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Electrochemical study of quercetin–DNA interactions Part II. In situ sensing with DNA biosensors

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Abstract

Quercetin interaction with dsDNA was investigated electrochemically using two types of DNA biosensor in order to evaluate the occurrence of DNA damage caused by oxidized quercetin. The results showed that quercetin binds to dsDNA where it can undergo oxidation. The radicals formed during quercetin oxidation cause breaks of the hydrogen bonds in the dsDNA finally giving rise to 8-oxoguanine since the DNA guanosine and adenosine nucleotides in contact with the electrode surface can easily be oxidized. A mechanism for oxidized quercetin-induced damage to dsDNA immobilized onto a glassy carbon electrode surface is proposed and the formation of 8-oxoguanine is explained. The importance of DNA-electrochemical biosensors in the determination of the interaction mechanism between DNA and quercetin is clearly demonstrated.

Keywords: DNA-electrochemical biosensors; Quercetin; Oxidation; Electrochemistry; Radical scavenging activity; Flavonoids

1. Introduction

There is much evidence that reactive oxygen species (ROS) produced in vivo react with cellular DNA, modifying it, leading to changes in the genetic information that induce mutagenesis and carcinogenesis. Therefore, attention has been paid into discovering free radical scavengers and antioxidants that inhibit such DNA lesions. Owing to their polyphenolic nature, flavonoids often exhibit strong antioxidant properties [1], but in contrast with this commonly accepted role there is considerable evidence that flavonoids are mutagenic and have DNA damaging ability [1,2].

One striking example reported in the literature is that of quercetin which, under certain circumstances, acts as a prooxidant and has mutagenic activity [3,4] and intercalates into the double-stranded DNA [5–12], predominantly at thymine and cytosine sites [5,10], even if binding occurs also for single-stranded DNA [11,12]. Although the mechanism of interaction of quercetin with DNA is still unknown, it is generally accepted that quercetin induces DNA strand scission [2,11,12]. It is proposed that quercetin can directly reduce transition metals, thus providing all the elements necessary to generate the highly oxidizing radical OH. Also, there is experimental support that the formation of quercetin radicals via auto-oxidation leads to the generation of superoxide radicals. Therefore, quercetin can promote oxidative damage to DNA through the generation of these highly reactive oxygen species [5,13,14]. High levels of 8-oxoguanine, a known biomarker of oxidative stress, were also detected in these studies [5]. The generation of this guanine oxidation product within the DNA double helix is strongly mutagenic and can contribute to cell dysfunction [15,16].

This detrimental effect should be carefully studied to use flavonoids for reducing cancer risk in humans. Although the fact that quercetin and its metal ion complex exhibit different antitumor activities is known for a long time, its mode of in vivo action is not yet fully understood. Efforts towards establishing its mechanism of action are still an important goal to explain its antioxidant activity.

It is therefore important to develop analytical methods and electrochemical transducer devices capable of fast and sensitive detection of both DNA damage and DNA damaging agents. An electrochemical sensor for DNA damage consists of an electrode with DNA immobilized on its

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surface. The strategy is based on dsDNA immobilization at a GCE surface, the new interface providing a model for the processes occurring in the living cell where DNA interacts with charged surfaces. Interactions of the surface-confined DNA with a DNA damaging agent are converted, via changes in electrochemical properties of the DNA recognition layer, into measurable electrical signals [17]. The interaction of several substances with dsDNA has been successfully studied using such kind of biosensor and the interpretation of results contributed to the elucidation of the mechanisms by which DNA is damaged by hazardous compounds [17–20].

In this context, the aim of the present paper is concerned with the electrochemical study of the in situ interaction of quercetin with dsDNA at a glassy carbon modified electrode. Several situations were studied using a dsDNA-modified glassy carbon electrode, i.e. a DNA-electrochemical biosensor. The experimental results presented here will give strong evidence that the radicals formed during quercetin oxidation can damage DNA. A possible mechanism of DNA damage by oxidized quercetin radicals is proposed.

2. Experimental

2.1. Substances and stock solutions

Quercetin, sodium salt calf thymus single-stranded (ss)DNA, double-stranded (ds)DNA, polyadenilic acid (poly[A]) from Sigma and $CuSO_4$ obtained from Merck were used without further purification. Stock solutions of 500 μM saturated quercetin, 1 mM $CuSO_4$, 100 μg ml^{-1} ssDNA, dsDNA and poly[A], and 35 mg ml^{-1} gel dsDNA were prepared in pH 4.3 0.1 M acetate buffer electrolyte. All stock solutions were stored at −4 °C and solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤0.1 μS cm^{-1}). All experiments were done in pH 4.3 0.1 M acetate buffer at room temperature (25 ± 1 °C).

2.2. Apparatus, software's and data treatment

All voltammetric measurements were carried out using a PGSTAT 10 Autolab running a GPES software version 4.9, Echo-Chemie, Utrecht, Netherlands. A glassy carbon working electrode (GCE) (d = 1.5 mm), a platinum wire counter electrode and an Ag/AgCl (sat KCl) as reference were used in a 0.5-ml one-compartment electrochemical cell. During DNA–quercetin interaction, the solutions were continuously stirred. The experimental conditions for differential pulse voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}. All the voltammograms obtained were smoothed using Savitsky–Golay algorithm and base line corrected by the moving average method (peak width of 1 mV) with which the GPES software is equipped.

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pipettes (Rainin Instruments, Woburn, USA). The pH measurements were carried out with a GLP 21 Crison pH meter.

All the graphs are treated and presented in Microcal Origin Version 6.0.

2.3. Electrode modification

The thick layer dsDNA-modified electrodes were prepared by covering the glassy carbon surface with 5 μl of 35 mg ml^{-1} dsDNA dissolved in pH 4.3 0.1 M acetate buffer and allowed it to dry. Thin layer dsDNA, ssDNA and poly[A]-modified electrodes were prepared by successively covering the glassy carbon surface with three drops of 5 μl each containing 50 μg ml^{-1} dsDNA. After placing each drop on the surface, the biosensor was allowed to dry.

3. Results and discussion

The interaction between dsDNA and quercetin or quercetin–Cu(II) complex has been undertaken at a dsDNA-modified GCE, referred as a DNA biosensor. Two different types of biosensors have been prepared in order to study the interaction and observe modifications in the DNA film: a thick layer dsDNA-modified GCE that requires a longer time of preparation and a thin layer dsDNA-modified GCE obtained by successive addition of dsDNA solution. In both cases, a uniform coverage of the electrode surface has been achieved and, therefore, the new peaks that arise are due only to the quercetin intercalated into the DNA films without any contribution from the diffusion process from quercetin in solution.

3.1. Thick layer dsDNA-modified GCE

A thick layer of dsDNA (5 μl of 35 mg ml^{-1} dsDNA) was placed on top of a GCE surface, previously polished and electrochemically conditioned in supporting electrolyte, and allowed to dry [18]. Also, the electrochemical conditioning of the dsDNA-modified GCE was carried out in acetate buffer, which was essential to obtain reproducible results. This thick layer dsDNA-modified GCE was used to study electrochemically the changes in the dsDNA film caused by quercetin–Cu(II) complex ions.

A solution containing 100 μM quercetin and 50 μM $CuSO_4$ was stirred during 3 h. Afterwards, the thick layer dsDNA-modified GCE was placed in the solution for different time intervals and then transferred to acetate buffer. Before introducing into supporting electrolyte, the modified electrode was gently washed with deionized water to assure the removal of unbounded quercetin or quercetin–Cu(II) complexes. In this way, the observed differential pulse voltammetric peaks could only arise from quercetin complex incorporated into dsDNA thick film without any contribution

from diffusion, as the results were obtained in supporting electrolyte.

Fig. 1 shows the differential pulse voltammograms obtained with this DNA biosensor previously immersed into the quercetin–$CuSO_4$ solution for 30 min and for 6 h. During immersion of the electrode, the solution containing quercetin and Cu(II) was continuously stirred. For comparison, a differential pulse voltammogram obtained with the dsDNA-modified GCE in acetate buffer is also presented in Fig. 1.

After 30 min of dsDNA-modified GCE immersion, a typical quercetin oxidation peak 1 was 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. Also, it could be observed that big changes occurred inside the dsDNA layer. Two new anodic peaks that could be identified with oxidation of the guanosine and adenosine residues appeared and their currents increased with incubation time. Considering that quercetin interacts with DNA especially at pyrimidinic residues [5] oxidizing them, the thymine and cytosine oxidized products would not be able to form hydrogen bonds with adenine and guanine residues, respectively, which are now more accessible to the electrode surface leading to the increase on their oxidation peak currents.

Quercetin adsorbs irreversibly at the glassy carbon surface. When using the dsDNA-modified electrodes after removal of the DNA film, no oxidation peak 1 due to quercetin was observed. This confirms that the peaks observed are due to quercetin–Cu(II) complex ions, intercalated into the DNA thick film and which do not reach the electrode surface.

Using the thick layer dsDNA-modified GCE, long periods of incubation with quercetin–Cu(II) complex were necessary for the detection of dsDNA modification. As DNA damage occurring with time was observed, it suggests that quercetin–Cu(II) complex intercalates with DNA and slowly interacts with it causing some breaking of the hydrogen bonds.

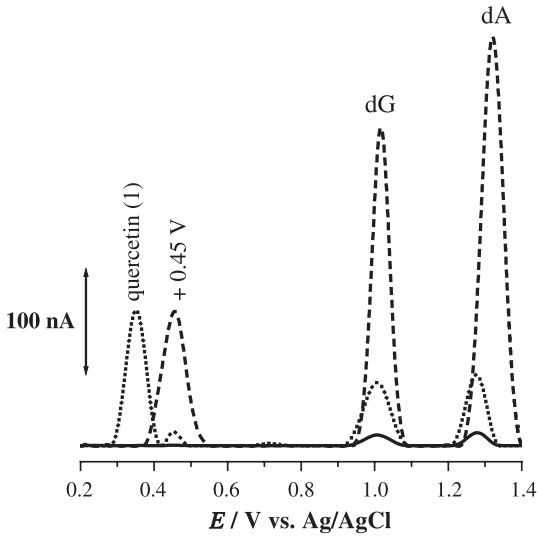


Fig. 1. Differential pulse voltammograms obtained in pH 4.3 0.1 M acetate buffer with a thick layer dsDNA-modified GCE after: (▬) 0 min, (• • •) 30 min and (- - -) 6 h incubation in a mixture of 100 μM quercetin with 50 μM $CuSO_4$. Scan rate 5 mV s^{-1}, pulse amplitude 50 mV, pulse width 0.07 s.

3.2. Thin layer dsDNA-modified GCE

A thin layer dsDNA-modified GCE was prepared by placing on a GCE surface previously polished and electrochemically conditioned in supporting electrolyte 5 μl of dsDNA 50 μg ml^{-1} and then the electrode was allowed to dry. The modified electrode was incubated for 10 min in solutions of quercetin. Following this, the DNA film was removed with a piece of adsorbent paper and the GCE surface was washed with a jet of deionized water. The electrode was place in supporting electrolyte, the differential pulse voltammogram recorded (not shown) and the quercetin oxidation peaks were obtained. Therefore, it was concluded that a non-compact thin layer of dsDNA is formed leaving the GCE with uncovered regions [21]. To avoid this and to assure a complete electrode surface coverage, the same modification procedure was repeated two more times. This thin layer dsDNA-modified GCE was rinsed with deionized water to wash the unbounded DNA strands and was conditioned in acetate buffer by scanning the potential between 0 and +1.4 V in order to obtain reproducible results. Finally, the DNA biosensor was kept for 10 min in either quercetin or quercetin–Cu(II) complex solutions. During this period of time, the solutions were under continuous stirring. After this period, the DNA film was removed, the electrode surface was washed with a jet of deionized water and the electrode placed in pure supporting electrolyte where a differential pulse voltammogram was recorded. No quercetin oxidation peak was obtained, so it was considered that complete coverage of the electrode surface had been achieved and this procedure was used in all the experiments described.

The changes occurring in the dsDNA during the interaction with quercetin or quercetin–Cu(II) complex ions were followed by differential pulse voltammetry, Fig. 2A. When the DNA biosensor was kept in quercetin and then transferred to acetate buffer, the oxidation peak 1 of quercetin occurs followed by the two small peaks due to guanosine and adenosine base oxidation, and this clearly proves that quercetin can undergo oxidation even after being intercalated into the DNA double strands. For comparison, a differential pulse voltammogram obtained with the dsDNA-modified GCE in acetate buffer is also presented in Fig. 2A.

The results obtained after dsDNA–quercetin–Cu(II) interaction are also in Fig. 2A and show different features. When the DNA biosensor was kept in the mixture of quercetin–Cu(II) ions and after transferred to supporting electrolyte, the quercetin peak 1 still occurs but with a smaller current followed by another peak at + 0.45 V. On the other hand, the peaks due to guanosine and adenosine bases are present and about 10 times higher than in the previous case. This clearly shows that greater modifications in the

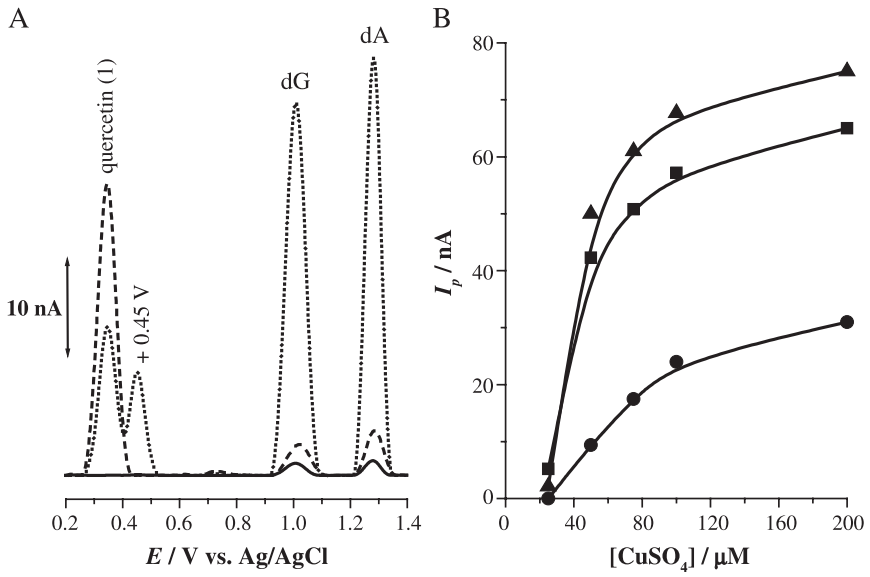


Fig. 2. (A) Differential pulse voltammograms in pH 4.3 0.1 M acetate buffer obtained with a thin layer dsDNA-modified GCE (▬) before and after being immersed for 10 min into (- - -) 100 μM quercetin or (• • •) a mixture of 100 μM quercetin with 50 μM $CuSO_4$. (B) Effect of concentration of $CuSO_4$ incubated with 100 μM quercetin on the currents of: (●) the oxidation peak at +0.45 V, (■) guanosine and (▲) adenosine oxidation peaks obtained at a thin layer dsDNA-modified GCE. Scan rate 5 mV s^{-1}, pulse amplitude 50 mV, pulse width 0.07 s.

dsDNA after interaction with quercetin–Cu(II) complex have occurred. The appearance of the peak at +0.45 V as well as guanosine and adenosine peaks has been monitored as function of Cu(II) ion concentration, Fig. 2B. All three peaks gradually increase with $CuSO_4$ concentration up to 100 μM when their currents reach constant values.

As mentioned, quercetin is auto-oxidizing in presence of Cu(II) ions, a process during which superoxide radicals are formed and are able to damage dsDNA [5,13]. Also, it has been demonstrated that quercetin alone interacts with dsDNA and quercetin can undergo oxidation after being intercalated into the dsDNA film. Thus, it is interesting to consider further the irreversible interaction of quercetin with dsDNA and the possibility of quercetin forming radicals during its oxidation process.

The thin layer dsDNA-modified GCE was kept for 10 min in a stirred quercetin solution. After this period, the DNA biosensor was rinsed with deionized water to ensure the removal of unbounded molecules and then immersed in acetate buffer where a potential of +0.400 V was applied for 300 s and a differential pulse voltammogram was recorded, Fig. 3A. During the conditioning time, the quercetin mole-

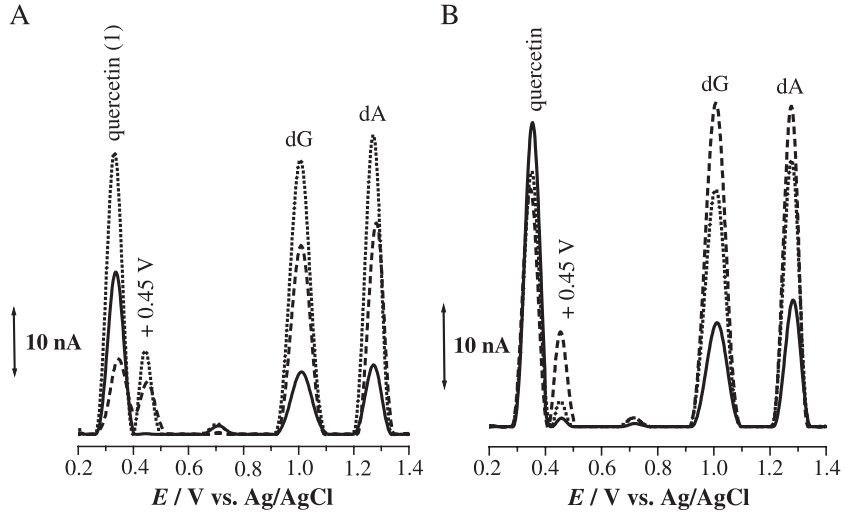


Fig. 3. Differential pulse voltammograms in pH 4.3 0.1 M acetate buffer obtained with a thin layer dsDNA-modified GCE incubated for 10 min in: (A) 100 μM quercetin after applying +0.400 V for 300 s (▬) with and (• • •) without bubbling N_2 in the solution, (- - -) a mixture of 100 μM quercetin with 50 μM $CuSO_4$. (B) 100 μM quercetin for 10 min and after applying a potential of +0.400 V for: (▬) 150 s, (• • •) 300 s and (- - -) 450 s. Scan rate 5 mV s^{-1}, pulse amplitude 50 mV, pulse width 0.07 s.

cules were oxidized, a process that leads to the formation of superoxide radicals. These radicals might damage dsDNA films and this is detected by the occurrence of the big peaks for guanosine and adenosine, respectively. On the other hand, the results obtained show still large oxidation peak 1 of quercetin and a small peak at +0.45 V.

In order to prove the involvement of superoxide radicals in the process of DNA damage during quercetin oxidation, the experiment described above was repeated and a potential of +0.400 V applied for 300 s whilst bubbling nitrogen in the previously N_2-saturated buffer electrolyte solution. In this way, the superoxide radicals formed during oxidation of quercetin react with nitrogen and should not damage the DNA film. Also, during the recording of the differential pulse voltammogram, a constant flux of nitrogen was made to flow over the electrolyte surface in the electrochemical cell. The differential pulse voltammogram obtained showed only a small oxidation peak of guanosine and adenosine proving that no DNA damage had occurred, Fig. 3A. Also, no additional peak, specifically at + 0.450 V, was observed although the quercetin oxidation peak 1 occurred with a small current.

Then, another thin layer dsDNA-modified GCE was kept for 10 min in a stirred quercetin–Cu(II) complex solution. After this period, the DNA biosensor was immersed into acetate buffer where differential pulse voltammogram was registered, Fig. 3A. The results obtained show a smaller oxidation peak 1 of quercetin followed by a peak at +0.45 V and bigger peaks could be observed for guanosine and adenosine oxidation. This means that the damage produced by intercalated quercetin at an applied potential of +0.40 V and the damage produced by quercetin–Cu(II) complex without applied potential are practically the same, Fig. 3A.

This experiment was repeated varying the time during which the potential of +0.400 V was applied, and always using a newly prepared DNA biosensor which was kept for 10 min in the quercetin solution. The differential pulse voltammograms obtained after applying +0.400 V during 150, 300 and 450 s are shown in Fig. 3B. The quercetin oxidation peak 1 shows a continuous decrease with time of applied potential probably due to quercetin consumption in its oxidation at + 0.400 V. On the other hand, the peak at + 0.450 V and the peaks corresponding to oxidation of guanosine and adenosine residues show a gradual increase with the potential application time, corresponding to the modification in the dsDNA structure that had occurred during the interaction with quercetin radicals.

At the end of each experiment, the dsDNA film was removed and the electrode was placed in acetate buffer where a differential pulse voltammogram was recorded. No oxidation peak of quercetin was observed, confirming that all the peaks are due to DNA-intercalated quercetin or quercetin–Cu(II) complex ions that do not reach the electrode surface.

Nevertheless, when using either a thick or a thin layer dsDNA-modified GCE, increased oxidation peak currents of quercetin compared with those when quercetin was adsorbed to the GCE surface were observed, Figs. 5–7, due to pre-concentration of quercetin molecules at the biosensor surface [18–20].

3.3. Control experiments

Control experiments were carried to check possible experimental errors that could lead to misinterpretation of the results. In order to clarify the importance of Cu(II) ions in the quercetin-induced dsDNA damages, the interaction between quercetin and $CuSO_4$ was briefly examined using both a bare GCE, a NAFION®-modified GCE, and a ssDNA or poly[A]-modified GCE.

Fig. 4 shows the differential pulse voltammograms obtained at a bare GCE. In the first experiment, the electrode was kept for 10 min in a solution of 100 μM quercetin incubated during 2 h with 50 μM $CuSO_4$ and then washed with water and transferred to acetate buffer where the differential pulse voltammogram was recorded. After this procedure, only one main peak at +0.35 V corresponding to quercetin oxidation peak 1 was observed. A binding reaction between quercetin and Cu(II) ions occurs [22,23] but it leads to the formation of a complex which does not adsorb at the GCE surface. Moreover, for the concentrations used in this study, there are still uncomplexed quercetin molecules that can be oxidized, which are responsible for peak 1.

In the second experiment, a clean electrode was kept for 10 min in a solution containing only 100 μM quercetin then the electrode was washed with deionized water and

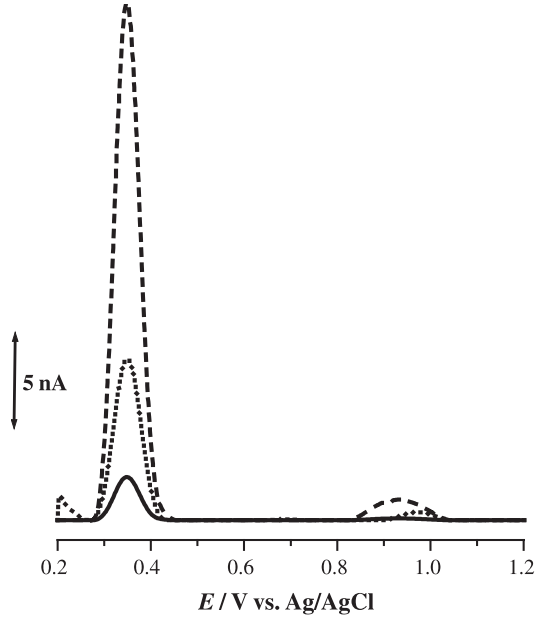


Fig. 4. Differential pulse voltammograms with GCE in pH 4.3 0.1 M acetate buffer after 10 min of adsorption in: (▬) quercetin–Cu(II) complex prepared from a solution of 100 μM quercetin incubated with 50 μM $CuSO_4$, (- - -) 100 μM quercetin solution and (• • •) 100 μM quercetin solution after applying +0.400 V for 450 s in acetate buffer.

transferred to acetate buffer. A differential pulse voltammogram recorded with quercetin adsorbed at the GCE surface was also shown in Fig. 4.

In a third experiment, after 10 min of adsorption in the same 100 μM quercetin solution, the electrode was washed with deionized water and transferred to acetate buffer and a potential of +0.400 V applied during 450 s. Both voltammograms obtained in acetate buffer with quercetin adsorbed at the electrode surface show the same feature that is the occurrence of quercetin oxidation peaks 1 and 4. No other additional signal at +0.45 V was observed.

In order to gain a better understanding of the dsDNA-modified GCE results obtained, the electrode surface was covered with a NAFION® film and the procedures described above repeated. All voltammograms showed the same features as at the bare GCE, the only difference being smaller peak currents. Since NAFION® covers the glassy carbon surface, a smaller electrode area is available for the oxidation reaction to occur and smaller peaks are expected.

The behaviour of only dsDNA in the presence of Cu(II) ions was also studied using a dsDNA-modified GCE. Thick or thin dsDNA-modified GCE were incubated for different periods of time in 50 μM $CuSO_4$ solutions and then transferred to acetate buffer where differential pulse voltammograms were recorded. No differences in the dsDNA oxidation behaviour were observed during these experiments.

It must be stressed that quercetin adsorbed at the GCE surface does not give any peak at +0.450 V in any of the cases studied, Fig. 4. Therefore, the peak at +0.450 V must be the product of oxidized quercetin or quercetin–Cu(II) complex interaction with DNA and the value of potential strongly suggests that it is due to the formation of 8-oxoguanine [24].

Differential pulse voltammograms, Fig. 5A were also recorded at the ssDNA-modified GCE after incubation for 10 min in quercetin solution, showing quercetin oxidation peak 1 followed by big guanosine and adenosine oxidation peaks. A new ssDNA biosensor was prepared and the incubation with quercetin was followed by the application of +0.400 V during 300 s. As in the case of dsDNA biosensor, a decrease of quercetin peak 1 occurs and a new peak at +0.450 V appeared. Also, a small increase of guanosine and adenosine currents is observed due to degradation of ssDNA after interaction with superoxide radicals produced during quercetin oxidation.

Differential pulse voltammograms, Fig. 5B recorded at the poly[A]-modified GCE after incubation for 10 min in quercetin solution showed quercetin oxidation peak 1 followed by the adenosine oxidation peak. After incubation, a potential of +0.400 V was applied during 300 s, a decrease of peak 1 being observed, due to consumption of quercetin molecules. No influence of the applied potential upon the adenosine peak current was observed. Also, no additional peak at +0.450 V occurs although the height of quercetin peak 1 decreased after application of +0.400 V. Nevertheless, smaller quercetin peaks were obtained when the GCE surface was modified with polyadenilic acid.

The behaviour of dsDNA only in the presence of Cu(II) ions was also studied using a dsDNA-modified GCE. Thick or thin dsDNA-modified GCEs were incubated for different periods of time in 50 μM $CuSO_4$ solutions and then transferred to acetate buffer where differential pulse voltammograms were recorded. No differences in the dsDNA oxidation behavior were observed during these experiments.

3.4. Interaction mechanism between dsDNA and oxidized quercetin

The results presented here give experimental evidence that the radical formed by oxidation of the catechol groups

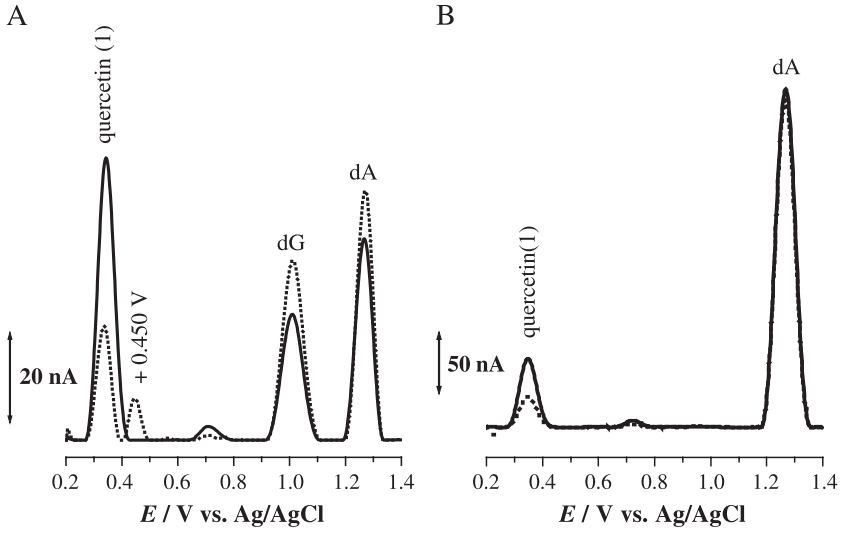
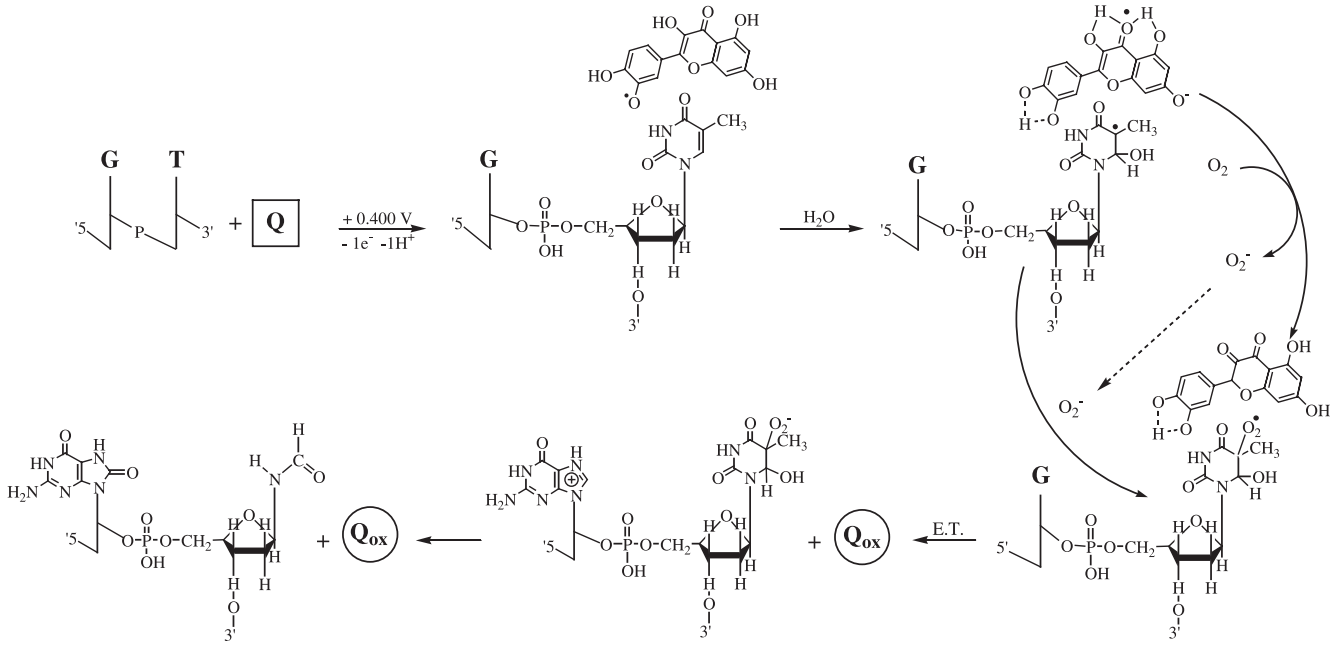


Fig. 5. Differential pulse voltammograms in pH 4.3 0.1M acetate buffer obtained at a thin layer: (A) ssDNA-modified GCE and (B) poly[A]-modified GCE, incubated for 10 min in 100 μM quercetin: (▬) before and (• • •) after applying +0.400 V for 300 s.



Scheme 1. Proposed interaction mechanism between quercetin and DNA.

of quercetin interacts with dsDNA and causes oxidative damage to dsDNA breaking hydrogen bonds between complementary strands and leading to the detection of guanosine, adenosine and 8-oxoguanine. Electrochemical in situ generation of quercetin radicals by applying a potential of +0.400 V enables the study of this interaction mechanism. Therefore, a redox reaction between the quercetin radical and thymine or cytosine residues could be considered in order to explain the experimental results.

Scheme 1 is a proposed mechanism of dsDNA damage induced by quercetin radicals. Quercetin molecules intercalate [5–12] to dsDNA and this process has, most probably, a hydrophobic nature. It has been demonstrated that quercetin molecules can undergo oxidation even after binding to dsDNA. Therefore, when a potential of +0.400 V is applied, quercetin is oxidized leading to formation of the quinone radical. Simultaneously, a redox process between oxidized quercetin and a pyrimidine molecule [5] occurs within the dsDNA. In this way, an electron transfer from a thymine or cytosine moiety to the quinone without hydrogen abstraction is likely to be the predominant reaction. Fast hydrolysis leads to oxidation of thymine and the quinone undergoes further rearrangements and oxidation processes during which superoxide radicals are formed [5,13]. The addition of the O_2 radical to the oxidized thymine residues leads to the formation of peroxyl radicals. It is proposed that the electron needed to reduce the peroxyl radical comes from a neighbouring guanine. Lesions generated by a single free radical initiating event have been studied using DNA oligomers containing different numbers of bases and then the mechanism of formation often involves the participation of guanine [25]. The π-stacked base pairs characteristic to dsDNA might be a determinant factor and can serve as a pathway reaction for charge transport between oxidized pyrimidinic base and guanine residues. The hydroxylic attack on thymine leads finally to the formation of a formamide remnant that would not form hydrogen bridges with adenine and could disrupt the double helix enabling guanine to be oxidized to 8-oxoguanine. Therefore, contact between DNA bases and electrode surface could easily be achieved.

4. Conclusions

This work has shown experimental evidence of interaction of radicals formed in oxidation of quercetin with dsDNA and may contribute to the understanding of the mechanism of action of this flavonoid. It was observed that quercetin–Cu(II) complex intercalates with dsDNA and slowly interacts with it, causing breaks of the hydrogen bonds. Electrochemical in situ sensing of dsDNA oxidative damage caused by oxidized quercetin incorporated into dsDNA was possible using a thin layer DNA-modified electrode. The results indicate that quercetin binds to the dsDNA and can undergo oxidation, a process during which quercetin and superoxide radicals are formed. These radicals readily attack the dsDNA, disrupting the helix and leading to the formation of 8-oxoguanine. A model for this electrochemically observed in situ oxidative damage has been proposed and may be used to explain the oxidant–antioxidant effect of quercetin.

The approach described here can be used advantageously for the understanding of dsDNA interactions with various complex agents and individual chemicals of environmental,

food and medical interests. The use of voltammetric techniques for the in situ generation of reactive intermediates is, in a promising way, a complementary tool for the study of biomolecular interaction mechanisms. The importance of DNA-electrochemical biosensors in the determination of the interaction mechanism between DNA and different compounds is clearly demonstrated.

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