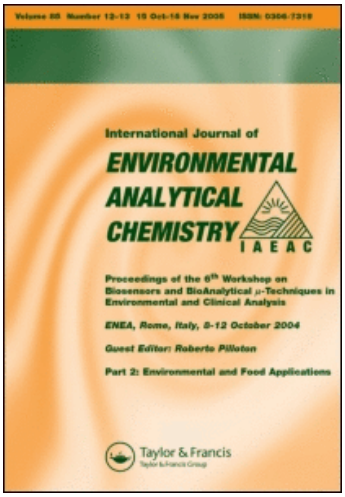

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S. Tombelli[a]; G. Marrazza[a]; M. Mascini[a]
[a] Dipartimento di Sanità Pubblica, Epidemiologia e Chimica Analitica Ambientale, Sez. Chimica Analitica, Firenze, Italy

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RECENT ADVANCES ON DNA BIOSENSORS

S. TOMBELLI, G. MARRAZZA and M. MASCINI*

Dipartimento di Sanità Pubblica, Epidemiologia e Chimica Analitica Ambientale, Sez. Chimica Analitica; Via G. Capponi, 9; 50121 Firenze, Italy

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DNA biosensors are realised immobilising a DNA structure on a suitable transducer to obtain selective information. In this paper we show how the determination of low-molecular weight compounds with affinity for DNA was measured by their effect on the oxidation signal of the guanine peak of calf thymus DNA immobilised on the electrode sensor and investigated by chronopotentiometric analysis. The DNA biosensor is able to detect known intercalating and groove binding compounds. Applicability to river water samples was demonstrated.

Moreover, a piezoelectric sensor coupled to a short oligonucleotide can be used as detector of the hybridisation reaction. We show as a model the detection of a specific mutation in apolipoprotein E (ApoE) gene.

Biotinylated 23-mer probes were immobilised on the streptavidin coated gold surface of a quartz crystal; the protein was covalently bound to the thiol/dextran modified gold surface. The device was able to distinguish different synthetic oligonucleotides. The hybridisation reaction was also performed using real samples of DNA extracted from human blood and amplified by Polymerase chain reaction PCR. The extension of such procedure to samples of environmental interest is discussed.

Keywords: DNA; biosensor; electrochemical; piezoelectric; PCR; hybridisation

INTRODUCTION

DNA biosensors are devices that combine a biological recognition element, which confers selectivity and a transducer, which provides sensitivity and converts the recognition event into a measurable signal.

The biological recognition element can be a single-stranded (ss) or a double-stranded (ds) DNA structure or a simple synthetic oligonucleotide (20–40 bases)[1].

The events to be recognised are of two kinds; in the first case, the event can be the interaction (intercalation, complexation, etc.) of a small molecule with one or

* Corresponding author: Fax: +39–55–2476972. E-mail: mascini@cesit1.unifi.it

more bases forming the DNA or with the whole DNA structure[2–4]. In this case, we look for a possible DNA damage and we call it a toxicity (or genotoxicity) test. In the second case, the reaction is the hybridisation reaction; the sequence of bases forming the oligonucleotide on the sensor (probe) will match its complementary form in solution (target)[5,6]. The probe is generally immobilised on a selected surface and the target is free in solution. The DNA hybridisation biosensors rely on the ability of an immobilised ssDNA probe to seek out and hybridise to its complementary target strand in the contacting solution. The probes are usually short oligonucleotides (20–40 bases) coupled to an electrochemical, optical or mass sensitive transducer.

In this paper we report some results of the two approaches using as example two different transducers, electrochemical and piezoelectric devices.

The electrochemical sensor is coupled with ss and dsDNA obtained from calf thymus and we will show how such a sensor can be used as a toxicity detector or as a rapid screening device for environmental use. Several compounds interact with the DNA and give a signal variation alerting that the structure has been modified.

The piezoelectric (mass sensitive) device is used as hybridisation detector and it is shown how such a coupling can be exploited for detection of specific DNA sequences. The latter holds a great promise for rapid testing for genetic or infectious diseases (cancer, cystic fibrosis, HIV, TB etc.), for criminal investigations or environmental monitoring.

RESULTS AND DISCUSSION

Calf thymus DNA electrochemical biosensors for toxicant screening

DNA electrochemical biosensors are realised by immobilising on a suitable electrode surface an oligonucleotide sequence or the calf thymus DNA; they are simple to assemble and can provide reliable results.

A major application 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[2–4,7–10].

The objective of our work was to develop a disposable electrochemical DNA sensor 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[11]. Moreover, single-use sensors are particularly useful for *in situ* analysis.

This biosensor has been realised by immobilising calf thymus DNA onto the carbon surface of a disposable strip[9,12–13]. The DNA biosensor is then immersed in the sample solution containing the analyte. After 2 min of interaction the DNA sensor is washed, immersed in acetate buffer and a chronopotentiometric analysis (PSA) is 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 low molecular weight pollutants.

Experimental results

The procedure consisted of the following steps: calf thymus DNA immobilisation on the electrode surface, dipping the electrode in the sample/blank solution and electrochemical interrogation of the surface[13].

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

The guanine peak appears very sharp in acetate buffer; the baseline at this high potential value was lower than in other buffers, then this is the buffer used for the interrogation of the surface.

The modified electrode performance was tested in buffer solutions containing methanol up to 10% v/v and no variation of the electrochemical signal of the guanine peak was observed. 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 immobilised when the sample contained different compounds of environmental interest. Table I summarises these experiments showing that the guanine peak is higher for ssDNA; the guanine base in single stranded DNA is more readily available for oxidation than in double stranded DNA.

TABLE I Compounds tested with single stranded or double stranded calf thymus DNA immobilised on screen printed electrodes. Calf thymus DNA immobilisation: 20 mg l^{-1} of single stranded 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 pH 5.0 with 10% v/v of methanol

Compounds tested	*Guanine peak area (ms) using calf thymus dsDNA immobilized (n=4)*	*Guanine peak area (ms) using calf thymus ssDNA immobilized (n=4)*
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
PCR mixture (Aroclor 1016) (20 mg l^{-1})	43 ± 8	95 ± 17
Aflatoxin B1 (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

Compounds behaving as intercalators, like daunomycin, showed an increase in the guanine peak area when using dsDNA (Figure 2). In this case we observed a linear increase with the daunomycin concentration in the range 1–5 mg l^{-1}.

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

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[14].

Figure 3 demonstrates the applicability of the DNA sensor to the analysis of these compounds. We obtained the gradual decrease of the guanine peak area in the presence of increasing levels of the aflatoxin B1. Such suppression of the guanine response results in a well defined concentration dependence. The signal was observed within the 10–30 mgl^{-1} range (Figure 3). Any effect in this concentration range was observed when dsDNA was used. We have to point out how

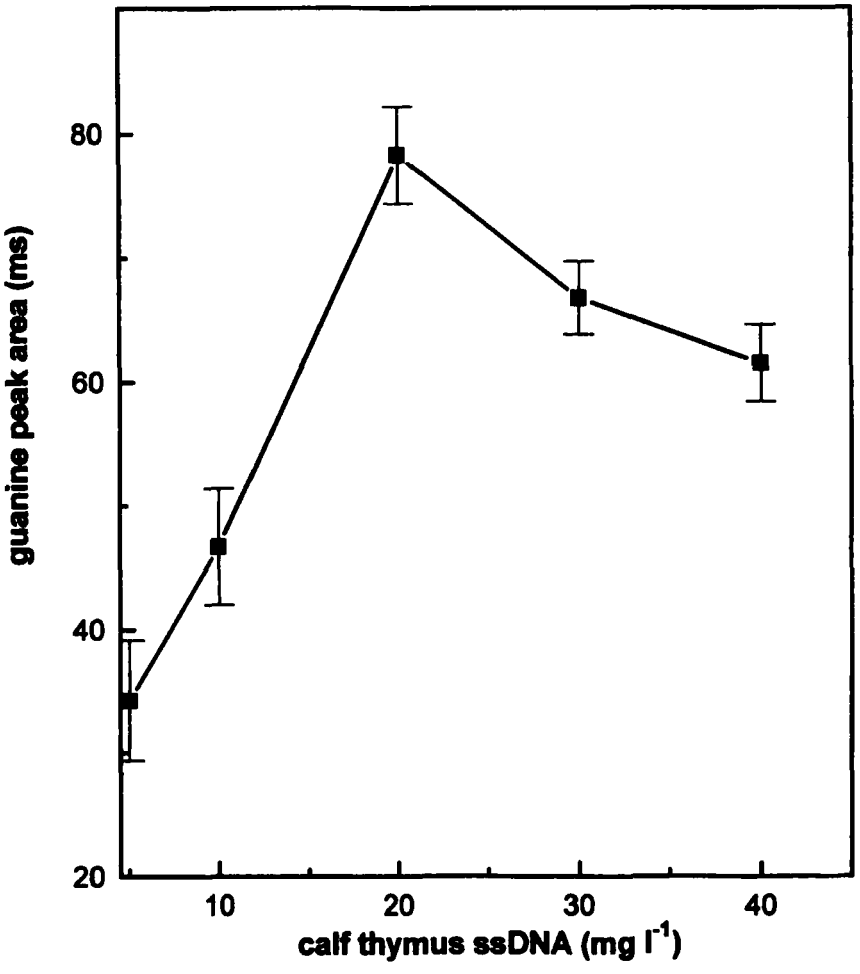

FIGURE 1 Anodic peak of the guanine obtained increasing the concentration of calf thymus ssDNA for immobilisation. Calf thymus ssDNA immobilisation: 2 min at +0.5 V vs SCE in 0.2 M acetate buffer pH 5.0. PSA transduction: in 0.2 M acetate buffer pH 5.0 with a stripping constant current of +6 μA and an initial potential of +0.5 V

for such compounds the ppm range is not relevant for environmental interest being often present at ppb level. However, this effect is considered as model for the class of toxins, which could be present in some waste water.

PCBs have been recognised for several years as ubiquitous environmental pollutants. The high toxicity of some of the PCB congeners represents a risk for the public health as these molecules are still present in the environment, even though

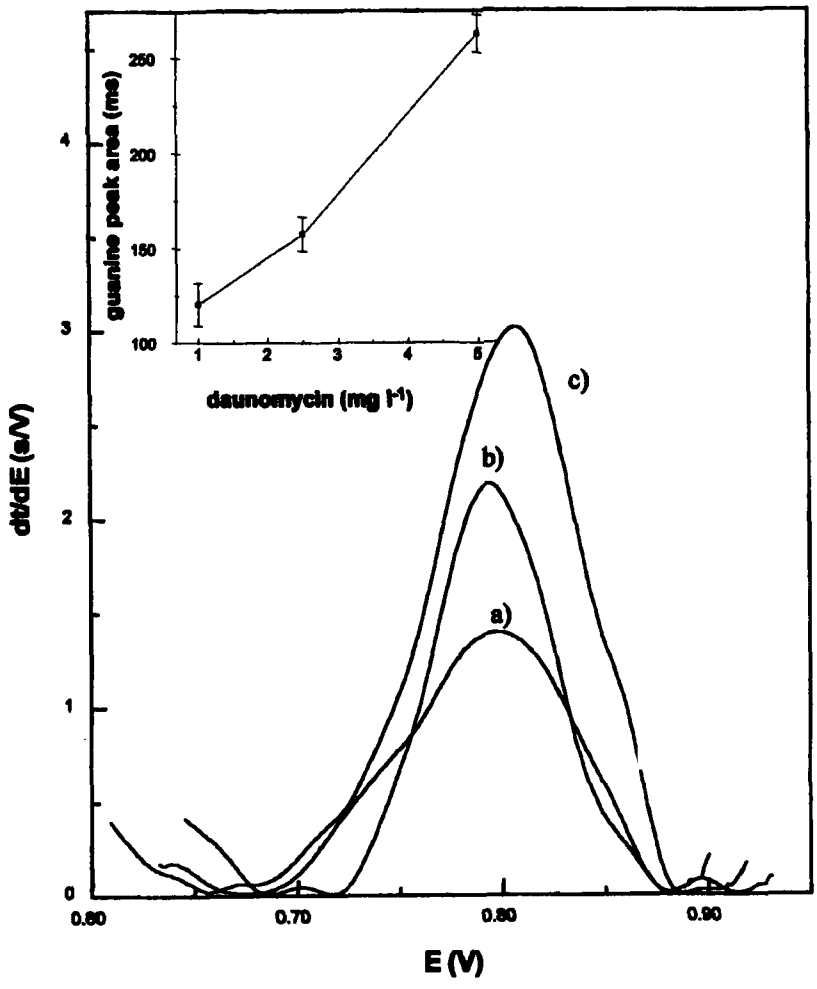

FIGURE 2 Chronopotentiograms for modified screen printed electrodes with calf thymus dsDNA obtained increasing the daunomycin concentrations a) 1 mg l^{-1}, b) 2.5 mg l^{-1}, c) 5 mg l^{-1}. Calf thymus ds DNA immobilisation: 10 mg l^{-1} dsDNA for 2 min at +0.5 V vs Ag/AgCl. PSA transduction: in 0.2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

the production of PCB has been banned. The screen printed electrodes modified with single stranded calf thymus DNA were used to detect PCB 105 and PCB mixtures (Arochlor 1260, 1016). The chronopotentiometric response of calf thy-

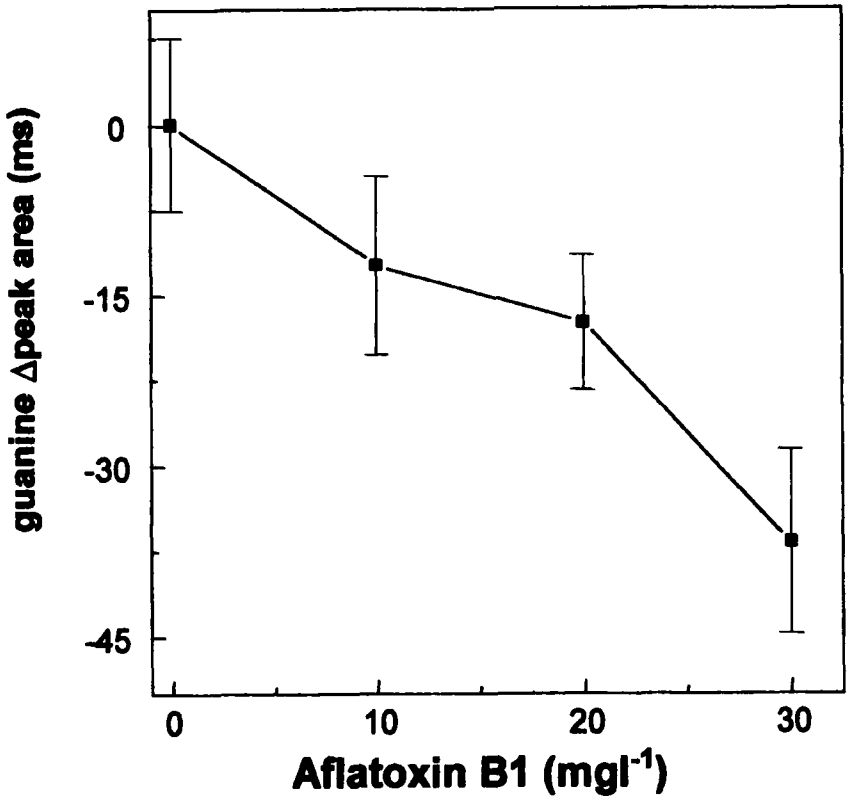

FIGURE 3 Calibration curve obtained increasing the concentration of aflatoxin B1. The results correspond to the difference between the guanine peak area after interaction with aflatoxin B1 minus that obtained for the buffer solution

mus ssDNA decreases with increasing PCB 105 concentration in the range 0.1–1.0 ppm.

Applicability to analysis of river water samples (River Arno during period April-June 1999) is also illustrated in Table II. The results obtained suggested to concentrate such samples in order to obtain clear responses. One of the first results is depicted in Table II where some samples (b, c, d) are compared, with ssDNA sensor giving different and clear results. The area of sample b is similar to the buffer while c and d samples gave measurable smaller areas. Analysis by HPLC of some pesticides in the same samples gave the results reported in Table II, where we can notice how samples c and d are heavily polluted in comparison with sample b.

TABLE II Guanine peak area detected with some river water samples (b, c, d) (River Arno, during the period April-June 1999) and results of the analysis 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). The peak area relative to blank solution is 89±13 ms

	b	*c*	*d*
Peak area (ms)	94 ± 7	61 ± 8	48 ± 9
Desethyl-terbuthylazine (ng l^{-1})	0	12	21
Carbofuran (ng l^{-1})	0	210	101
Simazine (ng l^{-1})	0	0	23
Terbuthylazine (ng l^{-1})	79	28	81
Ethofumesate (ng l^{-1})	6	184	83
Alachlor (ng l^{-1})	0	0	27
Metolachlor (ng l^{-1})	7	14	225

Some of these compounds, revealed by HPLC analysis, did not give any effect at the DNA sensor at such low level of concentration and we concluded that the DNA sensor revealed a cumulative effect.

Therefore the sensor is not able to distinguish specific compounds of environmental concern but it could be conveniently used as a screening tool of toxicity (or genotoxicity) due to the short time involved in the measuring step.

Oligonucleotide piezoelectric biosensors for hybridisation detection

The analysis of gene sequences plays a fundamental role in rapid detection of genetic mutations, in the detection of bacteria and in the identification of pathogenic strains. The detection of hybridisation forms the basis for such determinations: the classical methods are by labelling the probe with a radioisotope (^{32}P), or a fluorescent tag, but these procedures require long and tedious steps and present numerous disadvantages. Biosensor technology offers the possibility of monitoring hybridisation in real time with high selectivity and without the use of labels. Optical biosensors[15–18] and piezoelectric biosensors[19–24] are two main approaches; however, their sensitivity is usually quite low, therefore only seldom have real samples been considered. The use of Polymerase Chain Reaction (PCR) allows the concentration of selected portions of DNA to be increased by several orders of magnitude.

Coupling of PCR with piezoelectric biosensors has been demonstrated as possible way to solve the concentration problem[25]. In a specific application the

hybridisation detection allows to locate a point mutation in apolipoprotein E (apoE) gene in human beings[24]. This mutation is responsible for cardiovascular and nervous diseases. This application can be considered as a model; further determinations in the environmental field can be performed by only changing the immobilised probe. The gold surface of a piezoelectric quartz crystal was modified with 11-mercaptoundecanol and carboxylated dextran to immobilise streptavidin and then the biotinylated oligonucleotide (probe). This immobilisation procedure was selected in a previous work where several procedures were compared in immunochemical reactions[26]. The analytical signal is the difference in the frequency of the crystal in contact with buffer before and after the hybridisation reaction.

Hybridisation experimental results

Experiments performed with 23-mer oligonucleotides to examine the ability of the procedure to distinguish between the complementary sequence and the mismatch are reported in Table III. The immobilised probes had a sequence characteristic of the region of the ApoE gene around one of the polymorphic codons. We use the two probes identical apart from one base (mutated) in the middle of the sequence (shaded letter in the sequence).

TABLE III Results obtained in the experiments with the synthetic oligonucleotides. In the first column we report the probe immobilised on the crystal; in the second column we report the oligonucleotide with which we perform the hybridisation and the degree of complementarity with respect to the probe immobilised. In the other columns the average values of frequency shift (Hz) and the standard deviation (Hz) are detailed. The total concentration of the oligonucleotides is 2 μM

Immobilised Probe	*Target*	*Average (Hz)*	*SD (Hz)*
Probe 1	100% complementary (oligo 3)	46	6
	100% mismatch (oligo 4)	20	3
	50% complementary+ 50% mismatch (oligo 3+oligo 4)	36	1
	Non complementary	1	1
Probe 2	100% complementary (oligo 4)	46	3
	100% mismatch (oligo 3)	22	3
	50% complementary+ 50% mismatch (oligo 3+oligo 4)	32	5
	Non complementary	1	2

The base sequences of the 5'-biotinylated probes (23-mer), of the complementary oligonucleotides (23-mer) targets and of the non-complementary oligonucleotide (23-mer) are as below:

Probes:	5' biotin-CAGGCGGCCGCACACGTCCTCCA 3'	(probe 1)
	5' biotin-CAGGCGGCCGCGCACGTCCTCCA 3'	(probe 2)
Targets:	5' TGGAGGACGTGTGCGGCCGCCTG 3'	(oligo 3)
	5' TGGAGGACGTGCGCGGCCGCCTG 3'	(oligo 4)

Non-complementary strand: 5' TGCCCACACCGACGGCGCCCACC 3'

The results of table III clearly indicate how the complementary sequence can be distinguished from the mismatch (one base different over 23) and also from the mixture of complementary and mismatch provided the same total concentration is in solution. Experiments with real samples were performed with DNA extracted from human blood and amplified with PCR. The amplification step is necessary to reach a higher concentration of the DNA fragments.

The amplification products of the PCR were fragments of 244 base pair (bp) containing the two polymorphic codons of apoE gene[27].

For the investigation of the hybridisation reaction with the real samples, 40 μl of the solution of the DNA fragments obtained from the amplification by PCR were diluted with 60 μl of hybridisation buffer (final total volume, 100 μl). The sample was then denaturated by heating at 95°C for 5 minutes and then freezing the sample in ice for 30 seconds. The hybridisation reaction was allowed to proceed for 20 minutes, and then the crystal was washed with the hybridisation buffer. The values reported as results are the differences in the frequency of vibration of the piezoelectric crystal between this final value and the one recorded before the hybridisation step and are reported in Table IV. The different samples are listed according to the sequences present in the genes (ε2, ε3 or ε4); each sample contains two sequences derived from the two chromosomes inherited in humans.

When probe 1, which has the base adenine in the middle of the sequence, is immobilised, the 100% complementary sequence (Table IV) is the one containing the base thymine (samples ε2/ε2, ε2/ε3, ε3/ε3) whereas the mismatch is the one with cytosine (samples ε4/ ε4), while 50% of complementarity is when the two sequences are present (samples ε2/ ε4 and ε3/ ε4). With probe 2, we have the inverse situation. Results of Table IV indicate how the biosensor is able to distinguish among 3 types of genes present in the human blood samples.

TABLE IV Experiments with real samples obtained after the amplification of PCR and already characterised by column we report the probe immobilised on the crystal. In the following two columns we indicate the sequence of bases and the genotypes corresponding to each sample with the number of samples that we have analysed. The average values of frequency shift (Hz) (corresponding to three cycles of hybridisation) and the standard deviation are reported in the other columns. The sequences of the two probes are:
Probe 1 CAGGCGGCCGCACACACGTCCTC
Probe 2 CAGGCGGCCGCGCACACGTCCTC

Immobilised Probe	*Target*	*Samples*	*Average (Hz)*	*SD (Hz)*
Probe 1	100% fully complementary	ε2/ε2 (n=5)	29	5
		ε3/ε3 (n=8)	30	6
		ε2/ε3 (n=5)	29	5
	50% fully complementary + 50% mismatch	ε3/ε4 (n=5)	17	2
		ε2/ε4 (n=5)	18	2
	100% mismatch	ε4/ε4 (n=6)	11	2
		Blank (n=6)	1	1
Probe 2	100% mismatch	ε2/ε2 (n=5)	11	2
		ε3/ε3 (n=8)	12	2
		ε2/ε3 (n=5)	11	2
	50% fully complementary + 50% mismatch	ε3/ε4 (n=5)	18	2
		ε2/ε4 (n=5)	17	3
	100% fully complementary	ε4/ε4 (n=6)	32	4
		Blank (n=6)	2	2

All samples were characterised by a reference method based on restriction isotyping and polyacrylamide gel electrophoresis[27].

In Fig. 4 the frequency variation is reported for the real samples during a hybridisation-regeneration cycle. The frequency differences are reported as they appear after each step. From analytical interest the difference of frequency from the A and B level is that one reported in table IV and it corresponds to the frequency difference taken in the same buffer conditions.

CONCLUSIONS

The potential of DNA biosensors for DNA hybridisation and for detection of toxic compounds have been reported. This kind of procedure offers a sensitive,

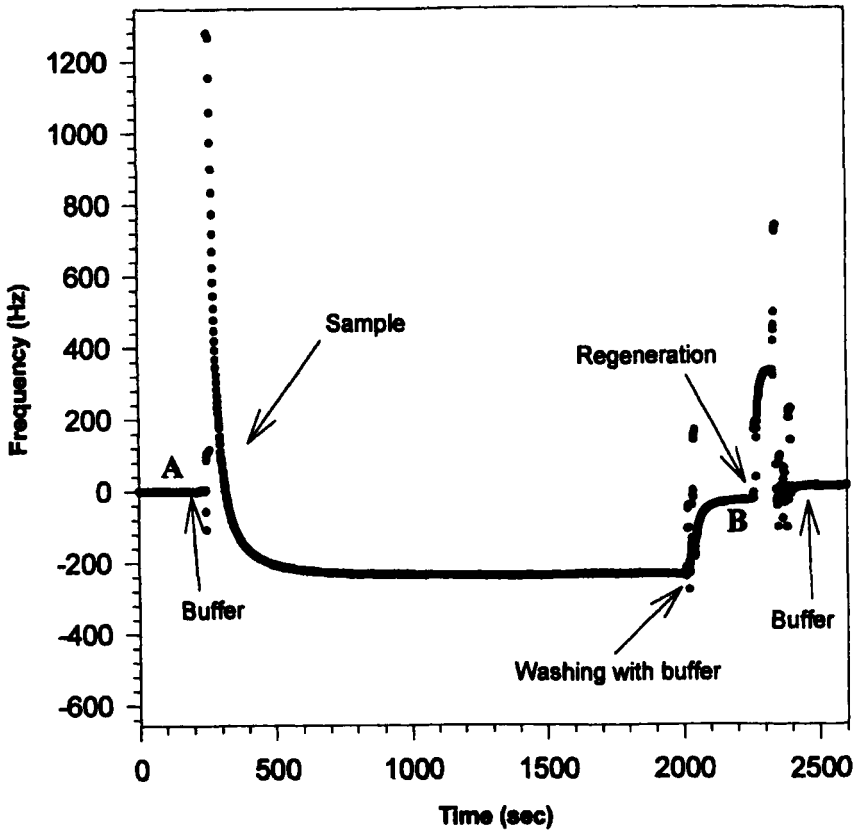

FIGURE 4 Frequency variations during a hybridisation-regeneration cycle performed with a real sample. The difference of frequency between A and B level is the one reported as analytical result

rapid and portable tool for field monitoring of several environmentally and toxicologically significant compounds.

Research has clearly demonstrated the feasibility of developing a wide array of specific probes for organisms and genes of environmental interest through hybridisation detection. However, environmental applications up to now require an amplification step by PCR.

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 of toxicity.

With the piezoelectric biosensor, using the procedure reported, we could perform a typing of apolipoprotein E distinguishing between three different groups

of samples. The system can be applied to the detection of other polymorphisms or, in environmental analysis, to the identification of the pathogen strains of various virus and bacteria.

References

[1] J. Wang, X. Cai, G. Rivas, H. Shiraishi, and N. Dontha, *Biosens. Bioelectron.*, **12**, 7, 587–599 (1997).

[2] J. Wang, G. Rivas, X. Cai, H. Shiraishi, P.A.M. Farias, N. Dontha, and D. Luo, *Anal. Chim. Acta*, **332**, 139–144 (1996).

[3] J. Wang, M. Chicharro, G. Rivas, X. Cai, N. Dontha,, P.A.M. Farias, and H. Shiraishi, *Anal. Chem.*, **68**, 13, 2251–2254 (1996).

[4] J. Wang, G. Rivas, D. Luo, X. Cai, F.S. Valera, and N. Dontha, *Anal. Chem.*, **68**, 24, 4365–4369 (1996).

[5] S. Palanti, G. Marrazza, and M Mascini, *Anal. Lett.*, **29**, 2309–2331 (1996).

[6] M. Rodriguez, and M.T. Carter, *Anal. Chem.*, **62**, 2658–2662 (1990).

[7] F. Jelen, M. Fojta, and E Palecek, *J. Electroanal. Chem.*, **427**, 49–56 (1997).

[8] F. Jelen, M. Tomoschik, and E Palecek, *J. Electroanal. Chem.*, **423**, 141–148 (1997).

[9] J. Wang, G. Rivas, E. Palecek, P. Nielsen, H. Shiraishi, N. Dontha, D. Luo, C. Parrado, M. Chicharro, P.A.M. Farias, F.S. Valera, D.H. Grant, and M. Ozsoz, *Anal. Chim. Acta*, **347**, 1–8 (1997).

[10] M. Mecklenburg, A. Grauers, B. Rees Jonsson, A. Weber, and B. Danielsson, *Anal. Chim. Acta*, **347**, 79–86 (1997).

[11] M. Del Carlo, I. Lionti, M. Taccini, A. Cagnini, and M. Mascini, *Anal. Chim. Acta*, **342**, 189–197 (1996).

[12] G. Marrazza, I. Chianella, and M. Mascini, *Biosens. Bioelectron.*, **14**, 43–51 (1999).

[13] G. Marrazza, I. Chianella, and M. Mascini, *Anal. Chim. Acta*, **387**, 297–307 (1999).

[14] S. Gopalakrishnan, S. Byrd, M.P. Stone, and T.M. Harris, *Biochemistry*, **28**, 726–734 (1989).

[15] N.J.C. Strachan, and D.I. Gray. *Lett. Appl. Microbiol.*, **21**, 5–9 (1995).

[16] T. Vo-Dinh, K. Houck, and D.L. Stokes, *Anal. Chem.*, **66**, 3379–3383 (1994).

[17] H.J. Watts, D. Yeung, and H. Parkes, *Anal. Chem.*, **67**, 4283–4289 (1995).

[18] E. Kai, S. Sawata, K. Ikebukuro, T. Lida, T. Honda, and I. Karube, *Anal. Chem.*, **71**, 796–800 (1999).

[19] N.C. Fawcett, J.A. Evans, L. Chien, and N. Flowers, *Anal. Lett.*, **21**, 7, 1099–1114 (1988).

[20] Y. Okahata, Y. Matsunobu, K. Ijiro, M. Mukae, A. Murakami, and K. Makino, *J. Am. Chem. Soc.*, **114**, 8299–8300 (1992).

[21] Y. Okahata, M. Kawase, K. Niikura, F. Ohtake, H. Furusawa, and Y. Ebara, *Anal. Chem.*, **70**, 1288–1296 (1998).

[22] F. Caruso, E. Rodda, D.N. Furlong, K. Niikura, and Y. Okahata *Anal. Chem.*, **69**, 2043–2949 (1997).

[23] S. Yamaguchi, and T. Shimomura, *Anal. Chem.*, **65**, 1925–1927 (1993).

[24] S. Tombelli, M. Mascini, L. Braccini, M. Anichini, and A.P.F Turner, *Biosens. Bioelectron.*, in press.

[25] S. Tombelli, M. Mascini, C. Sacco, and A.P.F. Turner, *Anal. Chim. Acta*, **418**, 1–9 (2000).

[26] S. Storri, T. Santoni, M. Minunni, and M. Mascini, *Biosens. Bioelectron.*, **13**, 3–4, 347–357 (1998).

[27] J.E. Hixon, and D.T. Vernier, *Journal of Lipid Research*, **31**, 545–549 (1990).