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Dedicated to prof. dr. I. C. Popescu
on the occasion of his 70th anniversary

DNA-ELECTROCHEMICAL BIOSENSORS: A MINI-REVIEW

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ABSTRACT. DNA-electrochemical biosensors allow rapid detection of hazard compounds interaction with dsDNA with great sensitivity. The application of the DNA-electrochemical biosensor for the detection of DNA oxidative damage by hazard compounds and pharmaceutical drugs, namely by hydroxyl free radicals, *in situ* generated at the boron doped diamond electrode surface, the hepato-toxins microcystine-LR and nodularine, the temozolomide, the nucleoside analogue gemcitabine, the anticancer disubstituted triazole-linked acridine compounds, and the anticancer antibody rituximab, showing current directions and strategies, will be revisited.

Keywords: *DNA, oxidative damage, electrochemistry, biosensor, cancer.*

INTRODUCTION

DNA is a major target for interaction with many hazard compounds. These interactions induce changes to dsDNA structure and base sequence, damaging the genetic information code, and are the main cause of health anomalies including cancer [1-3]. Hence, the development of technologies and methodologies to detect dsDNA-hazard compound interactions is very important.

The electrochemical transducers for the characterization of dsDNA-hazard compound interaction received a particular interest due to their rapid detection and great sensitivity, representing an attractive solution in many different fields of application such as the investigation and the

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evaluation of dsDNA-hazard compound interaction mechanisms, detection of DNA base damage in clinical diagnosis, or detection of specific DNA sequences in human, viral, and bacterial nucleic acids [4-9].

Among the electrochemical transducers, carbon and especially glassy carbon electrodes (GCE) exhibit several unique properties. The extensive potential window in the positive direction allows sensitive electrochemical detection of dsDNA conformational changes and oxidative damage caused to DNA, by monitoring the appearance of the oxidation peaks of the DNA components such as, nucleotides, nucleosides, purine and pyrimidine bases, and guanine (Gua) oxidation product 8-oxoguanine (8-oxoGua), and adenine (Ade) oxidation product 2,8-dyhydroxyadenine (2,8-oxoAde), biomarkers of DNA base oxidative damage [5, 7, 9].

The DNA-electrochemical biosensors design is essential to understand the DNA electrochemical properties and the performance of the DNA-electrochemical biosensor is related to the DNA immobilization procedure. Therefore, a full understanding of the surface morphology of the DNA-electrochemical biosensor is necessary to guarantee the correct interpretation of the experimental results.

This review presents the electrochemical behaviour of DNA, its adsorption morphology, and applications of the DNA-electrochemical biosensor, prepared by three different procedures on glassy carbon surfaces, for the detection of DNA damage by reactive oxygen species, toxins, anticancer drugs and antibodies.

ELECTROCHEMISTRY OF DNA

The electrochemical properties of all DNA bases, nucleosides and nucleotides were studied at a glassy carbon electrode [10].

Differential pulse (DP) voltammograms (**Figure 1**) recorded at the GCE in solutions of DNA bases guanine (Gua), adenine (Ade), thymine (Thy) and cytosine (Cyt) have shown that all DNA bases undergo electrochemical oxidation at different potentials.

The oxidation of purine bases Gua and Ade occurs at more negative values than the oxidation of pyrimidines bases Thy and Cyt. Gua being the easiest oxidised base. At the same time, DP voltammograms in nucleoside solutions, of the sugar purine bases, deoxyguanosine (dGuo) and deoxyadenosine (dAdo) (**Figure 1**), have shown that their oxidation occurs at ~ 200 mV more positive potential than the corresponding purine base oxidation.

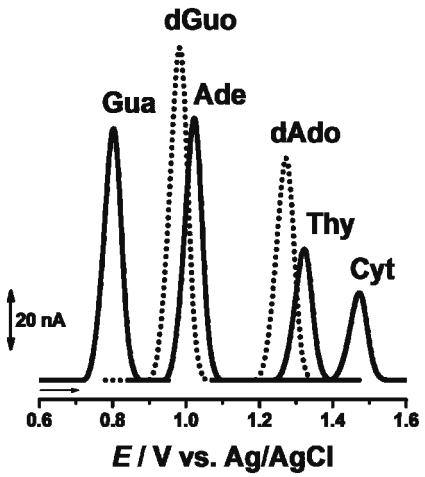


Figure 1. DP voltammograms base-line corrected at the GCE in solutions of: 10 μM guanine (Gua), guanosine (dGuo), adenine (Ade), adenosine (dAdo), and 50 μM thymine (Thy) and cytosine (Cyt) in pH = 4.5 0.1 M acetate buffer.

The electrochemical oxidation of DNA at the GCE shows two anodic peaks corresponding to the oxidation of Gua and Ade residues (**Figure 2**). The DP voltammogram of single stranded DNA (ssDNA) shows higher currents for both bases oxidation when compared to the voltammogram in the solution of double stranded DNA (dsDNA).

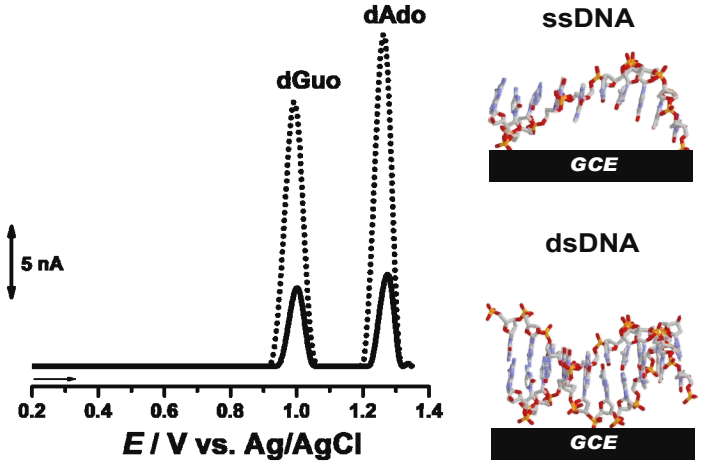


Figure 2. DP voltammograms base-line corrected at the GCE in solutions of 60 μg mL^{-1} (•••) ssDNA and (—) dsDNA in pH = 4.5 0.1 M acetate buffer.

The electrochemical behaviour obtained for dsDNA and ssDNA illustrates the greater difficulty for the transfer of electrons from the inside of the rigid DNA double-strand helix to the electrode surface, than from the flexible DNA single strand where the bases are in close proximity to the electrode surface. Thus, DP voltammetry allows the detection and characterisation of DNA structure in different samples.

DNA-ELECTROCHEMICAL BIOSENSORS PREPARATION

The immobilization of the dsDNA probe on the electrode surface is done in the pH range 4.5 - 5.5 due to the better adsorption of dsDNA at this pH on the GCE surface, consequently leading to an enhanced electrochemical response (**Figure 3**).

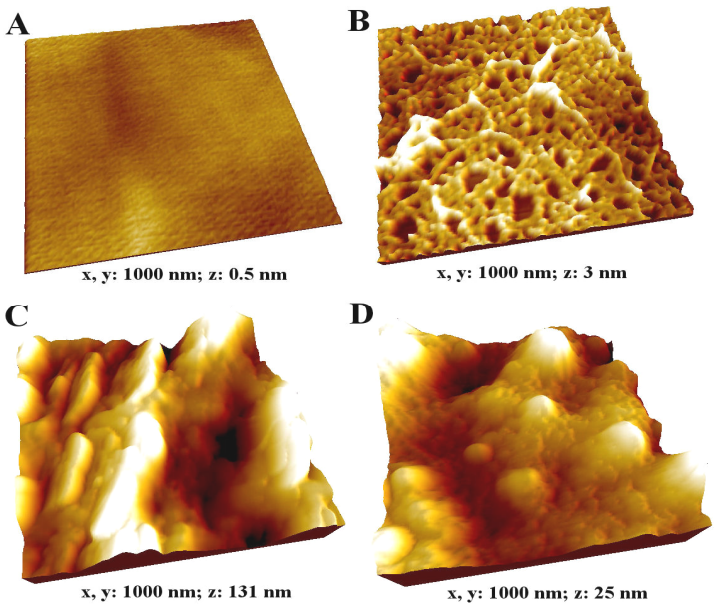


Figure 3. MAC Mode AFM three-dimensional images in air of:
A) clean HOPG electrode;
B) thin-layer dsDNA-electrochemical biosensor surface, prepared onto HOPG by 3 min free adsorption from 60 μg mL^{-1} dsDNA in pH 4.5 0.1 M acetate buffer;
C) multi-layer dsDNA-electrochemical biosensor, prepared onto HOPG by evaporation of 3 consecutive drops each containing 5 μL of 50 μg mL^{-1} dsDNA in pH 4.5 0.1 M acetate buffer;
D) thick-layer dsDNA-electrochemical biosensor, prepared onto HOPG by evaporation from 37.5 mg mL^{-1} dsDNA in pH 4.5 0.1 M acetate buffer.
From reference [5] with permission.

Three different procedures have been followed in the DNA-electrochemical biosensor construction:

1. A thin-layer dsDNA-electrochemical biosensor: prepared by immersing the GCE surface in a 60 μg mL^{-1} dsDNA solution during 3 min (**Figure 3B**).

2. A multi-layer dsDNA-electrochemical biosensor: prepared by successively covering the GCE surface with three drops, of 5 μL each of 50 μg mL^{-1} dsDNA solution. After placing each drop on the electrode surface the biosensor is dried under a constant flux of N_2 (**Figure 3C**).

3. A thick-layer dsDNA-electrochemical biosensor: prepared by covering the GCE surface with 10 μL of 35 mg mL^{-1} dsDNA solution and allowing it to dry overnight in a normal atmosphere (**Figure 3D**).

ADSORPTION OF DNA

The development of DNA-electrochemical biosensors is associated with an adsorption processes of dsDNA on charged electrode surfaces [5, 7, 9].

The immobilization of dsDNA, to form the DNA-electrochemical biosensor, and the morphological characterization of the modified electrode surface is essential for the correct evaluation of all factors that may influence the electrochemical response.

The surface characteristics of dsDNA-electrochemical biosensors on highly oriented graphite (HOPG) visualized by *ex situ* MAC mode AFM showed different patterns of adsorption dependent on polynucleotide primary and secondary structures, concentration and potential applied during immobilization procedure (**Figure 3**).

The complete coverage of the electrode surface is crucial for the robustness of the dsDNA film and reduction of non-specifical adsorption.

The AFM studies enabled the correct evaluation of factors influencing the development of DNA-electrochemical biosensors for detection of DNA-hazard compound interactions.

APPLICATIONS OF THE DNA-ELECTROCHEMICAL BIOSENSOR

Free radical damage

Reactive oxygen species (ROS) such as superoxide (O_2^{-}•), peroxyl (ROO•), and hydroxyl (OH•) radicals are generated inside cells as products of metabolism, by leakage from mitochondrial respiration, and also under the influence of exogenous agents such as ionizing radiation, quinones, and peroxides.

Excess ROS are responsible for causing DNA oxidative modifications and mutations, which can initiate carcinogenesis and may play a role in the development of several age-correlated degenerative diseases [11].

The *in situ* interaction and oxidative damage caused by hydroxyl radicals to dsDNA was investigated using a thick multilayer DNA-electrochemical biosensor prepared onto the oxidized boron doped diamond electrode (BDDE) surface [11]. The BDDE allows the generation of OH• at approximately + 3.00 V (vs. Ag/AgCl in pH = 4.5 0.1 M acetate buffer) in agreement with the reaction

$$\mathbf{BDD + H_2O \rightarrow BDD(OH\bullet) + H^+ + e^-}$$

The DNA-electrochemical biosensor on the BDDE enabled preconcentration of the OH• electrogenerated at the BDDE surface. Controlling the applied potential, different concentrations of OH• were electrochemically generated *in situ* on the BDDE surface.

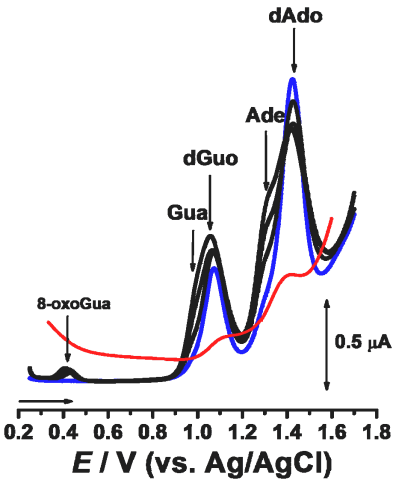


Figure 4. DP voltammograms in pH = 4.5 0.1 M acetate buffer with a thick multi-layer dsDNA-BDDE biosensor: (—) control and (—) first scan and (—) subsequent scans after applying + 3.0 V during 2 h to the BDDE surface causing electrogeneration of hydroxyl radicals. From reference [11] with permission.

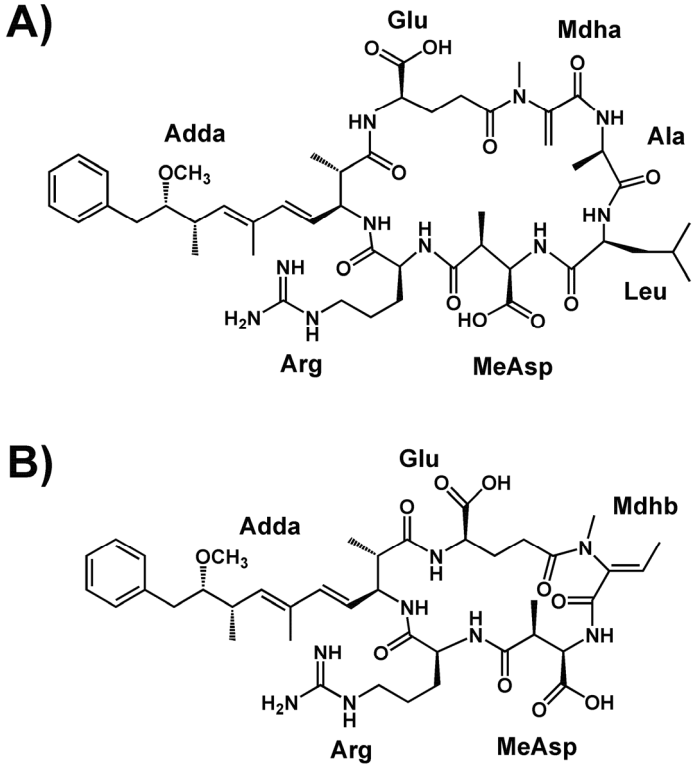
After monitoring the modification of the oxidation peak currents of the purine deoxynucleoside residues (**Figure 4**), it was found that OH• oxidatively damaged the immobilized dsDNA on the BDDE surface, leading to modifications in the dsDNA structure, exposing more purinic residues to the electrode surface and facilitating their oxidation. The dsDNA structural

modifications were confirmed by electrophoresis and the voltammetric results demonstrated the occurrence of the 8-oxoGua oxidation peak, a biomarker of DNA oxidative damage.

The electrochemical transduction is dynamic in that the electrode is itself a tuneable charged reagent as well as a detector of all surface phenomena, which greatly enlarges the DNA-electrochemical biosensing capabilities.

Toxins

Microcystine-LR (MC-LR) and nodularine (NOD) (*Scheme 1*) are among the most commonly reported toxins produced by cyanobacteria [12-14]. Several previous studies have brought evidence for the possibility of direct induction of dsDNA damage *in vitro* and *in vivo* upon interaction with any of these toxins whereas other studies suggested that MC-LR and NOD genotoxicity and carcinogenicity arise mainly from the secondary effects of these toxins rather than direct toxin-DNA interaction.



Scheme 1. Chemical structures of A) MC-LR and B) NOD.

The interaction between dsDNA and MC-LR or NOD was investigated using the DNA-electrochemical biosensors and in DNA incubated with MC-LR or NOD solutions [15]. The results have shown the decrease with time of the dsDNA oxidation peaks (**Figure 5A**).

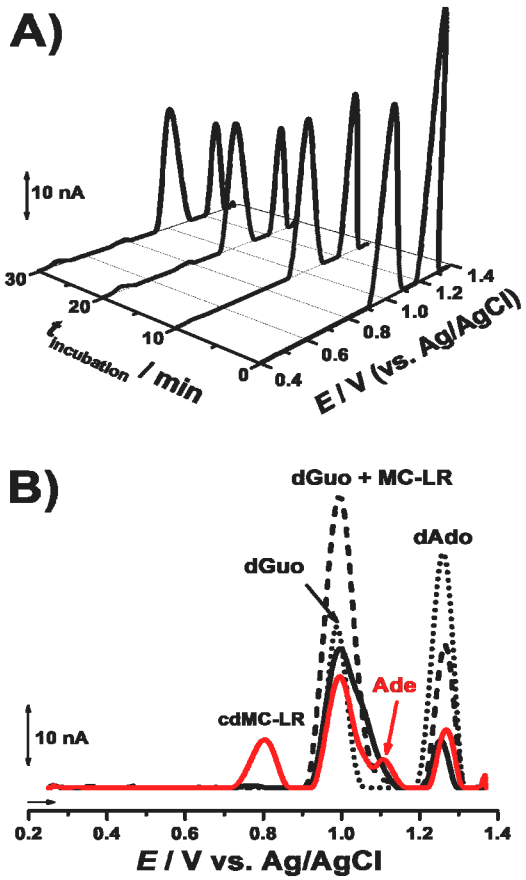


Figure 5. DP voltammograms with: **A)** the dsDNA-electrochemical biosensor in buffer pH = 4.5 incubated for different times in 30 μM MC-LR and **B)** GCE in 50 μg mL^{-1} dsDNA solution in pH = 4.5 0.1 M acetate buffer (•••) before and after incubation with 30 μM MC-LR during (▪▪▪) 0, (▬) 6 and (▬) 24 h. From reference [15] with permission.

The analysis of dsDNA interaction with MC-LR or NOD in incubated solutions, where dsDNA strands are allowed to move freely and adopt the better conformation before and after the interaction, enabled the detection of free adenine residues (**Figure 5B**).

Experiments carried out with purine polyhomonucleotides of guanine, polyguanylic (poly[G]) acid, and adenine, polyadenylic (poly[A]) acid, demonstrated that the interaction between dsDNA and MC-LR or NOD, caused strand aggregation, and the interaction occurred preferentially at adenine residues. Free adenine molecules were liberated upon cleavage of the bond between phosphate-sugar backbone of dsDNA, leading to the formation of DNA abasic sites, a type of DNA damage which, if left unrepaired can lead to mutations during the replication process.

Anticancer Drugs

Temozolomide (TMZ) is an antineoplasic alkylating agent with activity against serious and aggressive types of brain tumours. It has been postulated that TMZ exerts its antitumor activity via its spontaneous degradation at physiological pH resulting first in 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) and finally in 5-aminoimidazole-4-carbox-amide (AIC) and methyldiazonium ion [16]

TMZ → MTIC → AIC + methyldiazonium ion

The *in vitro* evaluation of the interaction of TMZ and its final metabolites, AIC and methyldiazonium ion, with dsDNA was studied using DP voltammetry at a GCE [17]. The DNA damage was electrochemically detected following the changes in the oxidation peaks of guanosine and adenosine residues. The results obtained revealed the decrease of the dsDNA oxidation peaks with incubation time, showing that TMZ and AIC/methyldiazonium ion interacted with dsDNA causing its condensation (**Figure 6**).

Furthermore, the experiments using the DNA-electrochemical biosensor for the *in situ* TMZ and AIC/methyldiazonium ion–dsDNA interaction confirmed the condensation of dsDNA caused by these species and showed evidence of a specific interaction between the guanosine residues and the TMZ metabolites, since the free guanine oxidation peak was detected. The oxidative damage caused to dsDNA bases by TMZ metabolites was also detected electrochemically by monitoring the appearance of the 8-oxoguanine/2,8-dyhydroxyadenine oxidation peaks (**Figure 6**). Agarose gel electrophoresis of AIC/methyldiazonium ion–dsDNA samples confirmed the occurrence of dsDNA condensation and the DNA oxidative damage observed in the electrochemical results. The importance of the DNA-electrochemical biosensor in the *in situ* evaluation of TMZ–dsDNA interactions was clearly demonstrated.

Nucleoside analogs (NA) of nucleobases are a pharmacological class of compounds with cytotoxic, immunosuppressive and antiviral properties.

The interaction of dsDNA with purines NA cladribine [18] and clofarabine [19] was investigated in incubated solutions and using the DNA-electrochemical biosensor. Both compounds interacted with dsDNA causing structural modifications in a time-dependent manner confirmed using the purine homo-polynucleotide single stranded sequences of guanosine and adenosine, poly[G] and poly[A]-electrochemical biosensors. No DNA oxidative damage was observed.

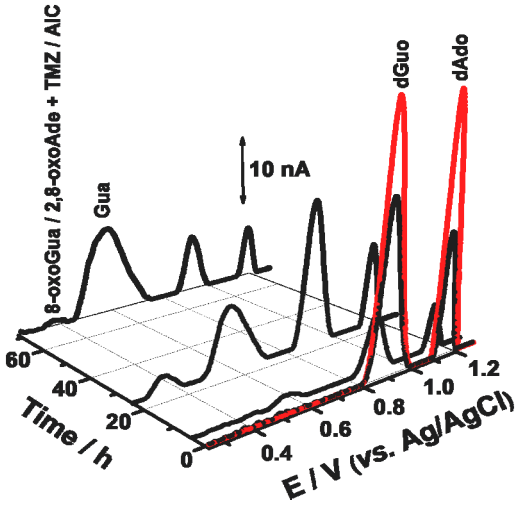
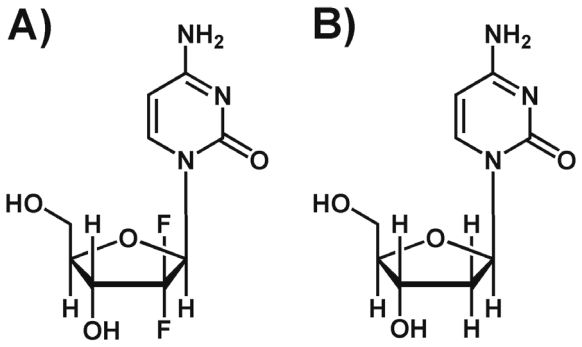


Figure 6. DP voltammograms baseline corrected in 0.1 M phosphate buffer pH = 7.0 using a multilayer dsDNA-electrochemical biosensor (—) control and after (—) 10 min, 4, 24 and 72 h in 250 μM TMZ solution. From reference [17] with permission.

The electrochemical behaviour of the cytosine NA and anti-cancer drug gemcitabine [20] was investigated at GCE, using cyclic voltammetry, differential pulse and square wave voltammetry in different pH supporting electrolytes and no electrochemical process was observed.

Gemcitabine (GEM), 2,2 difluorodeoxycitidine (*Scheme 2A*) is a pyrimidine NA of cytidine (*Scheme 2B*) and plays a major role in the treatment of several cancers.

The results obtained revealed that the interaction mechanism occurs in two sequential steps. The initial process is independent of the dsDNA sequence and leads to the condensation/aggregation of DNA strands. The formation of these rigid structures favours a second step during which the guanine hydrogen atoms participating in the C-G base pair and the fluorine atoms in the ribose moiety of GEM interacted provoking the release and/or exposure of guanine residues on the electrode surface.



Scheme 2. Chemical structures of A) gemcitabine (GEM) and B) cytidine.

The evaluation of the interaction between GEM and DNA was studied in incubated solutions using a GCE and with the DNA-electrochemical biosensor [20] (**Figure 7**).

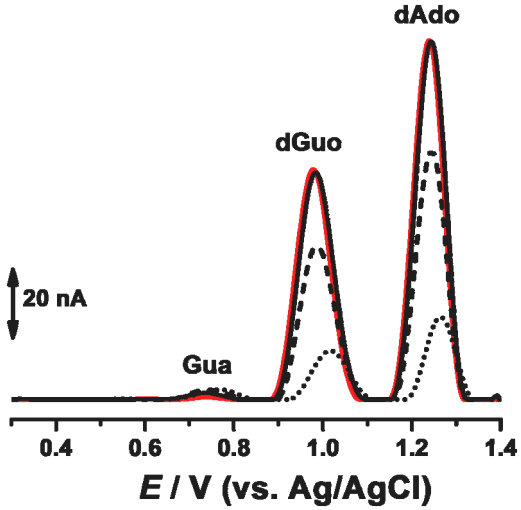


Figure 7. DP voltammograms baseline-corrected in pH = 4.5 0.1 M acetate buffer with dsDNA-electrochemical biosensor incubated with 10 μM GEM during (—) 0, (▬) 15 min, (▪▪▪) 2 h and (•••) 4 h. From reference [20] with permission

Redox mechanisms of two disubstituted triazole-linked acridine compounds (GL15 and GL7), previously reported as quadruplex DNA-binding molecules, and *in situ* electrochemical interaction with dsDNA using a DNA-electrochemical biosensor were investigated [21]. The redox properties of GL15 and GL7 involved a complex, pH-dependent, adsorption-controlled

irreversible process and were investigated using cyclic, differential pulse, and square wave voltammetry at a GCE. The interaction between dsDNA and GL15 or GL7 was investigated using the dsDNA-, poly[G]-, and poly[A]-electrochemical biosensors. It was demonstrated that the interaction is time-dependent, both GL15 and GL7 interacting with dsDNA, causing condensation of dsDNA morphological structure but not oxidative damage.

Anticancer Antibodies

Rituximab (RTX) is a chimeric human/mouse monoclonal antibody (mAb), which belongs to a class of anticancer drugs that targets specifically the CD20 antigen, a receptor expressed on the majority of malignant B-cells (more than 80%) and on normal differentiated B-lymphocytes (pre-B and mature B-lymphocytes) [22].

This antibody was the first Food and Drug Administration (FDA) approved genetically engineered mAb for use in indolent B-cell non-Hodgkin's lymphomas (B-NHLs), a type of lymphoma that affects B-lymphocytes. Currently, RTX is indicated in both indolent and aggressive B-NHLs, B-cell chronic lymphocytic leukemia (B-CLL) and some autoimmune diseases, administered as monotherapy or in combination with chemotherapy and immunotherapy [23]. Recently, it was demonstrated a relation between RTX and type 1 diabetes showing that patients treatment with a four-dose of this mAb, partially preserved beta-cell function over a period of one year and reduced the intake of insulin.

The interaction between RTX and dsDNA has great importance to predict its action mechanism as a genotoxic anticancer drug and to understand its biological activity and toxicity *in vivo*. The dsDNA-RTX interaction was investigated by DP voltammetry in incubated samples and using a multilayer DNA-electrochemical biosensor and gel electrophoresis, at pH 7.0 [23].

The DP voltammetric study showed a strong condensation of the dsDNA helical structure promoted by the dsDNA-RTX interaction, as well as the dAdo oxidation peak disappeared, the dGuo oxidation peak current decreased, and free Gua and Ade were released from DNA, but no DNA base oxidative damage was detected (**Figure 8**). This was confirmed by electrophoresis.

The sensitivity of the multilayer DNA-electrochemical biosensors offered the possibility to follow the interaction of RTX with DNA under different conditions, and the results enabled a better understanding of the dsDNA-RTX interaction molecular mechanism, with electroanalytical applications in clinical diagnostics.

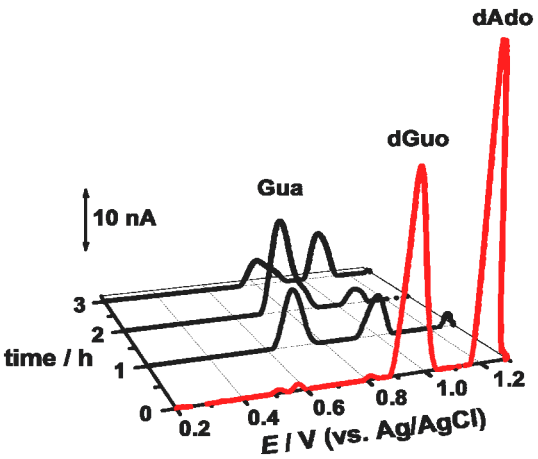


Figure 8. 3D plot of baseline subtracted DP voltammograms of multilayer (—) dsDNA-electrochemical biosensor control and (—) after incubation in 2.5 mg mL^{-1} RTX in phosphate buffer pH = 7.0 during 0, 1, 2 and 3 h. From reference [23] with permission.

CONCLUSIONS

The development of the DNA-electrochemical biosensor opened wide perspectives using a particularly sensitive and selective method for the detection of specific interactions. The possibility of predicting the damage that chemical compounds may cause to DNA arises from the preconcentration of either the starting materials or the redox reaction products on the DNA-electrochemical biosensor surface, thus allowing the electrochemical probing of the presence of short-lived intermediates and of their damage to DNA.

The use of DNA-electrochemical biosensors for the understanding of DNA interactions with molecules or ions exploits the use of voltammetric techniques for *in situ* generation of reactive intermediates and is a complementary tool for the study of biomolecular interaction mechanisms. The interpretation of the electrochemical data can contribute to the elucidation of the mechanism by which DNA is oxidatively damaged by such compounds, in an approach to the real action scenario that occurs in the living cell.

ACKNOWLEDGMENTS

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REFERENCES

1. M.S. Cooke, M.D. Evans, M. Dizdamglu, J. Lunec, *Faseb Journal*, **2003**, *17*, 1195.
2. M.D. Evans, M. Dizdaroglu, M.S. Cooke, *Mutation Research*, **2004**, *567*, 61.
3. S.I. GrivennikovI, F.R. Greten, M. Karin, *Cell*, **2010**, *140*, 883.
4. M. Fojta, "Electrochemistry of Nucleic Acids and Proteins-Towards Electrochemical Sensors for Genomics and Proteomics", Elsevier, Amsterdam, **2005**, chapter 12.
5. V.C. Diculescu, A.M. Oliveira Brett, "Biosensors and Cancer", CRC Press, London, **2012**, chapter 10.
6. E. Palecek, *Electroanalysis*, **2009**, *21*, 239.
7. A.M. Oliveira Brett, V.C. Diculescu, A.M. Chiorcea-Paquim, S.H.P. Serrano, "Electrochemical Sensor Analysis", Elsevier, Netherlands, **2007**, chapter 20.
8. S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman, M.A. Anwar, A.M. Khalid, *Journal of Pharmaceutical and Biomedical Analysis*, **2005**, *37*, 205.
9. V.C. Diculescu, A.M. Chiorcea-Paquim, A.M. Oliveira Brett, *Sensors*, **2005**, *5*, 377.
10. A.M. Oliveira Brett, J.A.P. Piedade, L.A. Silva, V.C. Diculescu, *Analytical Biochemistry*, **2004**, *332*, 321.
11. S.C.B. Oliveira, A.M. Oliveira-Brett, *Langmuir*, **2012**, *28(10)*, 4896.
12. P.V.F. Santos, I.C. Lopes, V.C. Diculescu, M.C.U. de Araújo, A.M. Oliveira-Brett, *Electroanalysis*, **2011**, *23*(10), 2310.
13. I.C. Lopes, P.V.F. Santos, V.C. Diculescu, F.M.P. Peixoto, M.C.U. Araújo, A.A. Tanaka, A.M. Oliveira-Brett, *Analyst*, **2012**, *137*(8), 1904.
14. I.C., Lopes, P.V.F. Santos, V.C. Diculescu, M.C.U. Araújo, A.M. Oliveira-Brett, *Analytical Letters*, **2012**, *45*(4), 408.
15. P.V.F. Santos, I.C. Lopes, V.C. Diculescu, A.M. Oliveira-Brett, *Electroanalysis*, **2012**, *24*, 547.
16. M. Ghalkhani, I.P.G. Fernandes, S.C.B. Oliveira, S. Shahrokhian, A.M. Oliveira Brett, *Electroanalysis*, **2010**, *22*, 2633.
17. I.C. Lopes, S.C.B. Oliveira, A.M. Oliveira Brett, *Analytical Bioanalytical Chemistry*, **2013**, *405*, 3783.
18. A.D.R. Pontinha, H.E. Satana, V.C. Diculescu, A.M. Oliveira-Brett, *Electroanalysis*, **2011**, *23*, 2651.
19. H. Eda Satana, A.D.R. Pontinha, V.C. Diculescu, A.M. Oliveira-Brett, *Bioelectrochemistry*, **2012**, *87*, 3.
20. R.M. Buoro, I.C. Lopes, V.C. Diculescu, S.H.P. Serrano, L. Lemos, A.M. Oliveira-Brett, *Bioelectrochemistry*, **2014**, *99*, 40.
21. A.D.R. Pontinha, S. Sparapani, S. Neidle, A.M. Oliveira-Brett, *Bioelectrochemistry*, **2013**, *89*, 50.
22. S.C.B. Oliveira, I.B. Santarino, A.M. Oliveira-Brett, Electroanalysis, **2013**, *25*, 1029.
23. I.B. Santarino, S.C.B. Oliveira, A.M. Oliveira Brett, *Electroanalysis*, **2014**, *26*, 1304.