appearance of a larger peak at +0.45 V, and big changes occurred in the dsDNA layer. Two new anodic peaks that could be identified with oxidation of the deoxyguanosine (dGuo) and deoxyadenosine (dAdo) residues appeared and their currents increased with incubation time. This can be explained, since quercetin interacts with DNA especially at pyrimidinic residues oxidizing them. The thymine and cytosine oxidation products will not be able to form hydrogen bonds with adenine and guanine residues, which now become more accessible to the electrode surface leading to an increase in their oxidation peak currents.

Using the thick-layer dsDNA-modified GCE, long periods of incubation with quercetin–Cu(II) complex are necessary for the detection of dsDNA interaction. The necessity of a faster response led to the construction of different types of biosensors such as the thin-layer dsDNA biosensors obtained by electrostatic adsorption of dsDNA strands at the

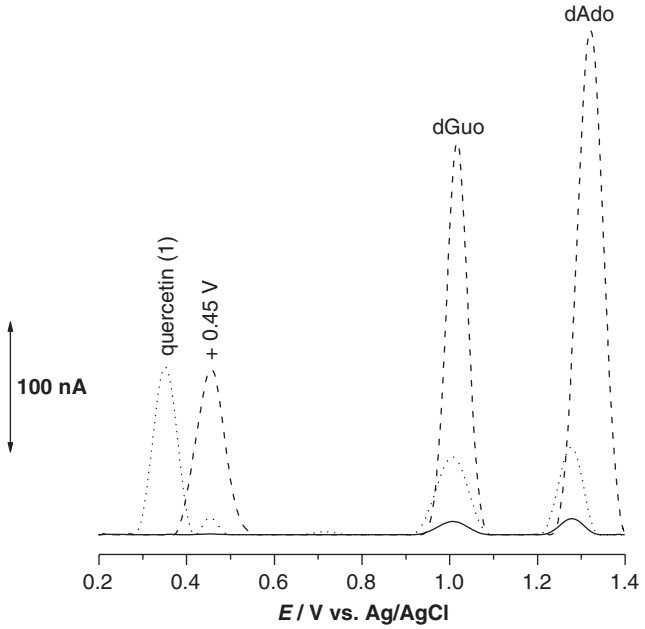


Fig. 20.2. DP voltammograms obtained in pH 4.3 0.1 M acetate buffer with a thick-layer dsDNA-modified GCE after (▬) 0 min, (▪▪▪) 30 min and (▪ ▪ ▪) 6 h incubation in a mixture of 100 μM quercetin with 50 μM $CuSO_4$. With permission from Ref. [35].

GCE surface. Such kinds of devices have been shown to be inappropriate since they do not ensure a complete coverage of the GCE surface allowing the non-specific adsorption of the compound. However, a new type of biosensor-multi-layer dsDNA-electrochemical biosensor obtained by successive additions of small quantities of dsDNA on the GCE surface has been developed (see Procedure 29 in CD accompanying this book) and further used to study the interaction between dsDNA and the quercetin–Cu(II) complex.

20.4.1.2 Quercetin–dsDNA interaction at a multi-layer DNA biosensor

To study the interaction between dsDNA and the quercetin–Cu(II) ion complex, a newly prepared multi-layer dsDNA biosensor was kept for 10 min in a mixture of quercetin and Cu(II) ions. The electrode was transferred to supporting electrolyte and a DP voltammogram recorded. The quercetin oxidation peak 1 occurs followed by another peak at +0.45 V (Fig. 20.3). Comparing with the voltammogram obtained before incubation, the peaks due to dGuo and dAdo base oxidation are several

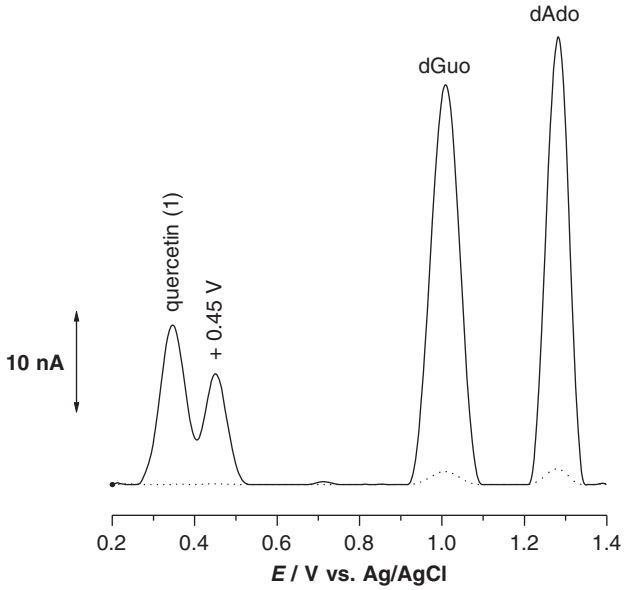


Fig. 20.3. DP voltammograms obtained in pH 4.3 0.1 M acetate buffer with a multi-layer dsDNA-modified GCE (▪▪▪▪) before and (▬) after 10 min of incubation in a mixture of 100 μM quercetin with 50 μM $CuSO_4$. With permission from Ref. [35].

times higher. This means that large modifications in the dsDNA have occurred after interaction with the quercetin–Cu(II) complex, and the presence of the peak at a potential of +0.45 V indicates the formation of a new product after DNA interaction with quercetin–Cu(II).

The same quercetin–DNA interaction occurs either at a thick or multi-layer dsDNA-electrochemical biosensor, but at the multi-layer DNA sensor it occurs more rapidly. Besides, the construction of a multi-layer dsDNA biosensor is faster than that of the thick-layer biosensor, it consumes less reagents, and so was further used to study the interaction between dsDNA and ROS produced by redox activation of quercetin.

20.4.1.3 The role of ROS in the dsDNA damage promoted by quercetin

Quercetin–Cu(II) complexes bind to dsDNA causing DNA oxidative damage. Several studies have shown that the hydroxyl groups of the catechol ring of quercetin is important for the Cu(II) ions chelation [45–47] and this reaction produces ROS, which can attack the dsDNA, disrupting the helix and leading to the formation of 8-oxodeoxyguanosine (8-oxodGuo). It is known that the electrochemical oxidation of quercetin occurs first at the hydroxyl groups of the catechol ring and

the quercetin radicals formed react with molecular oxygen thus producing ROS. Thus, it can be proposed that the oxidation of quercetin bonded to DNA could cause oxidative damage, and an electrochemical multi-layer biosensor was used to confirm the dsDNA damage produced by oxidation of quercetin.

A newly prepared biosensor was incubated for 10 min in a solution of quercetin; the electrode was washed with deionized water in order to remove the unbound quercetin molecules and transferred to electrolyte solution. The DP voltammogram recorded (Fig. 20.4) shows the quercetin peak 1 due to the oxidation of the hydroxyl groups of the catechol ring followed by small peaks of dGuo and dAdo residues confirming that quercetin interacts with dsDNA and even after interaction quercetin can still undergo oxidation.

The incubation procedure was repeated using a new biosensor and the electrode was transferred to pure buffer solution where a potential of +0.40 V was applied for 5 min. During this conditioning period, the quercetin molecules bound to DNA are oxidized leading to formation of ROS. The radical damages dsDNA, detected by the occurrence of high oxidation peaks of dGuo and dAdo residues (Fig. 20.4). Moreover, the DP voltammogram obtained in these conditions shows a peak at +0.45 V, confirming the formation of a new product.

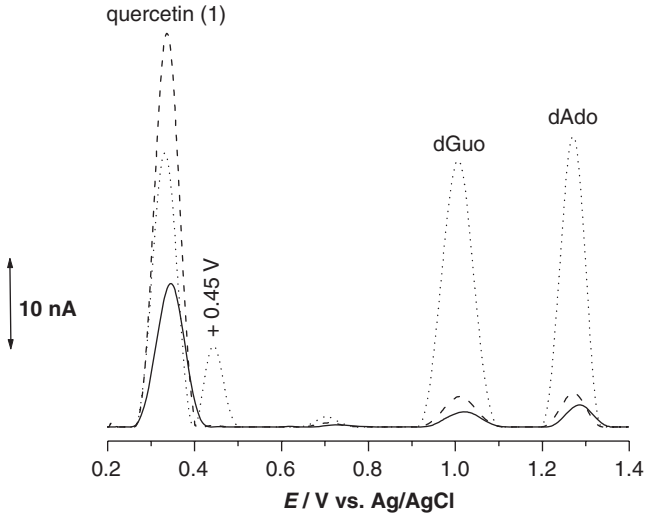


Fig. 20.4. DP voltammograms in pH 4.3 0.1 M acetate buffer obtained with a multi-layer dsDNA biosensor incubated for 10 min in 100 μM quercetin (▪▪▪) before and after applying +0.40 V for 300 s (▬) with and (▪▪▪▪) without bubbling N_2 in the solution. With permission from Ref. [35].

In order to obtain information about the origin of the peak at +0.45 V, the GCE surface was modified with DNA-like sequences (polyguanylic and polyadenylic acids) that contain or not guanine residues [35]. These new types of biosensors were incubated in a quercetin solution and then conditioned (see Procedure 29 in CD accompanying this book). In this way, it was shown that the peak at +0.45 V is directly related with the presence of guanine residues in the polynucleotidic chain and that it is due to the formation of 8-oxodGuo.

To prove the involvement of ROS in the process of DNA damage during quercetin oxidation, another experiment was carried out after bubbling nitrogen into the buffer electrolyte solution. After O_2 removal from the solution, the quercetin radicals formed during the oxidation of quercetin could not react with oxygen and no ROS were formed to damage the DNA film. The DP voltammogram obtained (Fig. 20.4) showed only a small oxidation peak of dGuo and dAdo proving that no DNA damage had occurred and no additional peak, at +0.45 V due to 8-oxodGuo, was observed although quercetin peak 1 occurred with a smaller current due to oxidation of quercetin during the conditioning procedure. It was demonstrated that the presence of oxygen is fundamental for extensive DNA damage, promoted by the highly reactive oxygen radicals. The interpretation of the results obtained enabled the understanding of the quercetin–dsDNA interaction mechanism [35].

20.4.2 Adriamycin

Adriamycin is an antibiotic of the family of anthracyclines with a wide spectrum of chemotherapeutic applications and anti-neoplasic action but it causes cardiotoxicity ranging from delayed and insidious cardiomyopathy to irreversible heart failure [48–52].

There was experimental evidence that adriamycin can promote oxidative damage to DNA in cancerous cells through the generation of ROS [50–52] and high levels of 8-oxoguanine (8-oxoGua), a known biomarker of oxidative stress, were detected in *in vitro* studies [53]. The generation of this main product of guanine oxidation within DNA is strongly mutagenic and can contribute to cell dysfunction [54]. There was ample evidence that adriamycin interacts with DNA through intercalation [55] but less was known as to whether it could directly oxidize DNA after intercalation has occurred.

Electrochemical-dsDNA biosensors were used to detect *in situ* adriamycin DNA oxidative damage [37]. The experimental conditions used during the investigation of *in situ* electrochemical DNA damage caused

by adriamycin are described in Procedure 29 (see in CD accompanying this book).

20.4.2.1 Adriamycin–DNA interaction at a thick-dsDNA electrochemical biosensor

The oxidation of adriamycin at the thick-layer DNA-electrochemical biosensor was investigated and the effect of the immersion time of the thick-layer DNA biosensor in 1 μM adriamycin solution (Fig. 20.5) was compared with the results obtained with a bare GCE [37,56,57]. Using the thick-layer DNA-electrochemical biosensor it was possible to preconcentrate adriamycin on the thick layer of DNA and the adriamycin oxidation peak current was found to increase with immersion time and to reach saturation after 1 h of immersion (see insert, Fig. 20.5A).

However, if a potential of −0.60 V was applied to the DNA biosensor during 120 s, big changes occurred inside the DNA layer (Fig. 20.6). Two new oxidation peaks appeared, which can be identified [2]: the first at +0.80 V, as guanine oxidation, and the second at +1.05 V, as adenine oxidation. Nevertheless, the oxidation peak potentials for guanosine and adenine are very close, making their identification difficult.

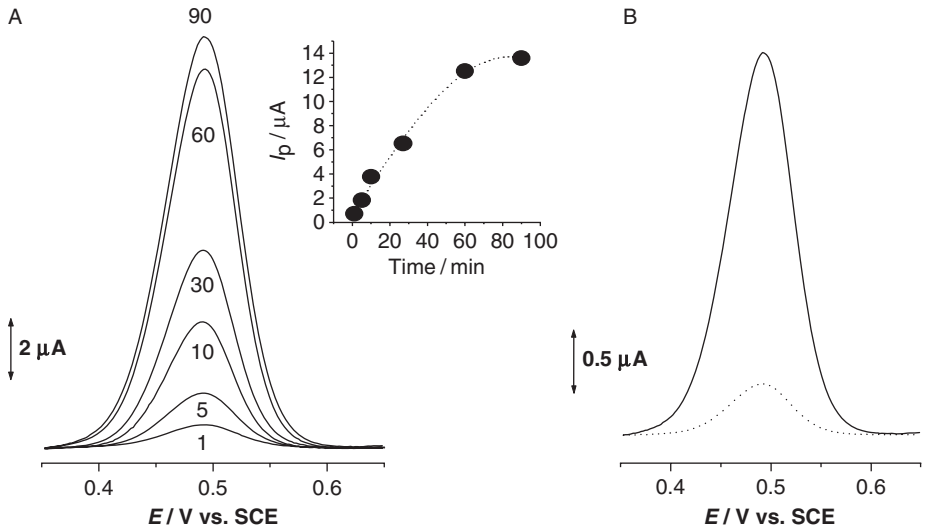


Fig. 20.5. Background-subtracted DP voltammograms of 1 μM adriamycin in 0.1 M pH 4.5 acetate buffer obtained with a thick-layer dsDNA-electrochemical biosensor: (A) Effect of immersion time, insert I_p vs. t; (B) Current decrease in successive differential pulse voltammograms: (▬) First voltammogram after 5 min immersion and (▪▪▪▪) Fifth voltammogram. With permission from Ref. [37].

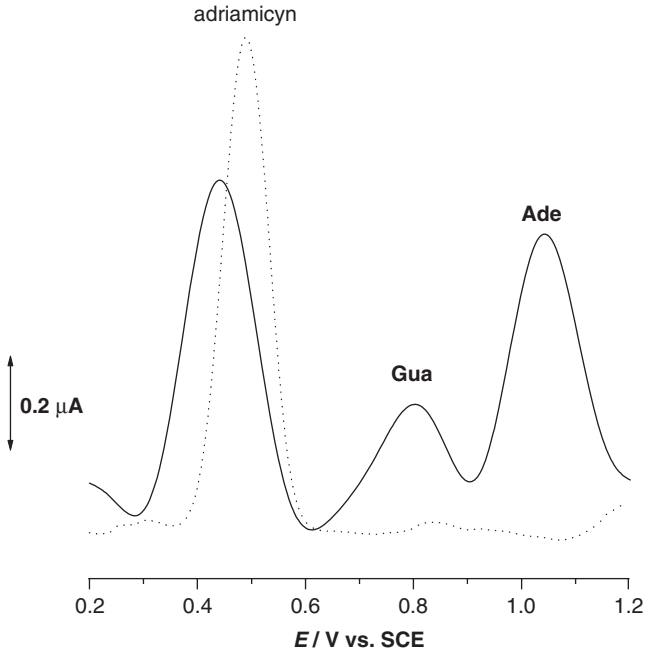


Fig. 20.6. Background-subtracted DP voltammograms in pH 4.5 0.1 M acetate buffer obtained with a thick-layer dsDNA-electrochemical biosensor GCE after being immersed during 10 min in a 1 μM adriamycin solution and rinsed with water before the experiment in buffer: (▪▪▪▪) without applied potential and (▬) subsequent scan after applying a potential of –0.6 V during 120 s. With permission from Ref. [37].

20.4.2.2 Adriamycin–dsDNA interaction at a thin-dsDNA electrochemical biosensor

The thin-layer DNA biosensor was immersed during 3 min in an adriamycin solution, rinsed with water, and later transferred to buffer, where a DP voltammogram was recorded. The peak for adriamycin oxidation occurs at +0.50 V, and only after applying the potential of −0.60 V during 60 s, the oxidation peak for guanine, at +0.84 V, and the oxidation peak for 8-oxoGua, at +0.38 V (Fig. 20.7) appeared.

These results are in agreement with those obtained with the thick-layer dsDNA-electrochemical biosensor. The clear separation of the adriamycin and 8-oxoGua peaks can be explained by the non-uniform coverage of the electrode surface by DNA and the adsorption of adriamycin [37] on the uncovered glassy carbon. This peak separation is

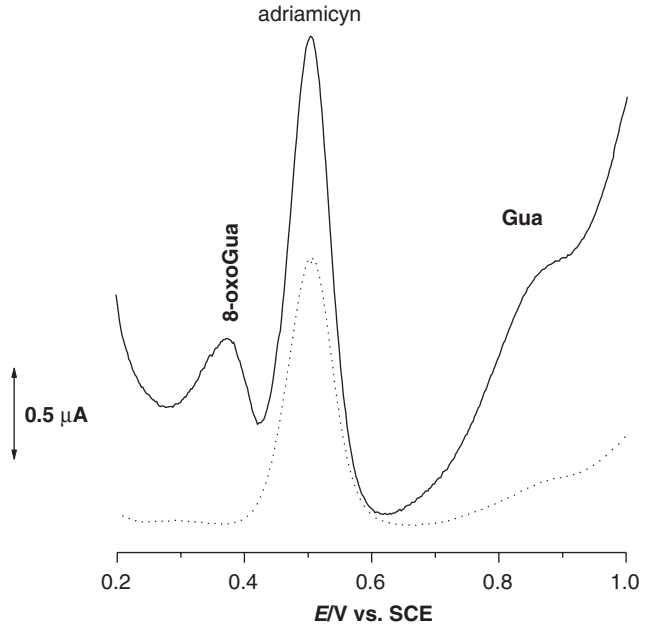


Fig. 20.7. DP voltammograms in pH 4.5 0.1 M acetate buffer obtained with a thin-layer dsDNA-electrochemical biosensor after being immersed in a 5 μM adriamycin solution during 3 min and rinsed with water before the experiment in buffer: (▪▪▪▪) without applied potential; (▬) applying a potential of −0.6 V during 60 s. First scans with permission from Ref. [37].

very important as it enables the use of the less positive 8-oxoGua oxidation peak to detect adriamycin damage to dsDNA.

Adriamycin intercalates within double helix DNA and can undergo a reaction beginning with the transfer of a single electron to the quinone portion of the adriamycin ring system, generating a free radical [57], which can interact with DNA *in situ* with the products of this interaction being retained in the DNA layer.

Adriamycin electroactive functional groups (the oxidizable hydroquinone group in ring B, and the reducible quinone function in ring C) are intercalated between the base pairs in the dsDNA [37]. The reducible quinone group in ring C protrudes slightly into the major groove, and this enables *in situ* (*in helix*) generation of an adriamycin radical within the double helix [58]. Therefore, a redox reaction between adriamycin and guanine residues inside the double helix of DNA can occur and 8-oxoGua is the main product of guanine oxidation. The mechanistic pathway involving two electrons and two protons [14] depends on the chemical environment surrounding guanine.

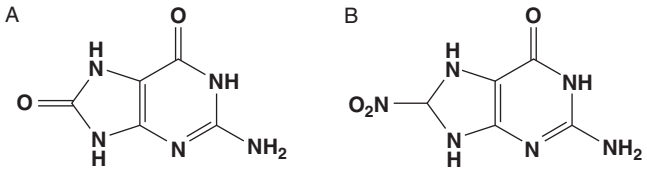
20.4.3 Nitric oxide

Nitric oxide (NO), the nitrogen monoxide, is a physiologically active molecule regulating numerous biological processes including vasodilatation, neurotransmission and cell-dependent immunity [59,60]. However, it was shown that in certain conditions (infected or inflamed tissues), large amounts of NO produce genotoxic effects that have been associated with carcinogenesis.

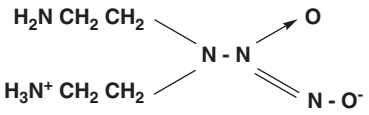
The whole spectra of DNA damage by NO and its derivatives include nitrosative deamination of DNA bases causing transition mutations during DNA replication, strand breakage and both oxidation and nitration of the bases [61]. For example, it was demonstrated that peroxynitrite ($ONOO^-$) formed during the interaction of NO and superoxide radicals ($O_2^{-\bullet}$) causes DNA cleavage at every nucleotide with a slight predominance for guanine sites and high amounts of 8-oxoGua (Scheme 20.3A) were measured [62]. There are conflicting reports concerning the formation of 8-oxoGua, this compound being a target for further oxidation by $ONOO^-$ giving rise to oxaluric acid; and other products could be generated by $ONOO^-$-mediated guanine modification. After interaction of $ONOO^-$ with guanine residues, 8-nitroguanine (8-nitroGua) (Scheme 20.3B) and 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2′-deoxyguanosine were also separated and characterized [63,64].

Due to the importance of nitric oxide in regulating various cell functions, an electrochemical study of the NO–DNA interaction has been undertaken (see Procedure 29 in CD accompanying this book).

The source of nitric oxide was diethylenetriamine/nitric oxide, DETA/NO (Scheme 20.4) a compound that has been used in studies of the cytostatic, vasodilatory and other pharmacological properties of NO [65–67]. DETA/NO is a 1-substituted diazen-1-ium-1,2-diolate containing the $[N(O)NO]^-$ functional group that has been proved to be useful for the reliable generation of nitric oxide in homogenous solutions [68]. When dissolved in blood, cell culture medium or buffer this compound dissociates to generate NO leaving the nucleophilic structure as a by-product.



Scheme 20.3. Chemical structures of (A) 8-oxoguanine and (B) 8-nitroguanine.



Scheme 20.4. Chemical structure of DETA/NO.

The electrochemical behaviour of DETA/NO was studied using a GCE [40]. One main oxidation peak at $E_{pa} = +0.80\,V$ independent of pH was observed. This potential corresponds to the oxidation of NO. The peak current is constant with time, meaning that the NO is dissolved in the solution after being liberated from the nucleophilic DETA/NO [69].

20.4.3.1 NO–DNA interaction at a thick-layer dsDNA biosensor

The DP voltammogram obtained in supporting electrolyte with the thick-layer dsDNA biosensor after incubation for 5 min in a solution of 100 μM DETA/NO (Fig. 20.8) shows the anodic peak specific to NO oxidation ($E_{pa} = +0.80\,V$) followed by the two small peaks of dGuo and dAdo, comparable with those obtained with the biosensor before incubation, meaning that the NO radicals themselves do not damage DNA.

After incubation in DETA/NO, a newly prepared biosensor was transferred to buffer and a potential of $-0.60\,V$ was applied during 3 min, causing the electrochemical reduction of oxygen and generation of $O_2^{-\bullet}$. The superoxide reacts with NO pre-concentrated into the thick DNA film to form peroxynitrite ($ONOO^-$), a highly reactive species that can cause oxidative damage to DNA [70]. The DP voltammogram (Fig. 20.8) shows increase of dGuo and dAdo peaks due to the cleavage of the dsDNA helix. The NO oxidation peak still appears but with a smaller current because of having been consumed, due to its reaction with superoxide radicals.

In order to produce the peroxynitrite radicals that damage DNA, the thick-layer biosensor has to be first incubated in the solution of DETA/NO and then conditioned at $-0.60\,V$ such that $O_2^{-\bullet}$ reacts with NO pre-concentrated into the thick DNA film. On the contrary, the production of peroxynitrite at the multi-layer dsDNA biosensor is carried out in a single step.

20.4.3.2 NO–DNA interaction at a multi-layer dsDNA biosensor

A multi-layer dsDNA biosensor was incubated in DETA/NO at $-0.60\,V$, and the $O_2^{-\bullet}$ radicals produced at the electrode surface reacted with the NO molecules that diffuse from solution towards the biosensor surface, giving rise to $ONOO^-$ that damage DNA. The DP voltammogram

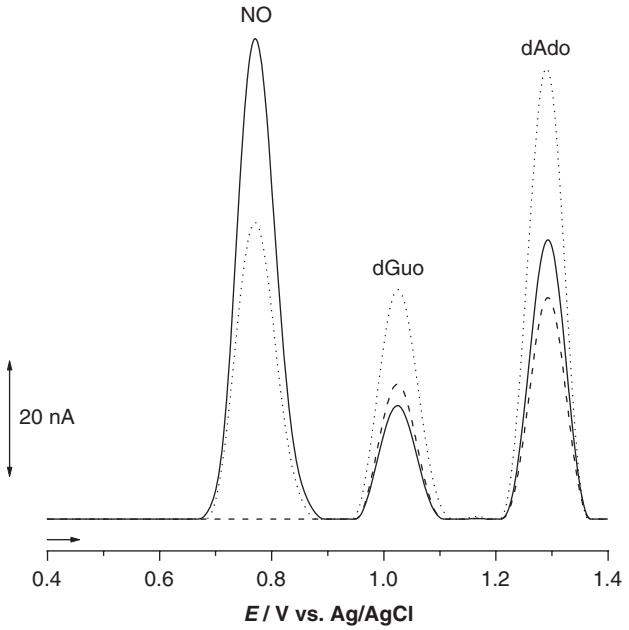


Fig. 20.8. DP voltammograms obtained in pH 4.5 0.1 M acetate buffer with a thick-layer DNA biosensor (▪▪▪▪) before and after incubation during 5 min in 100 μM DETA/NO (▬) without or (▪▪▪▪) with application of −0.60 V during 3 min. With permission from Ref. [40].

obtained after the transfer of the biosensor into buffer electrolyte (Fig. 20.9) shows the variation with time of the cleavage of DNA recognizable by the higher oxidation peaks of dGuo and dAdo.

For higher DETA/NO concentrations, the DP voltammogram showed two large oxidation peaks for dGuo and dAdo plus a third peak at a lower potential, $E_{pa} = +0.77$ V (Fig. 20.10). This peak is a consequence of the interaction between peroxynitrite radicals and DNA, corresponding to guanine (Gua) oxidation.

This result is in agreement with the fact that peroxynitrite induces DNA cleavage predominantly at the 5′-G of GG and GGG sequences [62]. Hence, the 5′ terminal guanine residues will be in direct contact with the electrode surface.

For longer incubation periods, 3 min at −0.60 V in concentrated DETA/NO, the dAdo peak current remained unchanged, and the dGuo peak became smaller whereas the Gua oxidation current increases (Fig. 20.10). This experiment showed that for longer incubation times, more $ONOO^-$ is available to damage DNA and, as a consequence, more guanine residues are accessible to the electrode surface.

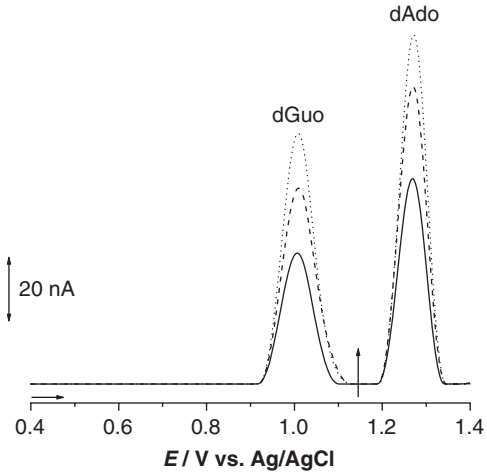


Fig. 20.9. DP voltammograms obtained in pH 4.5 0.1 M acetate buffer with a thin multi-layer DNA biosensor (▬) before and after incubation in 100 μM DETA/NO at −0.60 V during (▪▪▪▪) 3 or (▪▪▪▪▪) 5 min. With permission from Ref. [40].

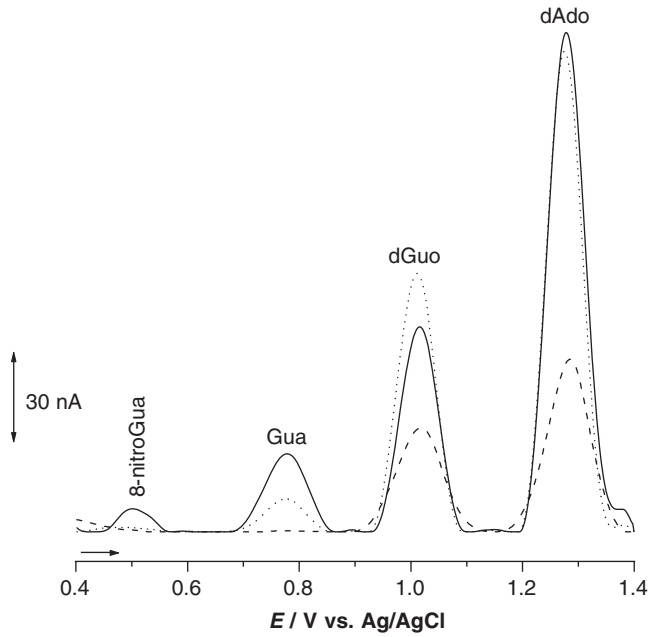


Fig. 20.10. DP voltammograms obtained in pH 4.5 0.1 M acetate buffer with a thin multi-layer dsDNA biosensor previously incubated in 1.5 mM DETA: (▪▪▪▪) during 3 min without applying any potential or during (▪▪▪▪▪) 2 and (▬) 3 min applying −0.60 V. With permission from Ref. [40].

Also, a new peak is observed at $E_{pa} = +0.49\,V$ (Fig. 20.10). This new peak is due to oxidative damage to immobilized DNA after interaction with peroxynitrite. Since guanine is the most easily oxidized base in DNA, it is expected that $ONOO^-$ readily interacts with guanine. Although 8-oxoGua is the main guanine oxidation product, literature reports 8-nitroGua as the principal oxidative damage to DNA treated with peroxynitrite. 8-nitroGua has a structure very similar to 8-oxoGua (Scheme 20.3) and it is expected that their oxidation potentials are very close to each other. Both products are highly mutagenic and can contribute to cell dysfunction.

20.5 CONCLUSION

The development of the electrochemical DNA biosensors has opened a wide perspective using particularly sensitive and selective methods for the detection of specific DNA damaging interactions. Electrochemical voltammetric *in situ* sensing of DNA oxidative damage caused by different compounds with or without therapeutic activity is possible using different types (thin, multi and thick layer) of electrochemical dsDNA biosensors. The choice of the best approach to be used depends on the drug and the time necessary to cause DNA damage. However, previous knowledge of the electrochemical DNA biosensor surface morphology and of the electrochemical behaviour of the drug at a bare electrode is most important to avoid possible misinterpretations.

The understanding of DNA interaction with molecules or ions using voltammetric techniques for *in situ* generation of reactive intermediates is a complementary tool for the study of these biomolecular interaction mechanisms. The electrochemical DNA biosensor enables the preconcentration of compounds on the biosensor surface. Controlling the potential, the *in situ* electrochemical generation of radical intermediates is possible. Monitoring the changes of dGuo and dAdo oxidation peak currents or the appearance of new redox peaks it is possible to conclude about the damaging and potential toxic effect of different compounds.

The understanding of the mechanism of action of health hazard compounds that interact with DNA can aid in explaining the differences in reactivity between similar moieties. This knowledge can and should be used as an important parameter for quantitative structure–activity relationships (QSAR) and/or molecular modelling studies, as a contribution to the design of new structure-specific DNA-binding drugs, and for the possibility of pre-screening the damage they may cause to DNA integrity.

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