Procedure 29

Electrochemical sensing of DNA damage by ROS and RNS produced by redox activation of quercetin, adriamycin and nitric oxide

Victor C. Diculescu, José A.P. Piedade, Sílvia H.P. Serrano and Ana M. Oliveira-Brett

29.1 OBJECTIVES

The electrochemical study of the *in situ* interaction of quercetin, adriamycin, DETA/NO and their metabolites with double-stranded DNA (dsDNA) immobilized on a glassy carbon electrode (GCE) surface.

29.2 MATERIALS AND INSTRUMENTS

Sodium salt calf thymus dsDNA (type II), quercetin, $CuSO_4$ and DETA/NO from Sigma and adriamycin from Pharma-APS are used without further purification.

Five hundred micromolar saturated quercetin, 1 mM $CuSO_4$ and different concentrations of adriamycin are prepared by solubilization of an adequate quantity of the reagents in pH 4.5 0.1 M acetate buffer electrolyte.

DETA/NO stock solution is prepared in a 10 mM NaOH solution achieving a final concentration of 10 mM DETA/NO. The stock solution was kept at $-18^{\circ}C$.

Stock solutions of 35 mg ml^{-1} and 50 μg ml^{-1} dsDNA are prepared in pH 4.5 0.1 M acetate buffer and are stored at $-4^{\circ}C$.

pH 4.5 0.1 M acetate buffer as supporting electrolyte is prepared in Milli-Q water (conductivity $\leqslant 0.1\,\mu S\ cm^{-1}$).

Nitrogen-saturated solutions are obtained by bubbling high-purity N_2 for a minimum of 10 min in the solution and continuing

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with a flow of pure gas over the solution during the voltammetric experiments.

Microvolumes are measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA).

The pH measurements are carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All the experiments are done at room temperature ($25 \pm 1^{\circ}C$).

To study the interaction between quercetin, quercetin–Cu(II) and nitric oxide with DNA, a GCE ($d = 1.5\,mm$) working electrode, a Pt wire counter electrode and a Ag/AgCl (sat. KCl) as reference electrode are used in a 0.5 ml one-compartment electrochemical cell.

To study the interaction between adriamycin and DNA, a GCE ($d = 6\,mm$) working electrode, a Pt wire counter electrode and a saturated calomel electrode (SCE) as reference electrode are used in a 5 ml one-compartment electrochemical cell.

Voltammetric experiments are carried out using an Autolab PGSTAT 10 running with GPES version 4.9 software, Eco-Chemie, Utrecht, The Netherlands.

29.3 CONSTRUCTION OF DNA-BIOSENSORS

Polish the GCE using diamond spray (particle size 1 μm) before every electrochemical assay. After polishing, rinse the electrode thoroughly with Milli-Q water for 30 s, then sonicate for 1 min in an ultrasound bath and again rinse with water. After this mechanical treatment, place the GCE in pH 4.5 0.1 M acetate buffer electrolyte and record various DP voltammograms until a steady-state baseline voltammogram is obtained. This procedure is fundamental in order to obtain reproducible experimental results.

- *Thick-layer* dsDNA biosensor: prepare by covering the GCE surface with 10 μL of $35\,mg\,mL^{-1}$ dsDNA solution and allow it to dry in normal atmosphere.
- *Multi-layer* dsDNA biosensor: prepare by successively covering the GCE ($d = 1.5\,mm$) surface with three drops of 5 μL each of $50\,\mu g\,mL^{-1}$ dsDNA solution. After placing each drop on the electrode surface of the biosensor, dry under a constant flux of N_2.
- *Thin-layer* dsDNA biosensor: prepare by immersing the GCE in a $60\,mg\,mL^{-1}$ dsDNA solution and apply a potential of +0.40 V during 10 min.

29.4 ELECTRICAL TRANSDUCTION OF DNA DAMAGE

Always monitor the electrical transduction of DNA damage by differential pulse voltammetry (DPV). Between recording voltammograms always keep the working electrode at a standby potential of 0 V. Before recording a voltammogram use an equilibration time of 5 s. The experimental conditions for DPV are as follows: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}.

29.5 ACQUISITION AND PRESENTATION OF VOLTAMMETRIC DATA

All the voltammograms presented are background-subtracted and baseline-corrected using the moving average application with a step window of 5 mV included in GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artefact, although the peak intensity is in some cases reduced ($<10\%$) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms is used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all graphs are determined from the original untreated voltammograms after subtraction of the baseline.

29.6 QUERCETIN–DSDNA INTERACTION

- After construction of the biosensor (thick- or multi-layer) run a DPV scan in pH 4.5 0.1 M acetate buffer to obtain the background response.
- To study the interaction between dsDNA and quercetin–Cu(II) ion complex, the newly prepared biosensor (thick- or multi-layer) is immersed for different periods in a solution of 100 μM quercetin previously incubated for 3 h with 50 μM $CuSO_4$. After the incubation of the biosensor in the quercetin–Cu(II) solution, the electrode is washed in water and transferred to pure supporting electrolyte where transduction is performed.
- To study the interaction between dsDNA and quercetin metabolites, a multi-layer newly prepared biosensor is immersed for 10 min in a solution of 100 μM quercetin. After incubation, the biosensor is washed in water and transferred to another electrochemical cell that contains only pure supporting electrolyte. A

potential of +0.40 V is applied for different periods of time, followed by transduction in the same electrochemical cell [1].

29.7 ADRIAMYCIN–DSDNA INTERACTION

- The thick-layer biosensor is immersed in a 0.1 μM of adriamycin for different times and the differential pulse voltammograms are recorded, in supporting electrolyte, after each immersion time.
- To evaluate the effect of the adriamycin reduction products on the dsDNA, the thin-layer dsDNA electrochemical biosensor is immersed for 3 min in an 0.1 μM adriamycin solution, rinsed with water and afterwards transferred to buffer, where DPV is performed. The procedure is repeated with a new biosensor, but after being transferred to buffer, the sensor is subjected to an applied potential of –0.60 V during 30 s [2].

29.8 DETA/NO–DSDNA INTERACTION

- After construction of the biosensor (thick- or multi-layer), run a DPV scan in pH 4.5 0.1 M acetate buffer to obtain the background response.
- The thick-layer biosensor is immersed for 5 min in a 100 μM solution of DETA/NO in pH 4.5 0.1 M acetate buffer. Then the biosensor is washed in deionized water and transferred to pure acetate buffer where a potential of –0.60 V is applied for 3 min. Transduction is then performed in the same cell containing only electrolyte.
- A multi-layer biosensor is immersed for different periods of time in solutions of DETA/NO of different concentration, depending on the experiment (for more details see Chapter 20, Section 20.4.3.2) whilst maintaining the potential at –0.60 V. Then the biosensor is washed in deionized water and transferred to pure acetate buffer where transduction is performed [3].

29.9 DISCUSSION

The structure of dsDNA makes access of the bases to the electrode surface difficult, hindering their oxidation. The occurrence of DNA damage causes the unwinding of the double helix. As the double helix unwinds, closer access of the bases to the surface is possible, hence they

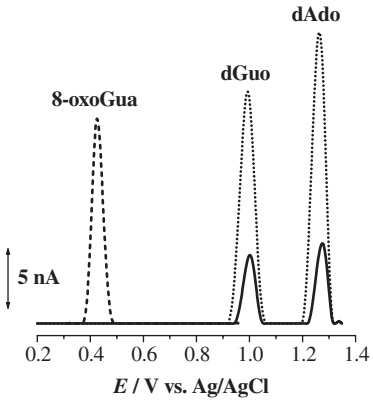

Fig. 29.1. Base line corrected DP voltammograms of (- - -) 5 μM 8-oxoGua and of 60 $\mu g\,mL^{-1}$ (···) ssDNA and (—) dsDNA in pH 4.5 0.1 M acetate buffer.

can be oxidized, leading to the possibility of voltammetric detection of DNA damage [4–6].

Following the increase in the height of the DNA bases' oxidation peaks and/or searching for new electrochemical signals such as of 8-oxoguanine, Fig. 29.1, the interaction of DNA with different compounds is evaluated.

SELECTED LITERATURE

1 A.M. Oliveira-Brett and V.C. Diculescu, Electrochemical study of quercetin–DNA interactions. Part II—*In situ* sensing with DNA-biosensors, *Bioelectrochemistry*, 64 (2004) 143–150.
2 A.M. Oliveira Brett, M. Vivan, I.R. Fernandes and J.A.P. Piedade, Electrochemical detection of *in situ* adriamycin oxidative DNA damage to DNA, *Talanta*, 56 (2002) 959–970.
3 V.C. Diculescu, R.M. Barbosa and A.M. Oliveira–Brett, *In situ* sensing of DNA damage by a nitric oxide-releasing compound, *Anal. Lett.*, 38 (2005) 2525–2540.
4 A.M. Oliveira-Brett, J.A.P. Piedade, L.A. Silva and V.C. Diculescu, Voltammetric determination of all DNA nucleotides, *Anal. Biochem.*, 332 (2004) 321–329.
5 A.M. Oliveira Brett, J.A.P. Piedade and S.H.P. Serrano, Electrochemical oxidation of 8-oxoguanine, *Electroanalysis*, 13 (2001) 199–203.
6 A.M. Oliveira Brett and S.H.P. Serrano, Development of DNA-based biosensors for carcinogens. In: P. Frangopol, D.P. Nikolelis and U.J. Krull (Eds.), *Current Topics in Biophysics, Vol. 2, Biosensors*, Al. I. Cuza University Press, Iaşi, Romania, 1997, pp. 223–238, Chapter 10.