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Affinity electrochemical biosensors for pollution control*

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Abstract: Disposable, electrochemical DNA-based biosensors have been exploited for the determination of low-molecular-weight compounds with affinity for nucleic acids. The application is related to the molecular interaction between the surface-linked DNA obtained from calf thymus and the target pollutants or drugs, in order to develop a simple device for rapid screening of genotoxic or similar compounds. The determination of such compounds was measured by their effect on the oxidation signal of the guanine peak of the DNA immobilized on the electrode surface and investigated by chronopotentiometric or square-wave voltammetric analysis. The DNA biosensor is able to detect known intercalating and groove-binding compounds such as daunomycin, polychlorinated biphenyls (PCBs), aflatoxin B1, and aromatic amines. Applicability to river and waste water samples is discussed and reported.

INTRODUCTION

DNA electrochemical biosensors, realized by immobilizing on a suitable electrode surface an oligonucleotide sequence of the calf thymus DNA, are simple to assemble and can provide reliable results; such DNA biosensors hold an enormous potential for environmental monitoring [11,12].

A major application of a DNA biosensor will be the testing of water, food, soil, and plant samples for the presence of analytes (carcinogens, drugs, mutagenic pollutants, etc.) with binding affinities for the structure of DNA. Binding of small molecules to DNA and generally DNA damage by ionizing radiation, dimethyl sulphate, etc. has been described through the variation of the electrochemical signal of guanine [5–10].

The objective of our work was to develop disposable, electrochemical DNA sensors to evaluate the presence of small DNA-binding compounds by measuring changes of the electrochemical signal of guanine in calf thymus DNA extract. Single-use sensors have several advantages, such as avoidance of contamination among samples, constant sensitivity and reproducibility, and ease of use [1].

This biosensor is realized by immobilizing calf thymus DNA onto the electrode surface [3,4,12]. The DNA biosensor was then immersed in the sample solution containing the analyte. After 2 min of interaction, the DNA sensor is washed, immersed in a suitable clean buffer, and a chronopotentiometric analysis (PSA) was carried out to evaluate the oxidation of guanine residues on the electrode surface. We report some preliminary experiments showing clear electrochemical effects due to the presence of genotoxic compounds. We can extrapolate and evaluate such electrochemical signals as resulting from potentially genotoxic compounds present in real water samples.

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PROCEDURES

Electrochemical measurements

All electrochemical measurements were carried out at room temperature in 2-mL Teflon beaker. Potentiometric stripping analysis at a constant current (PSA) was performed with the following parameters; the potentials were sampled at a frequency of 33 KHz, and the derivative signal (dt/dE) was recorded vs. the potential.

The electrodes modified by calf thymus ssDNA immobilization were examined in 0.2 M acetate buffer pH 5.0, using an initial potential of +0.5 V and a constant current of +6 µA. The guanine peak area following baseline fitting was used as the analytical signal.

DNA sensor for binding compounds with affinity for DNA

The procedure consisted of the following steps: calf thymus DNA immobilization on the electrode surface, dipping the electrode in the sample/blank solution, and electrochemical interrogation of the surface.

Calf thymus immobilization consisted of an electrochemical oxidation (+1.6 V vs. SCE for 1 min in 0.2 M acetate buffer pH 5.0), then the electrode was immersed in a stirred buffer solution containing 10–20 mg 1-1 of single-stranded or double-stranded calf thymus DNA. This immobilization step lasted for 2 min holding the electrode surface at a potential of +0.5 V vs. SCE. The electrode was then washed with buffer solution for 10 s. The modified electrode was placed in a sample solution for 2 min. A chronopotentiogram was carried out in 0.2 M acetate buffer pH 5.0 by using an initial potential of +0.5 V and a constant current of +6 µA; the guanine peak area is obtained at around +1 V (see Fig. 1).

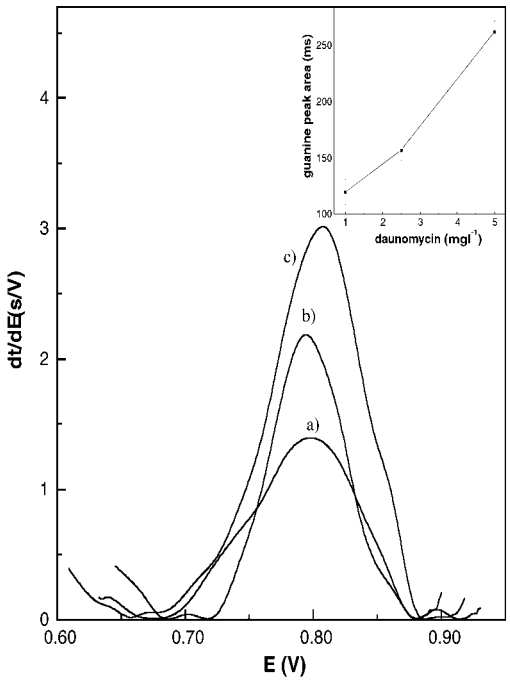


Fig. 1 Chronopotenotiograms for modified screen-printed electrodes calf thymus ssDNA obtained increasing the daunomycin concentrations a) 1 mg 1^{-1}, b) 2.5 mg 1^{-1}, c) 5 mg 1^{-1}. Calf thymus ssDNA immobilization: 10 mg 1^{-1} dsDNA for 2 min at +0.5 V vs Ag/AgCl. PSA transduction: in 0.2 M acetate buffer pH 5.0 with a stripping constant current of +1µA and an initial potential of +0.5 V.

This area is compared with the area obtained when the analyte concentration in the sample solution is zero. In some cases, the sample solvent was up to 10% of methanol to allow analyte dissolution.

Analysis of river waste sample

A preconcentration step of river water was found necessary to obtain some signals through this technique. The water samples were prefiltered through a 0.45-µm filter to clarify the water. Then 1 L of sample water was passed through a Isolute^{TM} column SPE. The organic compounds extracted were eluted using 500 µL ethyl acetate. The samples obtained were dried, then 10 ml of phosphate buffer containing 3% v/v methanol were added. The analysis was carried out as described before. The extracts of the water samples were also analyzed using a standard method with a Varian 3400 gas chromatograph coupled to a Finnigan Mat 800 ion trap detector mass spectrometer (GC-ITDMS).

RESULTS

DNA sensor for binding compounds with affinity for DNA

Preliminary studies were performed to identify general assay conditions which affected the electrochemical signal of the guanine oxidation peak, such as ionic strength, pH, buffer composition, DNA concentration, and form (single stranded and double stranded). Figure 2 reports the guanine peak area obtained as function of single-stranded calf thymus concentration. The area increases linearly with concentration up to 20 mg 1^{-1} then levels off. This value was generally used.

Table 1 reports the values of the guanine peak area as a function of the stripping oxidation current value. The area is smaller with larger currents, but the peak appeared very broad and not reproducible (a large RSD). 6 µA was then generally used.

The guanine peak appears very sharp in acetate buffer; the baseline at this high potential value was lower than in other buffer. The modified electrode performance was tested in buffer solution con-

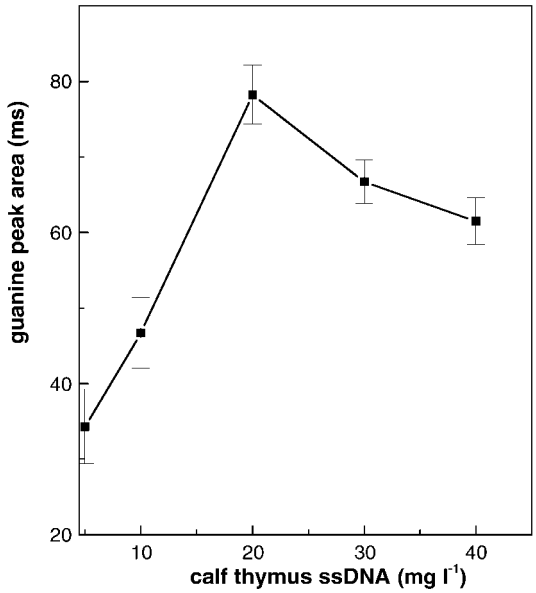


Fig. 2 Anodic peak of the guanine obtained increasing the concentration of calf thymus ssDNA for immobilization. Calf thymus ssDNA immobilization: 2 min at +0.5 V vs SCE in 0.2 M acetate buffer pH 5.0. PSA transduction: in 0.2 M acetate buffer pH 5.0 with a stripping constant current of +6 µA and an initial potential of +0.5 V.

Table 1 Effect of stripping current on guanine peak area using single-stranded calf thymus DNA. Single-stranded calf thymus DNA immobilization: 20 mg l^{-1} in 0.2 M acetate buffer solution pH 5.0 for 2 min. at +0.5 V vs. SCE. PSA conditions: in 0.2 M acetate buffer solution pH 5.0 with an initial potential of +0.5 V. The area is reported with each measurement repeated 4 times.

Stripping oxidation current (μA)	Guanine peak area (ms)
1	339 ± 60
2	268 ± 51
4	103 ± 33
6	78 ± 5
8	32 ± 7

Table 2 Compounds tested with single- or double-stranded calf thymus DNA immobilized on screen-printed electrodes. Calf thymus DNA immobilization: 20 mg l^{-1} of single- or double-stranded calf thymus DNA in 0.2 M acetate buffer solution pH 5.0 for 2 min at +0.5 V vs. SCE. PSA conditions: in 0.2 M acetate buffer solution pH 5.0 with a stripping current of +6 μA and an initial potential of +0.5 V. Buffer solution: 0.2 M acetate buffer acetate solution pH 5.0 with 10% v/v of methanol. The area is reported with each measurement repeated 4 times.

Compounds tested	Guanine peak area (ms) using calf thymus dsDNA immobilized	Guanine peak area (ms) using calf thymus ssDNA immobilized
buffer solution	36 ± 7	89 ± 13
Daunomycin	52 ± 6	86 ± 3
Phthalates mixture (20 mg l^{-1})	39 ± 16	80 ± 26
Atrazine (50 mg l^{-1})	66 ± 39	85 ± 11
Bisphenol (100 mg l^{-1})	76 ± 13	85 ± 17
PCB 105 (0.4 mg l^{-1})	54 ± 8	68 ± 7
PCB mixture (Aroclor 1260) (20 mg l^{-1})	39 ± 5	99 ± 7
PCB mixture (Aroclor 1016) (20 mg l^{-1})	43 ± 8	95 ± 17
Aflatoxin B_1 (10 mg l^{-1})	36 ± 8	77 ± 7
cisplatin (30 mg l^{-1})	58 ± 14	78 ± 6
Hydrazine (20 mg l^{-1})	22 ± 4	73 ± 6

taining methanol up to 10% v/v, and we observed any variation of the electrochemical signal of guanine peak. Methanol is useful for dissolving some organic compounds.

We performed several preliminary experiments for evaluating the variation of the area of the guanine peak obtained using single-stranded or double-stranded DNA immobilized when the sample contained different compounds of environmental interest.

Table 2 summarizes these experiments showing that the guanine peak is higher for ssDNA; the guanine base in single-stranded is obviously more readily available for oxidation than in double-stranded DNA.

Compounds behaving as intercalators, such as daunomycin or cisplatin, showed an increase in the guanine peak area when using dsDNA (Table 2). In this case, we observe a linear increase with the daunomycin concentration in the range 1–10 mg 1^{-1}.

Aflatoxins are metabolites produced by some strains of the mould *Aspergillus flavus*. They are among the most potent environment mutagens and are implicated as liver carcinogens. The binding of the aflatoxin B1 to both native and denatured DNA has been demonstrated [2].

Figures 3a–b demonstrate the applicability of the DNA sensor to the analysis of these pollutants. We obtained the gradual decrease of the guanine peak in the presence of increasing levels of the afla-

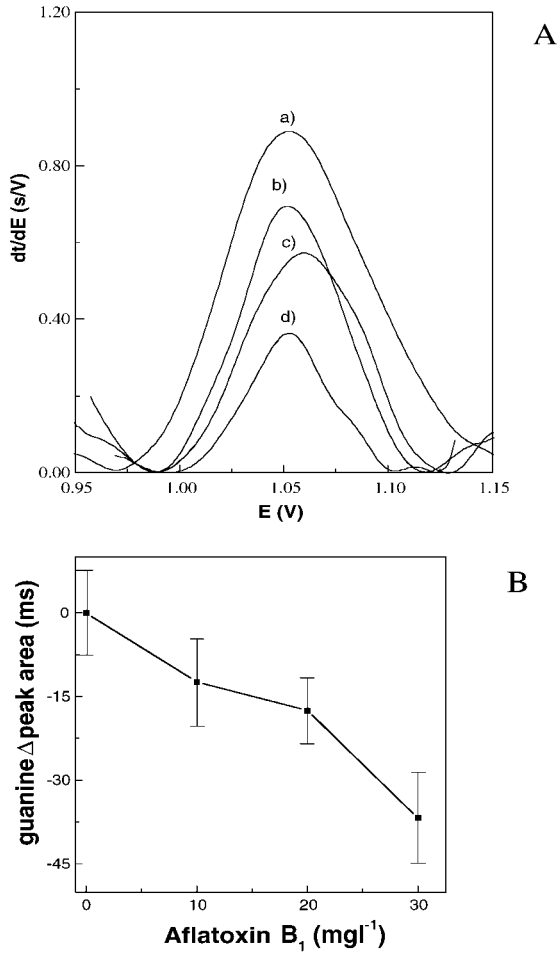


Fig. 3a Chronopotentiograms for a) calf thymus ssDNA-modified screen-printed electrodes and followed increasing aflatoxin B_1 concentrations, b) 10 mg 1^{-1}, c) 20 mg 1^{-1}, d) 30 mg 1^{-1}.

Fig. 3b Calibration curves obtained increasing the concentration of aflatoxin B_1. The results correspond to the difference between the guanine peak area after interaction with aflatoxin B_1 minus that obtained for the buffer solution. Calf thymus ssDNA immobilization: 20 mg 1^{-1} calf thymus ssDNA for 2 min at +0.5 V vs SCE. PSA transduction: in 0.2 M acetate buffer pH 5.0 with a stripping constant current of +6 μA and an initial potential of +0.5 V.

toxin B1. Such suppression of the guanine response results in a well-defined concentration dependence and offers convenient quantification of low levels of aflatoxin. The signal was observed within the 10–30 mg 1^{-1} (Fig. 3b).

In most of the compounds, ssDNA gave greater effects. The peak area of guanine decreased even when low concentrations were present (PCB 0.2 mg 1^{-1}). This can be explained by a binding of the compounds with guanine in short time (2 min) and a lower availability of guanine for oxidation at the electrode surface.

PCBs have been recognized for several years as ubiquitous environmental pollutants. The high toxicity of some of the PCB congeners represent a risk for the public health ,as these molecules are still present in the environment, even though the production of PCB has been banned. The screen-printed electrodes modified with single stranded calf thymus DNA were used to detect PCB 105. Figures 4a–b display the chronopotentiometric response of calf thymus ssDNA-modified electrode followed increasing PCB 105 concentrations.

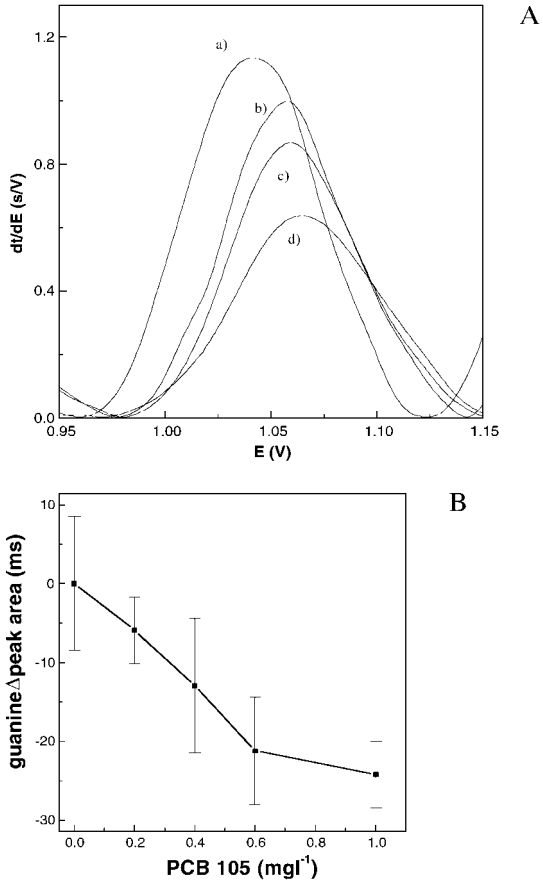
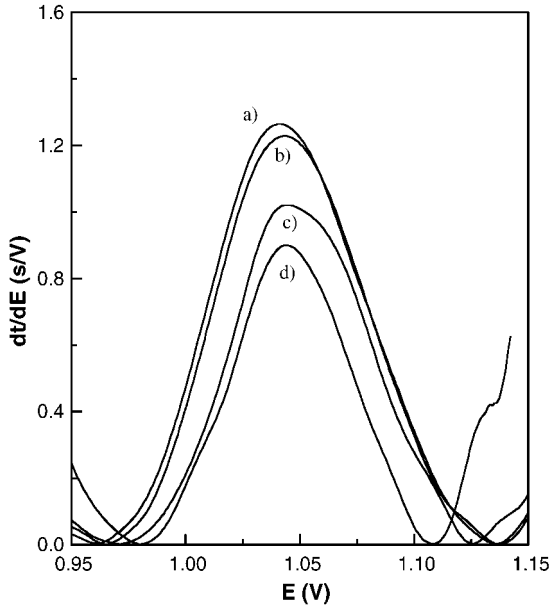


Fig. 4a Chronopotentiograms for a) calf thymus ssDNA-modified screen-printed electrodes and followed increasing PCB 105 concentration, b) 0.2 mg 1^{-1}, c) 0.6 mg 1^{-1}, d) 1 mg 1^{-1}.

Fig. 4b Calibration curves obtained increasing the concentration of PCB 105. The results correspond to the difference between the guanine peak area after interaction with PCB 105 minus that obtained for the buffer solution. Calf thymus ssDNA immobilization: 20 mg 1^{-1}, calf thymus ssDNA for 2 min at +0.5 V vs SCE. PSA transduction: in 0.2 M acetate buffer pH 5.0 with a stripping constant current of +6 μA and an initial potential of +0.5 V.



a) Peak area: 89 ± 13 ms
b) Peak area: 94 ± 7 ms
c) Peak area: 61 ± 8 ms
d) Peak area: 48 ± 9 ms

Fig. 5 Chronopotentiograms obtained using calf thymus ssDNA modified screen-printed electrode with: a) blank solution, b), c) and d) river water (100 times concentrated). Calf thymus ssDNA immobilization: 20 mg l^{-1} calf thymus ssDNA for 2 min at +0.5 V vs. SCE. PSA transduction: in 0.2 acetate buffer pH 5.0 with a stripping constant current of +6 µA and an initial potential of +0.5 V.

Table 3 Results of the water samples obtained using a standard method with a Varian 3400 gas chromatograph coupled to a Finningan Mat 800 ion trap detector mass spectrometer (GC-ITDMS).

	b (ng l^{-1})	c (ng l^{-1})	d (ng l^{-1})
desetil-terbutilazine	0	12	21
carbofuran	0	210	101
simazine	0	0	23
terbutilazine	79	28	81
etofumesate	6	184	83
alachlor	0	0	27
metolachlor	7	14	225

A decrease of guanine peak area could be detected with some water samples (Fig. 5, curves b, c). By HPLC analysis we obtained the results reported in Table 3.

It seems that there is an approximate relationship between the two sets of analysis. The sensor is not able to distinguish between compounds of environmental concern, but could be conveniently used as a screening tool of toxicity.

CONCLUSIONS

The potential of DNA biosensors for detection of toxic compounds has been demonstrated. This kind of procedure offers a sensitive, rapid, and portable tool for field monitoring of several environmentally and toxicologically significant compounds. Moreover, we found that calf thymus ssDNA biosensor interacts with low-molecular-mass substances of environmental concern, and it can be used as a general indicator.

The DNA biosensor, realized by immobilization of the calf thymus extract on suitable electrode surface, is a simple tool to assemble and obtains reliable results. This, therefore, opens the possibility of rapid evaluation as a screening tool of toxicity of water samples.

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