Flow-through electrochemical system with the DNA-based biosensor for the evaluation of deep DNA damage by chemicals and effect of antioxidants

Darina Šimková*, Ernest Beinrohr, Ján Labuda

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovakia

*simkovad@gmail.com, jan.labuda@stuba.sk

Abstract

In this work we present an electrochemical DNA biosensor arranged in a flow-through cell which was used for the evaluation of DNA damage. The DNA-based biosensor is created using a layer double stranded calf thymus DNA deposited on the surface of a screen-printed carbon electrode. The DNA damage by the Fenton reaction agents is detected by cyclic voltammetry parameters of the $[Fe(CN)_6]^{3-/4-}$ complex used as a redox indicator in the phosphate buffer solution. Electrochemical impedance spectroscopy is used for the verification of changes of the DNA layer. The biosensor was also used for the detection of antioxidative effects of standard chemicals and tea extracts.

Keywords: DNA damage, biosensor, flow-through electrochemical system, screen-printed carbon electrode

Introduction

Flow-through analytical systems represent a way to automation of work, fast analysis, reduction of errors caused by the exchange of solution and operator and thus to better accuracy and precision of the results than more or less tedious conventional sample treatment approach. The FIA principles are successfully utilized also in analysis with biosensors of various detection principles (Canh 1993). DNA-based biosensors belong to relatively new types of the biosensors. It is of great interest to couple also an electrochemical DNA-based biosensor within the electrochemical flow-through system.

According to the IUPAC document, an electrochemical biosensor is defined as a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is retained in direct spatial contact with an electrochemical transduction element (Thévenot et al. 1999). The biosensors based on DNA immobilized at the surface of an electrode are of particular interest for simple *in vitro* tests of (bio)chemical toxicity. As DNA belongs to substrates which change their structure *in vivo* under conditions of oxidative stress, it is also used to estimate an effect of antioxidants. For this purpose, various experimental arrangements including the biosensors are used (Ferancová et al. 2004).

Disposable electrochemical sensors, like screen printed electrodes (SPE), became of importance as mass-produced and simple, inexpensive, non-toxic and widely accessible analytical tools. The integrity of the nucleic acid layer at the screen printed carbon electrode (SPCE) surface can be simply indicated voltametrically using various indicators attached to the DNA or present in solution as well as impedimetrically. The cyclic voltammetry (CV) picture of a redox indicator like the $[Fe(CN)_6]^{3-/4-}$ anion is typically used for a simple electrode surface characterization (Maeda et al. 1992). In the case of DNA modified electrode, electrochemical reversibility of this redox system and thus the response are decreased due to a repulsion effect of the negatively charged DNA backbone towards $[Fe(CN)_6]^{3-/4-}$. On the other hand, they are improved with structural degradation of the DNA layer (Ovádeková et al. 2006).

There are chemical and biochemical reaction systems known to produce reactive oxygen species damaging DNA. The Fenton reaction is widely used to form hydroxyl radicals. Among antioxidants, phenolic compounds (polyphenols) represent one of the most important groups of compounds occurring in plants. Flavonoids and phenolic acids constitute classes of polyphenols with more than 5000 compounds already described. Rutin (glycoside of polyphenol quercetin) is the flavonoid with various biological activities beneficial to human health such as (Ferancova et al. 2004).and antiinflammatory effects (Kim et al. 2005). Caffeic acid is a natural standard antioxidant contaning both phenolic and acrylic functional groups. Chlorogenic acid is one of the phenolic compounds found in coffee as well as many other plants and known as an antioxidant. Ferulic acid is a phenolic phytochemical found in plant cell walls. Gallic acid is found both free and as part of tannins. Sinapinic or sinapic acid

is a member of the phenylpropanoid family. These compounds are used as antioxidant standards to test the test devices (Rice-Evans et al. 2003).

The development of a flow-through electrochemical system with the DNA-based biosensor for the detection of DNA damage and DNA protection was the aim of this study.

Materials and Methods

Reagents

Calf thymus dsDNA was purchased from Merck, Germany. Their stock solution (0.1 mg mL^{-1}) was prepared in 0.1 mol L^{-1} phosphate buffer solution (PBS) at pH 6.9 and stored at -4 °C. $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were purchased from Merck, Germany. Their stock solution of $1x10^{-3}$ mol L^{-1} of each of the compounds was prepared in PBS, pH 6.9. Caffeic, chlorogenic, ferulic, gallic, sinapic acids and rutin were purchased from Fluka, Germany. Their stock solutions were prepared in 0.1 mol L^{-1} phosphate buffer solution (PBS) at pH 6.9. Other chemicals used were of analytical reagent grade purity and used as received. Tea extracts were prepared by soaking one commercially tea bags in 250 ml of boiled (98 ˚C) or hot (70 ˚C) water for 5 min as time recommended by the tea producers. Tea extracts were tested after cooling to laboratory temperature and filtration through filter paper without any other pre-treatment. De-ionized and double-distilled water was used for measurements.

Apparatus

An automatic flow-through coulometer/ voltammeter EcaFlow Model 150 (from Istran Ltd., Bratislava, Slovakia) equipped with a flow-through cell for screen-printed electrodes was used in the experiments. The electrochemical DropSens flow-through cell (Oviedo, Spain) consists of the working electrode (4 mm diameter screen-printed carbon electrodes DropSens 110, SPCE), carbon auxiliary electrode and silver reference electrode. The DNA layer was formed at the SPCE by the application of 4 μL of the DNA stock solution and evaporation to dryness. The flow chart diagram of the instrument is depicted in Fig. 1. The system also facilitated an automatic calibration by making use of the standard addition technique. The aspirated sample solution was automatically segmented and mixed with the carrier electrolyte. The electrochemical impedimetric spectroscopy (EIS) measurements were carried out using the Autolab/FRA system with the potentiostat PGSTAT 12 and FRA-DSG, FRA-ADS

modules (Eco Chemie B.V., Netherland), version 4.9.006. All measurements were performed in 10 ml glass voltammetric cell.

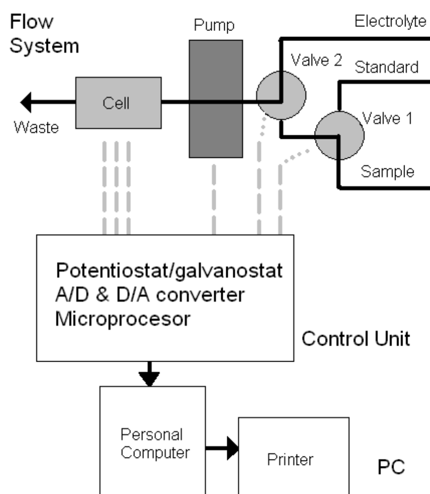

Fig. 1. The block diagram of the automatic flow-through analyser (reproduced by the courtesy of Istran Ltd., Slovakia)

CV measurement

The DNA modified electrode (DNA/SPCE) was placed into the flow-through cell, then, the cell was closed and connected to the ECA flow system via the input and contribution tubing. Finally, the electrode was connected with the device by using a specific cable. Under the manual control, the cell was first filled with 0.1 mol L^{-1} PBS pH 6.9 and, after recording the CV of blank solution, 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ indicator solution (in 0.1 mol L^{-1} PBS pH 6.9) was pumped using the flow rates of 2.5 and 6.0 ml min^{-1} and the CV record was obtained between 600 and -500 mV using the scan rate of 25 mV s^{-1} under static conditions with stopped flow. The attention was paid to have no air bubbles present in the measuring cell. The CV records were obtained in triplicate, for each measurement the cell was filled with new portion of the indicator solution.

Electrochemical impedance spectroscopy

The EIS measurements were carried out with DNA/SPCE placed in a voltammetric cell in 1 mmol L^{-1} $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ in the 0.1 mol L^{-1} PBS pH 6.9 at ambient temperature, at the polarization potential of 0 V in the frequency range of 0.1 – 104 Hz (in 51 frequency steps) and with the amplitude of 10 mV.

DNA damage and antioxidantive effect detection

Prior to the DNA damage, CV and EIS signals of DNA/SPCE were obtained as described above. After rinsing with distilled water, the cleavage agent solution without or with an addition of antioxidants was pumped through the cell and the same electrode was incubated in this solution at ambient temperature for 15 min. After rinsing with distilled water, the cell was filled with 0.1 mol L^{-1} PBS and the CV of blank solution was recorded. Then, the cell was filled with 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ indicator solution and the CV was recorded again.

The normalized signal values were obtained as follows:

$$\Delta I_{rel} = (I_{surv\ DNA} - I_{bare})/(I_{DNA} - I_{bare}) \qquad (1)$$

$$\Delta E_{p,rel} = (\Delta E_{p,surv} - \Delta E_{p,bare})/(\Delta E_{p,DNA}\ \Delta E_{p,bare}) \qquad (2)$$

$$\Delta R_{ct,rel} = (\Delta R_{ct,survDNA} - \Delta R_{ct,bare})/(\Delta R_{ct,DNA} - \Delta R_{ct,bare}) \qquad (3)$$

where I_{bare}, I_{DNA} and $I_{surv\ DNA}$ are the cathodic peak current values of 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ obtained for bare SPCE, DNA/SPCE before and after damage, respectively, all are measured at the peak potential of the redox probe at bare SPCE, ΔE_p are the anodic to cathodic peak potential separation measured at corresponding electrodes and $\Delta R_{ct,bare}$, $\Delta R_{ct,DNA}$ and $\Delta R_{ct,survDNA}$ are electron transfer resistance obtained for the SPCE without DNA, DNA/SPCE before and after damage.

Results and Discussion

First, an effect of the synthetic cleavage mixture composed of H_2O_2 and Fe^{2+} on the DNA/SPCE biosensor was tested using the CV and EIS measurements. These agents are known to produce OH radicals which result in multiple products of the deep DNA degradation such as base loss, base modification, and strand breaks (Ziyatdinova et al. 2008). On Fig 2, the typical CV picture of the redox indicator at the original DNA biosensor and bare SPCE electrode is shown together with records obtained for the biosensor with damaged DNA. The CV parameters and ΔR_{ct} data were evaluated as described in Material and Methods. With respect to differences between individual SPCE strips, the CV parameters and ΔR_{ct} data are expressed as normalized changes of the signals regarding to the parameters obtained for the original biosensor before its treatment in a cleavage agent. It was also confirmed that the individual components of this mixture have no effect on the biosensor signal values. The concentrations of 1.10^{-2} mol L^{-1} H_2O_2 and 1.10^{-4} mol L^{-1} Fe^{2+} were found

as optimum for the deep degradation of the immobilized DNA (about 0.25 portion of survived DNA). The CV results agree well with those for the ΔR_{ct} values obtained by EIS (Fig. 2) where the survived DNA portion of 0.30±0.09 was found.

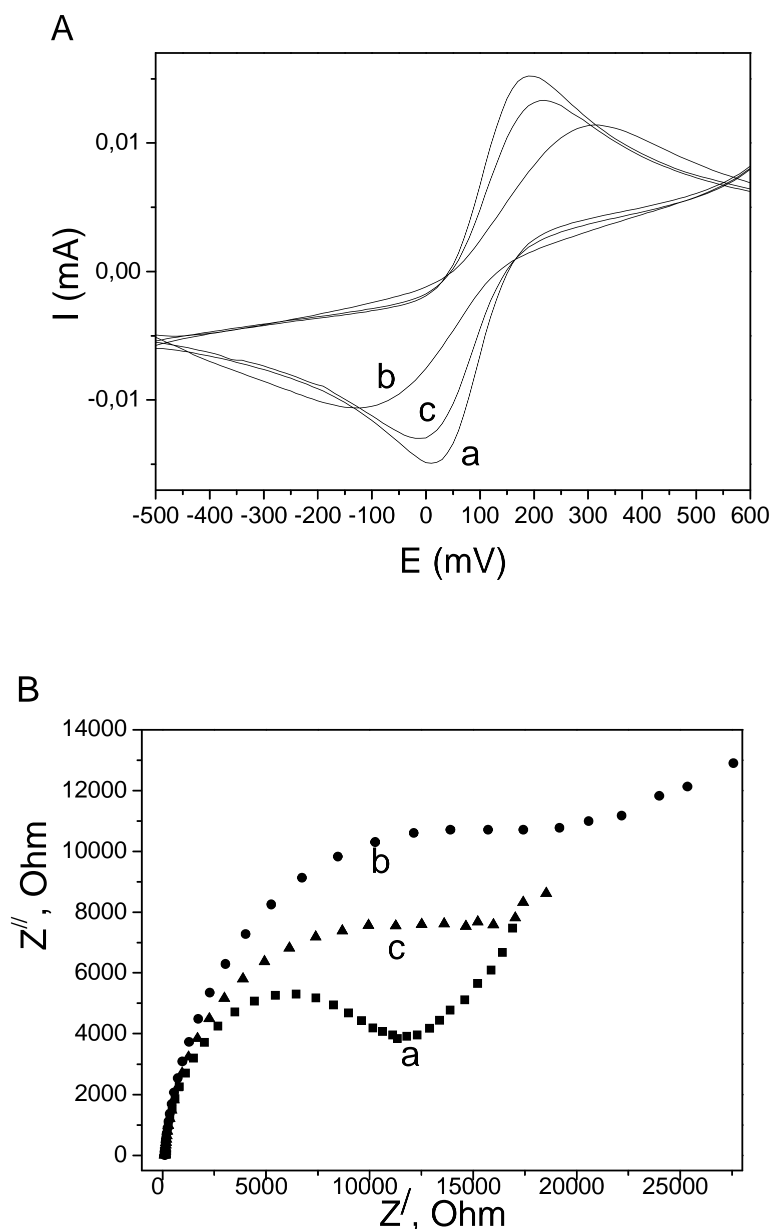

Fig. 2. A) Cyclic voltammogram of 1 mmol L^{-1} $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} PBS pH 6.9 at (a) bare SPCE electrode, (b) and (c) DNA/SPCE before and after the incubation in cleavage mixture of 1.10^{-2} mol L^{-1} H_2O_2 and 1.10^{-4} mol L^{-1} Fe^{2+}, resp., scan rate 25mV s^{-1},

B) Niquist plots in the presence of 1 mmol L^{-1} $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} PBS pH 6.9 for the same types of electrodes, frequency range (0.1-104) Hz, polarization potential 0 V and amplitude 10 mV.

Complex impedance spectra as the Niquist plots (the dependence of an imaginary part of the impedance Z'' *vs.* a real part of the impedance Z') represent semicircles at high frequencies corresponding to the electron transfer limiting process. For the bare SPCE and DNA/SPCE electrodes, there are short linear parts at low frequencies resulting from the diffusion limiting step of the electrochemical process (Lasia.et al.1999) It should be noted that this part of the spectrum represents the properties of the electrolyte solution and the diffusion of the redox indicator and, thus, are not affected by the modification of the electrode surface (Yang et al. 2005). The respective semicircles diameters at the high frequencies corresponding to the charge transfer resistance at the electrode surface increase in the presence of DNA on the electrode surface. Thus, the charge transfer resistance was used as a sensor signal. The impedance data were simulated using the Randles equivalent circuit (Fig. 3) consisting of a parallel combination of the capacitance (C) and charge transfer resistance by redox reactions (R_{ct}) in series with the supporting electrolyte resistance (R_{sol}). The fitting of spectra to the equivalent circuit has indicated a good agreement between the circuit model and the real experimental data, especially at the high frequency values.

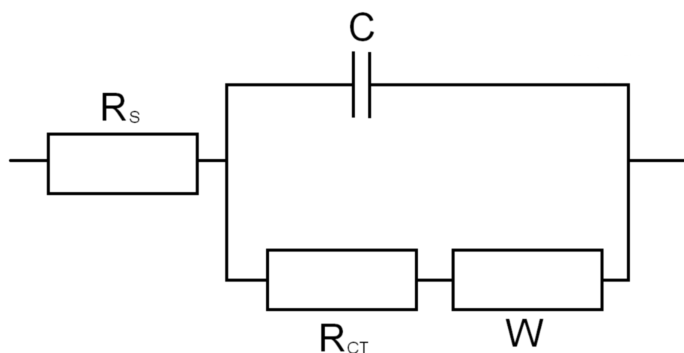

Fig. 3. Impedimetric equivalent circuit. R_{sol} – solution (supporting electrolyte) resistance, R_{ct} – charge transfer resistance, C – capacitance, W- Warburg impedance.

Standard chemicals such as rutin and phenolic acids as well as tee extracts added to this cleavage mixture were further tested (Tables 1 and 2). A great DNA protective effect can be seen leading to the portion of survived DNA between 0.7 and 0.8. This observation is similar to that reported in literature (Ferancova et al.2004)

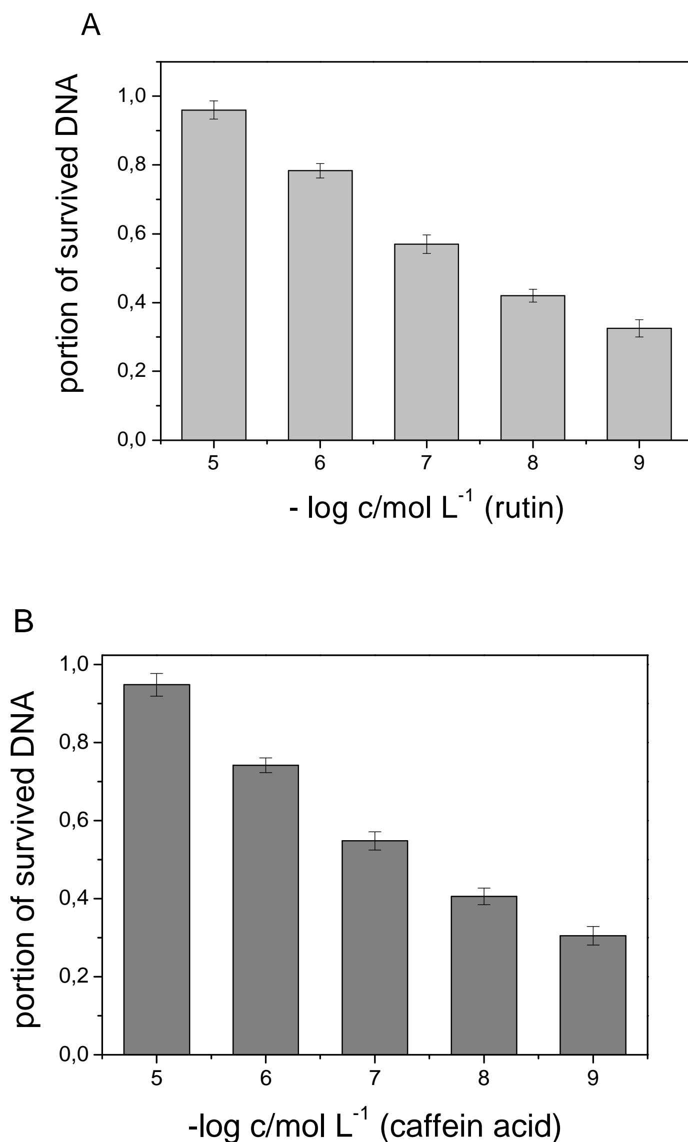

Fig. 4. Antioxidantive effect of rutin (A) and caffein acid (B) at DNA damage evaluated using the normalized CV peak current values. Conditions: 15 min incubation of DNA/SPCE in the mixture of 1.10^{-2} mol L^{-1} H_2O_2 and 1.10^{-4} mol L^{-1} Fe^{+2} with rutin or caffein acid in 0.1 mol L^{-1} PBS pH 6.9 at ambient temperature.

The biosensor was also able to detect a concentration dependence of the antioxidative effect of rutin and caffeic acid (Fig. 4a,b).

Table 1. Effect of phenolic acids as antioxidants added to the DNA cleavage mixture composed of 1.10^{-2} mol L^{-1} H_2O_2 and 1.10^{-4} mol L^{-1} Fe^{+2}. Conditions: 15 min incubation of the DNA/SPCE biosensor at ambient temp., CV measurement of 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ indicator in 0.1 mol L^{-1} PBS pH 6,9. Errors represent the RSD values for n=3.

1.10^{-6} mol L^{-1} antioxidant	**ΔI_{rel}**	**$\Delta E_{p,\,rel}$**	**$\Delta R_{ct,\,rel}$**
None	0.240±0.020	0.250±0.036	0.31±0.10
Rutin	0.790±0.016	0.544±0.026	-
Caffeic acid	0.715±0.018	0.631±0.022	-
Ferulic acid	0.761±0.016	0.550±0.027	-
Gallic acid	0.701±0.015	0.582±0.026	-
Chlorogenic acid	0.721±0.014	0.610±0.023	-
Sinapic acid	0.724±0.019	0.520±0.024	-

Table 2: Evaluation of antioxidative properties of various teas extracts using the DNA/SPCE. Conditions: Incubation of DNA/SPCE in the mixture of 1.0×10^{-4} mol L^{-1} Fe^{2+}, 1×10^{-2} mol L^{-1} H_2O_2 and the tea extract mixed in 1:1 volume ratio for 15 min, CV in 1mmol L^{-1} $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} PBS pH 6.9.

Tea sample	**ΔI_{rel} for extracts obtained at**	
	70 ˚C	**98 ˚C**
Green	0.761±0.017	0.865±0.019
Black	0.662±0.019	0.810±0.018
Ginkgo	0.365±0.021	0.592±0.020

There are significant differences between the effects of individual extracts. It can be concluded that, the data obtained follow typically reported antioxidant protective effects of teas depending on the extracts preparation. During the experiments it was also confirmed that the prepared DNA/SPCE sensor exhibits good storage stability in dry conditions and ambient temperature. The biosensors were stored in dry conditions and ambient temperature and used after 1 to 3 days of storage.

Conclusions

New bioanalytical system is described consisting of a commercial flow-through electrochemical cell and DNA-modified commercial screen-printed carbon electrode forming the DNA biosensor. Simple methods of cyclic voltammetry and impedimetry with the redox indicator in solution which can be performed in the same cell, are used to test chemical agents leading to oxidative *in vitro* damage to DNA as well as standard agents and tea extracts with antioxidative effect leading to the protection of DNA. Stability of the biosensor and availability of the system are promising for the wide application for tests of DNA damage and evaluation of antioxidants.

Acknowledgements

This work was supported by the Applied Research Project of the Ministry of Education of Slovak Republic (AV/4/0103/06).

References

Canh T M, Biosensors (1993), Chapman & Hall, London.

Ferancová A, Heilerová L, Korgová E, Šilhár S, Štepánek I, Labuda J (2004), European Food Research and Technology 219: 416- 419.

Kim H, Kong H, Choi B, Yang Y, Kim Y, Lim M J (2005), Pharmaceutical Research 22: 1499–1509.

Lasia A, in *Modern Aspects of Electrochemistry* (1999), Eds. Bockris J and White R E, Kluwer Academic/Plenum Publishers, New York.

Maeda M, Mitsuhashi Y, Nakano K, Takagi M (1992), Analytical Sciences 8: 83.

Ovádeková R, Jantová S, Letašiová S, Štepánek I, Labuda J (2006), Analytical and Bioanalytical Chemistry 386: 2055 - 2062.

Rice-Evans C, Parker L (2003), Flavonoids in Health and Disease, Marcel Dekker Inc, New York

Thévenot D R, Toth K, Durst R A, Wilson G S (1999), Pure Applied Chemistry 71: 2333 - 2348.

Yang L, Li Y(2005), Biosensors and Bioelectronics 20: 1407.

Ziyatdinova G, Galandová J, Labuda J (2008), International Journal of Electrochemical Science 3: 223 – 235.