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Electrochemical sensing of DNA–adriamycin interactions

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Abstract

Adriamycin, a cancerostatic anthracycline antibiotic, causes considerable death of tumour cells, together with the induction of breaks in DNA single and double strands. The interaction of this compound with DNA was investigated using an electrochemical DNA-biosensor. Adriamycin intercalation in DNA disrupts the double helix and the detection of guanine and 8-oxoguanine could mimic one possible mechanism for the in vivo adriamycin drug action. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Adriamycin; DNA-biosensor; Oxidative damage; 8-oxoguanine; Voltammetry

1. Introduction

The development of electrochemical DNA-biosensors to detect DNA–drug interactions and DNA oxidative damage has recently been investigated [1–3]. Adriamycin is an anthracycline antibiotic known for more than 30 years with a wide spectrum of antineoplasic action, although it causes cardiotoxicity. Its mode of action is not yet fully understood, although it is known that it intercalates strongly to the DNA double helix mainly at CG-GC steps, the aminosugar being determinant for intercalation to occur [4]. It interferes in several ways with DNA regulation machinery and promotes the generation of reactive oxygen species that ultimately cause oxidative damage to biomolecules [5,6]. In fact, high levels of 8-oxoguanine (a main biomarker of DNA oxidation) were detected in cancerous cells treated in vitro with adriamycin [7], but is not known whether it can directly oxidise DNA in vivo after intercalation has occurred.

The structurally analogous daunomycin is less toxic than adriamycin, and has been used as an electroactive probe for electrochemical detection of hybridization [2,3], but this work did not include an electrochemical study of the oxidative damage produced by daunomycin reduced after intercalation in dsDNA. Chronopotentiometric analysis of daunomycin–DNA interaction detected a signal due to the oxidation of DNA purine bases [8].

The interaction of adriamycin with DNA was investigated using a recently developed electrochemical DNA-biosensor [1] and it was possible to sense both intercalation of adriamycin and the generation of DNA oxidative adducts resulting from adriamycin–DNA interaction.

2. Experimental

Adriamycin (Doxorubicin hydrochloride, 2 mg/ml solution) from Pharma-APS, and sodium salt calf thymus DNA (type II) Sigma, were used without further purification. Adriamycin solutions were prepared directly in pH 4.5 0.1 M acetate buffer electrolyte solutions of ionic strength 0.1. All solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity<0.1 μS cm^{-1}). The DNA-biosensor was prepared by physical adsorption of 3 mg of dsDNA onto a glassy carbon electrode surface [1], previously polished and electrochemically cleaned in supporting electrolyte.

Voltammetric experiments were done using a μAutolab running with GPES version 4.8 software, Eco-Chemie, Utrecht, Netherlands. A one-compartment electrochemical cell with a volumetric capacity of 5 ml, a glassy carbon or DNA-biosensor [1] working electrode, a Pt wire counter electrode, and a saturated calomel electrode (SCE) as reference, were used in all experiments.

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3. Results and discussion

Adriamycin is a complex molecule and different groups can be oxidised and reduced. At pH 4.5, the reversible reductions of the 5,12-diquinone groups in the anthracycline chromophore occur at −0.4 and −0.6 V, Fig. 1a, and the oxidation of the 6,11-dihidroquinone-functionality at +0.5 and +0.6 V vs. SCE, Figs. 2 and 3, and both are pH-dependent.

Differential pulse voltammograms were obtained for the reduction and reoxidation of the diquinone group of adriamycin, Fig. 1a. They are different from those obtained using the DNA-biosensor, indicating that adriamycin has intercalated into DNA and that reactions are occurring between adriamycin and DNA bases. The formation of adducts between adriamycin and purine bases has occurred, Fig. 1b.

Using the DNA-biosensor, a constant potential of −0.6 V was applied for a period of time in order to pre-concentrate short-lived adriamycin radical intermediates formed during reduction of adriamycin, Fig. 2. These radicals, when in very close contact with DNA, can rapidly interact causing damage. The damage caused is detected by the occurrence of the oxidation peaks corresponding to the DNA purine bases. This enabled the electrochemical sensing and quantification of the damage caused by adriamycin to DNA. The appearance of a peak at +0.4 V is attributed to the oxidation of 8-oxoguanine [9], that is generated by the interaction between the adriamycin radical and a neighbouring guanine in the dsDNA during the cathodic polarization of the biosensor.

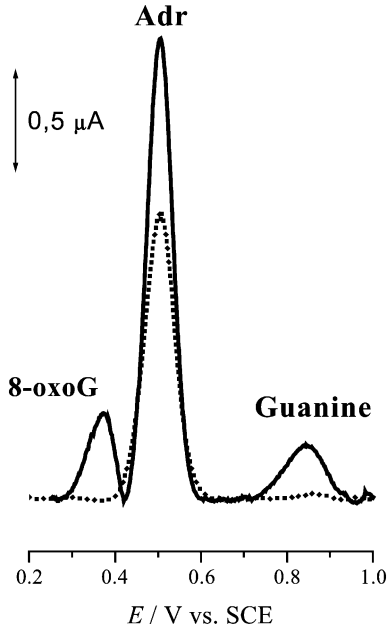

Fig. 2. Background-subtracted differential pulse voltammograms in pH 4.5 0.1 M acetate buffer in 5×10^{-6} M adriamycin, obtained with the DNA-biosensor: (••••) before and (——) after deposition at −0.6 V for 60 s.

Differential pulse voltammograms obtained with the DNA-biosensor in a more concentrated adriamycin solution

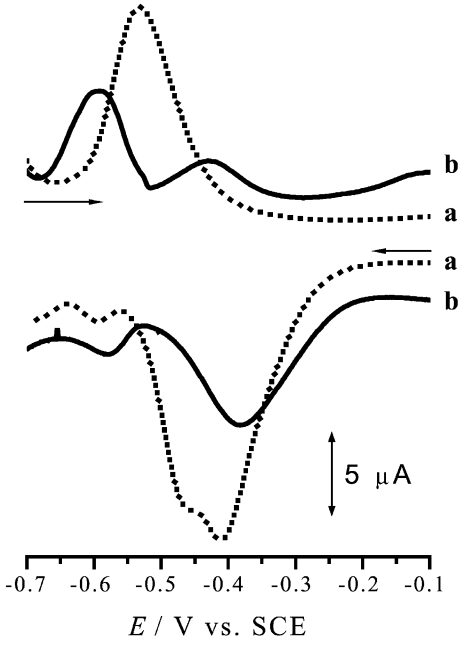

Fig. 1. Differential pulse voltammograms in pH 4.5 0.1 M acetate buffer: (a) bare glassy carbon electrode in a 1 μM adriamycin solution; (b) DNA-biosensor in buffer after being immersed in a 1 μM adriamycin solution during 10 min. Pulse amplitude 50 mV, pulse width 70 ms, scan rate 5 mV s^{-1}.

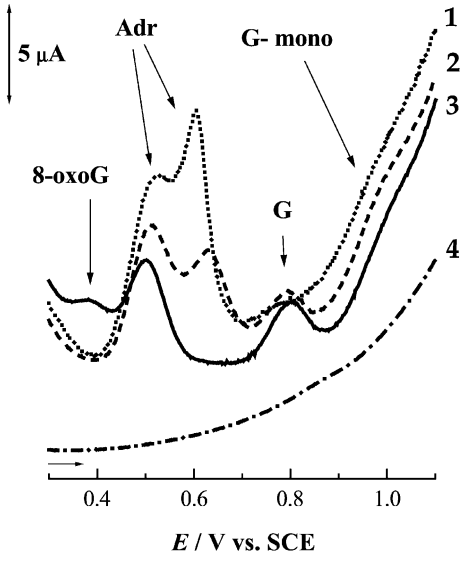

Fig. 3. Successive differential pulse voltammograms in a pH 4.5 0.1 M acetate buffer deoxygenated solution of 34 μM adriamycin, obtained with the DNA-biosensor: (1) 0, (2) 41 and (3) 100 min, E_d=0 V, t_d=120 s. (4) Differential pulse voltammogram obtained with the DNA-biosensor in a pH 4.5 0.1 M acetate buffer deoxygenated solution, E_d=0 V, t_d=120 s. Pulse amplitude 50 mV, pulse width 70 ms, scan rate 5 mV s^{-1}.

when no conditioning potential was applied, Fig. 3, showed two peaks for adriamycin oxidation corresponding to a different orientation of this molecule and intercalation took much longer to occur.

4. Conclusion

Electrochemical sensing of DNA oxidative damage caused by adriamycin was possible using the voltammetric DNA-biosensor. This is very important because the DNA–adriamycin interaction at charged interfaces is closer to the complex in vivo situation than in solution and shows that the electrochemical DNA-biosensor can enable a better understanding of DNA/molecule/ion interactions.

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