Electrochemical Nucleic Acid Biosensors for the Detection of Interaction Between Peroxynitrite and DNA

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Abstract
We studied the reactivity of peroxynitrite and different nucleic acid molecules using DNA electrochemical biosensors. SIN-1 (3-morpholinosydnonimine) has been used for the simultaneous generation of NO · and superoxide, i.e., as a peroxynitrite ($ONOO^-$) donor. Double strand DNA (dsDNA), single strand DNA (ssDNA) and 15 guanine bases oligonucleotide ($Oligo(dG)_{15}$) were immobilized on a carbon paste electrode to generate the biosensor and DPV was selected as the electroanalytical technique. Results showed that electrochemical biosensors were very sensitive for detecting interaction between $ONOO^-$ and DNA. A down/up effect was observed, i.e., at low $ONOO^-$ concentrations the guanine oxidation signal decreased while at high $ONOO^-$ concentrations the guanine oxidation current increased. $Oligo(dG)_{15}$ exhibited greater interaction at low $ONOO^-$ concentrations than the other DNA molecules. The reactivity between $ONOO^-$ and DNA was also evaluated in solution phase, showing the same down/up effect. Finally, the capacity of DNA to hybridize was prevented after interaction with $ONOO^-$.

Keywords: DNA damage, Peroxynitrite, Carbon paste electrode, Biosensors

1. Introduction

One of the greatest challenges in toxicological research is to develop sensitive, selective, rapid and inexpensive in vitro methods for detecting DNA damage. This damage may include breakage of one or both strands, chemical modification of the bases in DNA, or cross-linking [1].

DNA is the main target of physical and chemical toxics, e.g., alkylating compounds, polycyclic aromatic hydrocarbons, biphenyls, heterocyclic amines, ultraviolet (UV) light and ionizing radiation [2 – 4]. DNA is also the main target of most cytotoxic anticancer drugs that react either directly with DNA through reactive metabolites or indirectly through incorporation into DNA [5]. Finally, oxidative DNA damage by free radicals or other DNA-damaging agents is involved in mutagenesis, carcinogenesis, reproductive cell death, and aging [6, 7]. Among nucleic acid bases, guanine is the most easily oxidizable and is therefore a primary target of attack in DNA [8, 9].

In this sense, the electrochemical response of the guanine nucleobase is very sensitive and can be used for probing DNA damage or interactions. Changes in the guanine oxidation and or other intrinsic DNA redox signals have been used for both bioanalytical and chemical/physical damage detection purposes through the use of electrochemical DNA biosensors [10 – 13].

Anticancer drugs and DNA interaction have been profusely studied using electrochemical biosensors. A complete revision has recently been published by Khalid et al. [14]. On the other hand, oxidative DNA damage has also been studied using electrochemical biosensors based on mercury [15], carbon paste (CPE) [16], glassy carbon (GCE) [17 – 19], gold [22] and screen-printed [21] electrodes. For example, oxidative damage to DNA has been reported for arsenic trioxide (As_2O_3) using a CPE [16]. After interaction, the signal of guanine was found to decrease when accumulation time and concentration of As_2O_3 were increased. A GCE was also used by Oliveira-Brett et al. [17], demonstrating that the reduced thiophene-*S*-oxide interacts with dsDNA, causing damage with possible strand break, and that thiophene-*S*-oxide could form an adduct with dsDNA. The same group also studied the interaction between dsDNA immobilized on GCE and reactive nitrogen species released by a NO-releasing compound in pH 4.5 0.1 M acetate buffer [18]. The authors showed that it is possible to electrochemically generate NO metabolites such as peroxynitrite ($ONOO^-$), which damage dsDNA. The formation of modified DNA bases such as 8-nitroguanine was observed after interaction of DNA with peroxynitrite radicals electrochemically generated on the electrode surface.

Endogenous $ONOO^-$ has been implicated in a number of diseases. It displays a wide range of biochemical reactivity since it has been shown: 1) to nitrate proteins (tyrosine residues), carbohydrates, and nucleic acids; 2) to oxidize lipids, thiol groups, Fe/S and Zn/S centers, and oxyhemo-

globin to methemoglobin; 3) to freely cross the cytoplasmic membrane of red blood cells when protonated [22].

Peroxynitrite induces DNA base damage predominantly by guanine generation, i.e., 8-nitroguanine by $\cdot NO_2$ formed during peroxynitrite degradation and 8-oxoguanine (8-oxoG) by oxygen reactive species generated during a secondary degradation route of $ONOO^-$ [23, 24].

In this paper, a comparative study was conducted of the susceptibility of three DNA molecules, dsDNA, ssDNA and $Oligo(dG)_{15}$ to interact with $ONOO^-$ using a DNA biosensor. Also, the further capacity of $Oligo(dG)_{15}$ to hybridize was tested after $ONOO^-$ interaction. DNA modified CPE was selected due to the simplicity, reproducibility and to the stability of the adsorbed DNA. Interaction was performed in phosphate buffer at pH 7.40 to mimic physiological environment. The oxidation current of guanine was followed in acetate buffer pH 5.00 since below this condition the electrochemical DNA biosensors exhibit the most sensitive response.

2. Experimental

2.1. Reagents

Double stranded calf thymus DNA (dsDNA) (activated and lyophilized), single stranded calf thymus DNA (ssDNA) (lyophilized powder) and 3-morpholinosydnonimine (SIN-1) were from Sigma. $Oligo(dG)_{15}$ and $Oligo(dC)_{15}$ were obtained from Integrated DNA Technology. Stock solutions of nucleic acids (1000 and 250 ppm) were prepared with tris-EDTA (TE) buffer (1× concentrate, 20 mM Tris-HCl, 1 mM EDTA, pH 8.0).

All solutions were prepared with ultrapure water ($\rho =$ 18 MΩ) from a Millipore-MilliQ system (MQ water). 0.20 M, pH 5.00 acetate buffer solutions or 0.10 M, pH 7.40 phosphate buffer solution were employed as supporting electrolytes. All chemicals were used as received.

SIN-1, was used as a peroxynitrite generator. 10 mM stock solution was prepared just prior to experiments in a previously oxygenated 0.10 M pH 7.40 phosphate buffer solution. At physiological pH, 1 mM SIN-1 decomposition has been shown to generate about 1 μM/min $ONOO^-$ [25].

2.2. Apparatus

Differential pulse voltammetry (DPV) was performed with a CHI 440 setup (CH Instruments Inc., USA). A carbon paste electrode (CPE) 3 mm in diameter was used as a working electrode. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to the latter. A magnetic stirrer provided convective transport when necessary.

DPV experimental conditions were: potential increment, 0.04 V; pulse amplitude, 0.05 V; pulse width, 0.017 s; and pulse period, 0.2 s. Anodic current at around 1.0 V, corresponding to guanine oxidation, was used as an analytical signal.

2.3. Preparation of the Working Electrode

The carbon paste electrode (CPE) was prepared by mixing graphite powder (Fisher grade # 38) with mineral oil (DNAasa, RNAsa, protease free) (Aldrich). The paste was packed firmly into the cavity of an in-house made Teflon tube. The electrical contact was provided by a copper wire inserted into the carbon paste. The surface was polished on weighing paper before use.

2.4. Procedure

2.4.1. Interaction of DNA with Peroxynitrite Using the Biosensor

CPE was pretreated by applying 1.70 V for 3 min in 0.20 M, pH 5.00 acetate buffer solution containing the respective nucleic acid molecule without stirring. Then, the nucleic acids (ds- ssDNA or $Oligo(dG)_{15}$) were immobilized by applying a potential of 0.50 V for 3 min in 10 or 20 ppm (dsDNA and ssDNA) or 1.5 ppm $Oligo(dG)_{15}$ in 0.20 M acetate buffer solution with 750 rpm stirring. The electrode was then rinsed with 0.20 M, pH 5.00 acetate buffer solution for 10 s. The biosensor was then immersed into 0.10 M, pH 7.40 phosphate buffer solution containing different concentrations (0 – 2000 μM) of SIN-1 and incubated from 2.5 to 20 min at 37 °C. After interaction, the electrode was rinsed with 0.20 M, pH 5.00 acetate buffer solution for 10 s. Finally, guanine oxidation was assessed by DPV in 0.20 M, pH 5.00 acetate buffer.

2.4.2. Interaction of DNA with Peroxynitrite in Solution

30 μL of ssDNA stock solution (1000 ppm) was added to 270 μL of 0.10 M pH 7.40 phosphate buffer solution and incubated at 37 °C. Then, 1 – 5 μL of SIN-1 stock solution (10 mM) were added just as added to the ssDNA solution to reach from 100 to 500 μM of final [$ONOO^-$]. Next, the solutions were incubated for 5 min at 37 °C. After that, the solution was diluted with 1.2 mL of 0.20 M acetate buffer (pH 5.00). Finally, the protocol of (a) CPE pretreatment (b) ssDNA adsorption and (c) voltammetric transduction as described in point 2.4.1 was followed.

2.4.3. Hybridization Studies

The CPE was pretreated by applying 1.70 V for 3 min in 1.5 ppm $Oligo(dG)_{15}$ acetate buffer solutions 0.20 M pH 5.00 without stirring. Then, $Oligo(dG)_{15}$ was immobilized by applying a potential of 0.50 V for 3 min with 750 rpm stirring. The electrode was then rinsed with 0.20 M pH 5.00 acetate buffer solution for 10 s. The biosensor was

then immersed into 0, 300 or 1000 μM [$ONOO^-$] 0.10 M, pH 7.40 phosphate buffer solution containing different concentration and incubated for 2.5 or 5 min at 37 °C. After interaction, the electrode was rinsed with 0.10 M, pH 7.40 phosphate buffer solution for 10 s and transferred into 1.5 ppm Oligo$(dC)_{15}$ 0.10 M, pH 7.40 phosphate buffer solution for 20 min to perform the hybridization process. After hybridization, the electrode was rinsed with 0.20 M, pH 5.00 acetate buffer solution for 10 s. Finally, guanine oxidation was assessed by DPV in 0.20 M pH 5.00 acetate buffer.

3. Results and Discussion

3.1. Interaction of DNA with Peroxynitrite

The interaction of DNA with peroxynitrite using a dsDNA biosensor was studied. The biosensor was immersed into 0.10 M pH 7.40 solution containing different concentration (0 – 2000 μM) of SIN-1 and incubated for 5 min at 37 °C.

The stability of the adsorbed dsDNA was assessed by incubating the biosensor in phosphate buffer at pH 7.40 without peroxynitrite at 37 °C for up to 20 min. The electrochemical signals obtained after 5 measurements were (1.50 ± 0.12), (1.58 ± 0.11), (1.79 ± 0.09) and (1.66 ± 0.12) μA for 0, 5, 10 and 20 min incubation, respectively. These results indicated that the biosensor produces stable and reproducible signals, i.e., there was no desorption of the biomolecule from the carbon paste surface. These measurements were used as control values for further experiments.

As the assessment of $ONOO^-$ toxicity is difficult to perform because of the extremely short half-life of this molecule under physiological conditions ($t_{1/2} < 20$ ms), a system was selected, based on the continuous in situ synthesis of $ONOO^-$ from NO · and $O_2^{\bullet -}$. In fact, SIN-1 is very helpful as an experimental model of peroxynitrite donor, in chemical and biological systems [26, 27].

Figure 1 shows the DP voltammograms of 20 ppm dsDNA biosensor before (a) and after (b) 5-min interaction with 2000 μM $ONOO^-$. The oxidation signal of guanine decreased in almost 50% and no new signals were observed at any other potential. Inset of Figure 1 shows the response of the biosensors after incubation in the same conditions at different $ONOO^-$ concentrations. A decrease in guanine oxidation current was observed at low $ONOO^-$ concentrations down to 1000 μM where the guanine signal represents ca. 70% of the control experiment. At higher $ONOO^-$ concentrations (1000 – 1500 μM) a change in the tendency was clearly observed, with an increase in the guanine oxidation current. Finally, when $ONOO^-$ was concentrated even more in the reaction medium, a strong decrease in the signal was observed with a minimum value of ca. 50% of the control experiment at 2000 μM. A similar down/up effect on the oxidation signal of guanine was previously observed by Ozsoz et al. [16] when they tested the As_2O_3 oxidative damage over both ssDNA and dsDNA. The decrease in guanine signal could be attributed to DNA oxidative

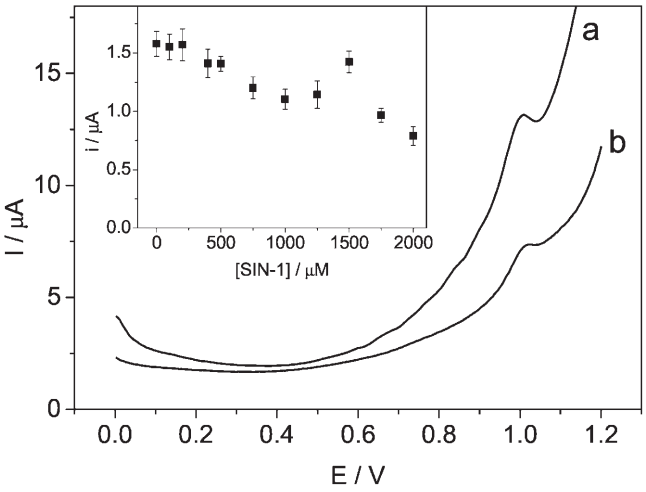

Fig. 1. DP voltammograms of 20 ppm dsDNA biosensor before (a) and after (b) interaction with 2000 μM of SIN-1. Inset: Effect of SIN-1 concentration on guanine oxidation after 5 min incubation in phosphate buffer pH 7.40 at 37 °C. Nucleic acid adsorption on CPE by applying 0.5 V potential during 3 min after pretreatment applying 1.70 V during 3 min. VPD were recorded in 0.20 M pH 5.00 acetate buffer solutions. Each error bar represents the standard deviation obtained for $n = 5$ independent experiments.

damage produced by $ONOO^-$, modifying the guanine bases present in DNA, as previously reported by Tannenbaum using chromatographic techniques [23, 24]. Otherwise, the increase observed in the peak current could be due to the capacity of $ONOO^-$ to react also with deoxyribose groups, giving rise to DNA strand breaks [28], implying a rearrangement of DNA on the electrode and more guanine bases exposed to oxidation. The increase in the guanine oxidation peak was always observed in the experimental conditions described and very good reproducibility of the results was obtained.

The effect of DNA concentration used to generate the biosensor was also assessed. In this sense, 10- and 20-ppm solutions of dsDNA at constant accumulation time (3 min) were selected. These concentrations represent values that are in the linear and in the saturated zones of the peak current vs dsDNA concentration plot. No significant differences were observed between both electrodes in a broad range of $ONOO^-$ concentrations (200 – 2000 μM), indicating that interaction between $ONOO^-$ did not depend on DNA concentration (data not shown).

Our findings are rather different from those previously obtained by Oliveira-Brett et al. [18]. There, an increase in the guanine oxidation signal when $ONOO^-$ interaction was studied and also the appearance of new signals due to reaction products such as 8-nitro- and 8-oxo-guanine were reported. First, methodological procedures about peroxynitrite generation could explain some the differences because Oliveira et al. generated $ONOO^-$ in the proximity of the electrode surface by preconcentration of NO and the application of an electrochemical pulse, which probably caused more DNA interaction than our method, which in turn implies the diffusion of $ONOO^-$ into adsorbed DNA.

Second, the biosensors were generated forming thin or thick layer of dsDNA by drying a higher quantity of nucleic acid on the electrode compared with our method, which implied adsorption of dsDNA from DNA solutions for a short time.

3.2. Interaction of Different Nucleic Acid Molecules with Peroxynitrite

The interaction of different nucleic acid molecules with peroxynitrite was also assessed. The response of the biosensor generated using dsDNA, ssDNA and Qligo$(dG)_{15}$ after 5 min interaction with different $ONOO^-$ concentrations is seen in Figure 2. The (i/i_0) vs. $[ONOO^-]$ plot shows that, at low peroxynitrite concentrations ($<300-500\,\mu M$), the oxidation currents decreased, suggesting that the guanine bases are being chemically oxidized by $ONOO^-$ with the corresponding decrease in the biosensor response. But when $ONOO^-$ concentrations were increased, the biosensors increased their responses, indicating that a more complex interaction mechanism is present.

Comparing the interactions between DNA molecules, ssDNA shows a lower reactivity than dsDNA in all the $ONOO^-$ concentrations under study. This fact has previously been reported by Ozsoz et al. [16], who postulated that the existence of a steric hindrance in the highly polymerized ssDNA that prevented the oxidative damage to guanine bases. On the other hand, Adamcik et al. [29] reported that after a process of adsorption, ssDNA can adopt not elongated form and have very compact structures with many kinks and nodes due to intrastrand base-pairing, while dsDNA is in almost 2D equilibrium conformation, i.e., elongated form. Then, adsorbed dsDNA would provide a better conformation for the interaction between guanine bases and $ONOO^-$.

On the other hand, the Oligo$(dG)_{15}$ biosensor exhibited a great interaction with $ONOO^-$, producing faster decay of the guanine oxidation current than the other DNA molecules. In this case, even though Oligo$(dG)_{15}$ is a single stranded molecule like ssDNA, it is a fact that guanine has the ability to form a well-known tetrameric structure [30, 31], which could have been destroyed by $ONOO^-$, producing an increase in the biosensor response.

3.3. Effect of Interaction Time

The effect of interaction time was assessed using the three biosensors. Figure 3 shows the effect of interaction time on the guanine signal using Oligo$(dG)_{15}$ biosensor at different $ONOO^-$ concentrations. As can be seen, at low interaction times (2.5 and 5 min) there is no difference in the response of the biosensor up to 500 μM $ONOO^-$, whereas at 10 min, faster decay was observed. However, at high $ONOO^-$ concentrations there is a correlation between time and the response of the biosensor. Thus, 1000 μM $ONOO^-$ produces changes of 0.88, 1.05 and 1.35 times in relation to the control signal when the interaction times are 2.5, 5 and 10 min respectively.

For both ds- and ssDNA the effect of interaction time was assessed using 1000 μM $ONOO^-$. At this concentration, the maximum decay was observed for both molecules before the current increased due to a rearrangement of the strands. Under this condition, no significant differences were observed at 5 and 10 min interaction time. Only when time was increased to 20 min for dsDNA was an increase detected in the signal.

Thus, a decrease in the biosensor response is observed when $ONOO^-$ concentration and interaction time are low, and an increase in the response of the DNA biosensor may

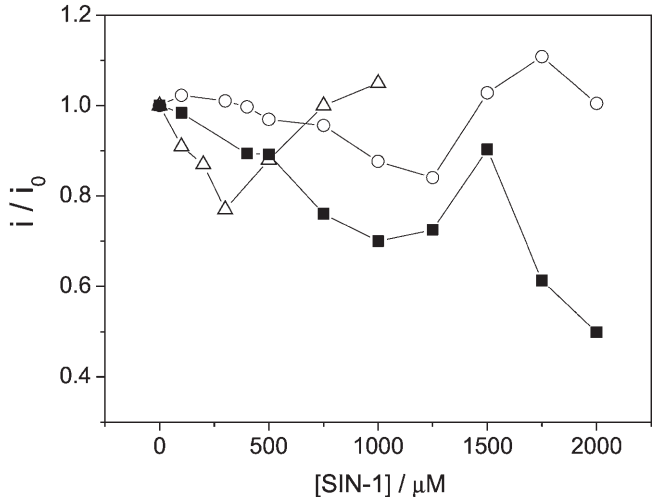

Fig. 2. Effect of SIN-1 concentration on normalized current of guanine oxidation of biosensor generated from 20 ppm dsDNA (■), 20 ppm ssDNA (○) and 1.5 ppm Oligo$(dG)_{15}$ (△). Other conditions as in Figure 1.

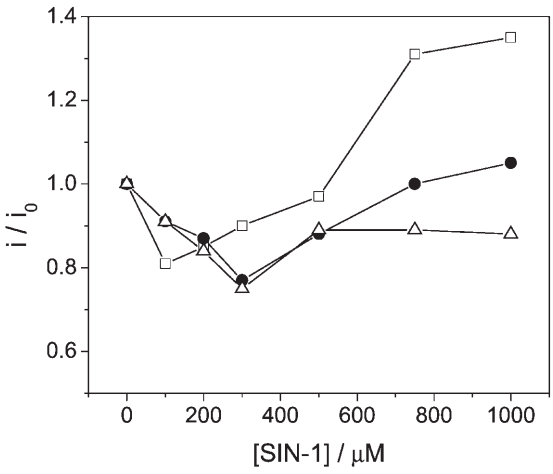

Fig. 3. Effect of incubation time on the dependence of normalized current of guanine oxidation on [SIN-1]. Incubation time: 2.5 (△), 5 (●) and 10 (□) min in phosphate buffer pH 7.40 at 37 °C. Biosensor generated from 1.5 ppm Oligo$(dG)_{15}$. Other conditions as in Figure 1.

be observed when (a) $ONOO^-$ concentration or (b) interaction time between the species is increased.

3.4. Interaction of DNA with Peroxynitrite in Solution

To make a comparison with the interaction of $ONOO^-$ and DNA using the biosensor, a series of experiments in solution phase were conducted. In this case ssDNA was added to the reaction medium (phosphate buffer at pH 7.40 at 37 °C) at the same time as the SIN-1 generated $ONOO^-$. After interaction time, an aliquot of this solution was transferred to 0.20 M, pH 5.00 acetate buffer solutions and the adsorption and oxidation protocol was performed.

Figure 4 shows the normalized current (black squares) obtained after interaction with different concentrations of peroxynitrite. Also, for comparison, the results obtained with the biosensor under the same experimental conditions are shown. As can be seen, at low $ONOO^-$ concentrations (100 – 300 μM) the response of the solution under study indicates that there is damage of the DNA guanine bases, whereas in the same conditions the biosensor response has shown no difference compared with control experiments. This can be explained considering that, in solution, DNA is freely dissolved and peroxynitrite can diffuse more readily into the molecule to produce oxidative damage. On the contrary, on the biosensor surface, DNA gets a more compact conformation, as a product of the adsorption process, which could make diffusion of $ONOO^-$ into the molecule difficult.

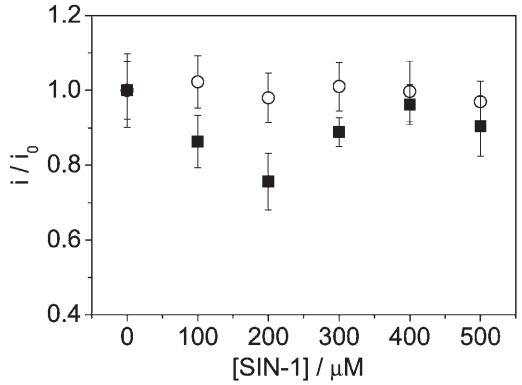

Fig. 4. Comparison of ssDNA/$ONOO^-$ interaction using an ssDAN biosensor (○) and in solution (■). Incubation time, 5 min in phosphate buffer pH 7.40 at 37 °C. Each error bar represents the standard deviation obtained for $n = 5$ independent experiments. Other conditions as in Figure 1.

Nevertheless, just as observed using the biosensor, at concentrated $ONOO^-$ solutions, an increase in the current value of the biosensor takes place, confirming that a change is occurring in the structure of DNA due to damage both at the guanine bases and at the sugar-phosphate of the DNA backbone. This damaged DNA permits that more guanine bases are in contact with the electrode for oxidation after adsorption.

3.5. Effect of Peroxynitrite Interaction on the Hybridization Process

The electrochemical DNA biosensors have been very useful tools to study the hybridization process [11]. The detection of hybridization can be done through direct, in situ detection of changes in the intrinsic redox activity of the nucleic acid target or probe [32]. In this way, we studied the capacity of hybridization that $Oligo(dG)_{15}$ and its complementary strand $Oligo(dC)_{15}$ exhibit when the $Oligo(dG)_{15}$ biosensor was previously incubated with $ONOO^-$ and when it was not.

Figures 5A and 5B show the DP voltammograms obtained with the biosensor generated from $Qligo(dG)_{15}$ incubated for 10 and 20 minutes, respectively, in the hybridization solution with (dotted lines) and without (solid lines) $Oligo(dC)_{15}$. As can be seen, there is a direct relationship between the guanine oxidation current and hybridization time. Thus, after 20 minutes, more hybridization took place since the electrochemical signal decays 40.1% with respect to the control instead of the 15.6% observed at 10 min. Decay in the oxidation signal is

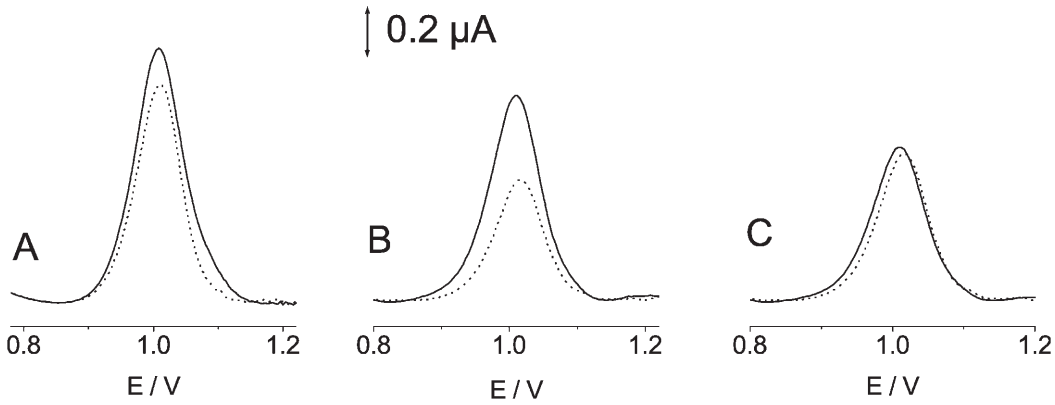

Fig. 5. Line base corrected DP voltammograms of $Oligo(dG)_{15}$ after 10 min (A) or 20 min (B – C) of hybridization in solution with (dotted lines) and without (solid lines) $Oligo(dC)_{15}$. (A) and (B) without previous interaction with $ONOO^-$ and (C) with 5 min of ONOO interaction. Other conditions as in Figure 1.

observed when the double strand is formed since guanine bases are not so available to be oxidized on the electrode. Later, 20 min was selected as hybridization time to study the hybridization capacity of Oligo$(dG)_{15}$ after its interaction with $ONOO^-$.

Figure 5C shows the DP voltammograms obtained with Oligo$(dG)_{15}$ biosensor, after 2.5 min interaction with 300 μM $ONOO^-$ solution and incubated for an additional 20 min in the hybridization solution with (dotted line) and without (solid line) the complementary Oligo$(dC)_{15}$. No differences were detected between the voltammograms, thus we conclude that Oligo$(dG)_{15}$ could not hybridize after interaction with $ONOO^-$. The same experiment was repeated changing the $ONOO^-$ concentration to 1000 μM with 5 min interaction, without any change in the biosensor response after incubation with Oligo$(dC)_{15}$ (data not shown).

4. Conclusions

We have shown that DNA-peroxynitrite interaction follows a complex mechanism; however, it may be studied using electrochemical biosensors. In all cases a down effect was observed when $ONOO^-$ concentration and interaction time were low, guanine oxidation being the predominant mechanism. On the other hand, an up effect was observed when either (a) $ONOO^-$ concentration or (b) time of interaction between the species was increased, the occurrence of DNA strand breaks, by $ONOO^-$/deoxyribose interaction, or Oligo$(dG)_{15}$ tetrameric disruption being significant in the interaction mechanisms. No new signals corresponding to 8-nitro or 8-oxo guanine were observed after $ONOO^-$ interaction, possibly due to low concentrations of DNA and/or low interaction times used. The capacity of hybridization, characteristic of DNA molecules, was lost after interaction with both low and high concentrations of $ONOO^-$. The DNA biosensor used in this work offers several advantages, such as fast response, low cost, sensitivity, reproducibility, and DNA stability and will be very useful for future assessments of antioxidant properties against peroxynitrite DNA damage.

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6. References

[1] R. S. Sodum, E. S. Fiala, *Chem. Res. Toxicol.* **2001**, *14*, 438.
[2] C. Bolognesi, *Mutat. Res. – Rev. Mutat.* **2003**, *543*, 251.
[3] G. Speit, L. Henderson, *Mutat. Res. – Rev. Mutat.* **2005**, *589*, 67.
[4] Y. Matsumura, H. N. Ananthaswamy, *Toxicol. Appl. Pharm.* **2004**, *195*, 298.
[5] D. E. Graves, L. M. Velea, *Curr. Org. Chem*. **2000**, *4*, 915.
[6] L. L. de Zwart, J. H. N. Meerman, J. N. M. Commandeur, N. P. E. Vermeulen, *Free Rad. Biol. Med.* **1999**, *26*, 202.
[7] G. Guetens, G. De Boeck, M. Highley, A. T. van Oosterom, E. A.de Bruijn, *Crit. Rev. Clin. Lab. Sci.* **2002**, *39*, 331.
[8] S. Steenken, S. V. Jovanovic, *J. Am. Chem. Soc.* **1997**, *119*, 617.
[9] J. Cadet, S. Bellon, M. Berger, A. G. Bourdat, T. Douki, V. Duarte, S. Frelon, D. Gasparutto, E. Muller, J. L. Ravanat, S. Sauvaigo, *Biol. Chem*. **2002**, *383*, 933.
[10] E. Palecek, *Talanta* **2002**, *56*, 809.
[11] J. Wang, *Anal. Chim. Acta* **2002**, *469*, 63.
[12] G. A. Rivas, M. L. Pedano, N. F. Ferreyra, *Anal. Lett.* **2005**, *38*, 2653.
[13] J. J. Gooding, *Electroanalysis* **2002**, *14*, 1149.
[14] S. Rauf, J. J. Gooding, K. Akhtar, M. A. Ghauri, M. Rahman, M. A. Anwar, A. M. Khalid, *J. Pharm. Biomed. Anal*. **2005**, *37*, 205.
[15] M. Fojta, L. Havran, E. Palecek, *Electroanalysis* **1997**, *9*, 1033.
[16] M. Ozsoz, A. Erdem, P. Kara, K. Kerman, D. Ozkan, *Electroanalysis* **2003**, *15*, 613.
[17] A. M. Oliveira-Brett, L. A. da Silva, H. Fujii, S. Mataka, T. Thiemann, *J. Electroanal. Chem.* **2003**, *549*, 91.
[18] V. C. Diculescu, R. M. Barbosa, A. M. O. Brett, *Anal. Lett.* **2005**, *38*, 2525.
[19] J. Kang, S. Dong, X. Lu, B. Su, H. Wu, K. Sun, *Bioelectrochem*. **2006**, *69*, 58.
[20] G.Chen, J.Zhao, X.Liu, G.Gao, J.Huang, G. Li, *J. Biotechnol.* **2007**, *127*, 653.
[21] G. Bagni, D. Osella, E. Sturchio, M. Mascini, *Anal. Chim. Acta* **2006**, *573–574*, 81.
[22] K. Kikugawa, K. Hiramoto, T. Ohkawa, *Biol. Pharm. Bull.* **2004**, *27*, 17.
[23] J. C. Niles, J. S. Wishnok, S. R. Tannenbaum, *Nitric Oxide* **2006**, *14*, 109.
[24] S. Burney, J. L. Caulfield, J. C. Niles, J. S. Wishnok, S. R. Tannenbaum, *Mut. Res.* **1999**, *424*, 37.
[25] J. P. E. Spencer, J. Wong, A. Jenner, O. I. Aruoma, C. E. Cross, B. Halliwell, *Chem. Res. Toxicol.* **1996**, *9*, 1152.
[26] J. S. Ravinder, N. Hogg, J. Joseph, E. Konorev, B. Kalyanaraman, *Arch. Biochem. Biophys.* **1999**, *361*, 331.
[27] V. M. Darley-Usmar, N. Hogg, V. J. O'Leary, M. T. Wilson, S. Moncada, *Free Rad. Res. Commun.* **1992**, *17*, 9.
[28] N. Y. Tretyakova, S. Burney, B. Pamir, J. S. Wishnok, P. C. Dedon, G. N. Wogan, S. R. Tannenbaum, *Mutat. Res.* **2000**, *447*, 287.
[29] J. Adamcik, D. V. Klinovc, G. Witza, S. K. Sekatskii, G. Dietlera, *FEBS Lett.* **2006**, *580*, 5671.
[30] J. L. Mergnya, A. T. Phan, L. Lacroix, *FEBS Lett.* **1998**, *435*, 74.
[31] L. H. Hurley, R. T. Wheelhouse, D. Sun, S,M. Kerwin, M. Salazar, O. Y. Fedoroff, F. X. Han, H. Han, E. Izbick, D. D. Von Hoff. *Pharm. Therapeut*. **2000**, *85*, 141.
[32] J. Wang, G. Rivas, J. R. Fernandes, J. L. Lopez-Paz, M. Jiang, R. Waymire *Anal. Chim. Acta* **1998**, *375*, 197.