

RECENT ADVANCES ON DNA BIOSENSORS

S. Tombelli, G. Marrazza, M. Mascini

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

e-mail Mascini@cesit1.unifi.it

Abstract: 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 immobilized on the electrode sensor and investigated by chronopotentiometric analysis. The DNA biosensor is able to detect known intercalating and groove binding compounds. Detection limits of 0.3, 0.2, 10 mg l⁻¹ were obtained for daunomycin, polychlorinated biphenils (PCBs) and aflatoxin B1, respectively. Applicability to river water samples was demonstrated.

Coupling of Polymerase Chain Reaction (PCR) with a piezoelectric biosensor for hybridization detection, to detect a specific mutation in apolipoprotein E (apoE) gene has been realized. Biotinylated 23-mer probes were immobilized 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 hybridization reaction was also performed using real samples of DNA extracted from human blood and amplified by PCR. The system was able to realize apoE typing distinguishing between different groups of genotypes.

Keywords: DNA, chronopotentiometry, piezoelectric biosensor.

ELECTROCHEMICAL BIOSENSORS FOR ENVIRONMENTAL SCREENING

The objective of the first part of such presentation is the disposable electrochemical DNA sensor for environmental screening. Such biosensor is able to evaluate the presence of small DNA binding compounds by measuring changes of the electrochemical signal of guanine in calf thymus DNA extract. This biosensor is realized by immobilizing calf thymus DNA onto the electrode surface [1,2]. The DNA biosensor was then immersed in the sample solution containing the analyte. After 2 min of interaction 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.

RESULTS

Different compounds of environmental interest were analyzed using modified electrodes by single stranded or double stranded DNA. Table 1 summarizes these experiments showing that the guanine peak is higher for ssDNA; the guanine base in single stranded DNA is obviously more readily available for oxidation than in double stranded DNA. The measurements were repeated 4 times.

Applicability to the analysis of some selected river water samples is also illustrated in Fig. 1. A decrease of the guanine peak area could be detected with some water samples (curves b, c). By liquid chromatography analysis we obtained the results reported in Table 2. It seems that there is an approximate relationship between the two sets of data. The sensor is not able to distinguish between compounds of environmental concern but could be conveniently used as a screening tool of toxicity.

Work in this direction will be expanded to obtain clear information on such effects.

Table 1.

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 (10mg l⁻¹)	52 ? 6	86 ? 3
Phthalates mixture (20 mg l⁻¹)	39 ? 16	80 ? 26
Atrazine (50 mg l⁻¹)	66 ? 39	85 ? 11
Bisphenol-A (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 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

Figure 1.

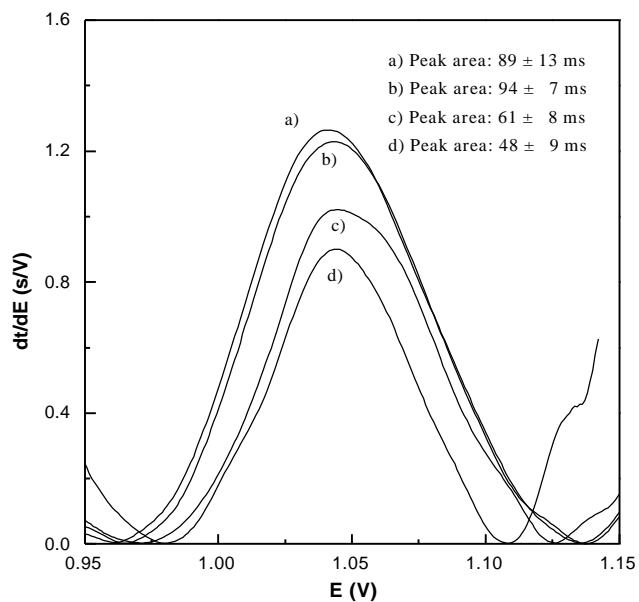


Table 2

	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

PCR and piezoelectric DNA biosensors

The analysis of gene sequences and the study of gene polymorphisms play a fundamental role in rapid detection of genetic mutations [3]. The detection of hybridization forms the basis for such diagnosis: biosensors technology offers the possibility of monitoring hybridization in real time with high selectivity and without the use of labels.

We report, for the first time to our knowledge, the coupling of PCR with piezoelectric biosensors. In this case, the hybridization detection allows to detect a point mutation in apolipoprotein E gene in human being. Such mutation is responsible of cardiovascular and nervous diseases.

Hybridization with DNA amplified real samples

The amplification products were fragments of 244 bp containing the two polymorphic codons 112 and 158 of apoE gene. In Fig. 2 the sequence characteristic of the region around codon 112 is reported.



Figure 2 Sequence of bases including codon 112 of the apoE gene: the polymorphic site is evidenced. The 23 bases included between the two arrows are the ones chosen for the experiments. The 3 polymorphic alleles are indicated near the corresponding sequences.

For the investigation of the hybridization reaction with the real samples, 40 μ l of the solution of the DNA fragments obtained from the amplification by PCR were diluted with 60 μ l of hybridization buffer (final total volume, 100 μ l). The sample was then denaturated by heating at 95°C for 5 minutes and then freezing the sample in ice for 30

seconds. The hybridization reaction was allowed to proceed for 20 minutes, then the crystal was washed with the hybridization buffer: the frequency value was recorded and the difference between this value and the one displayed before the hybridization was evaluated. After the hybridization reaction, the probe could be regenerated with 1 minute of HCl 1 mM as for the reaction with the standard solution of the oligonucleotides and another cycle of hybridization-regeneration could be performed again. In fig. 3 we report the frequency variations during a hybridization-regeneration cycle performed with a real sample.

In Table 3 we report the different possible genotypes and the corresponding base in the codon 112 of the gene. Immobilizing on the gold surface of the quartz crystal probe 1 or probe 2, we can detect the presence of thymine or cytosine, respectively, and realize in this way the apoE typing distinguishing between three different groups of genotypes.

Table 3 The six different genotypes of apoE gene: for each genotype the base present in the codon 112 in the sequence of the two alleles is reported.

Genotypes	Base present in codon 112 in the two alleles of the genotype		
?2/?2	Thymine	?2 Thymine	?2
?2/?3	Thymine	?2 Thymine	?3
?2/?4	Thymine	?2 Cytosine	?4
?3/?3	Thymine	?3 Thymine	?3
?3/?4	Thymine	?3 Cytosine	?4
?4/?4	Cytosine	?4 Cytosine	?4

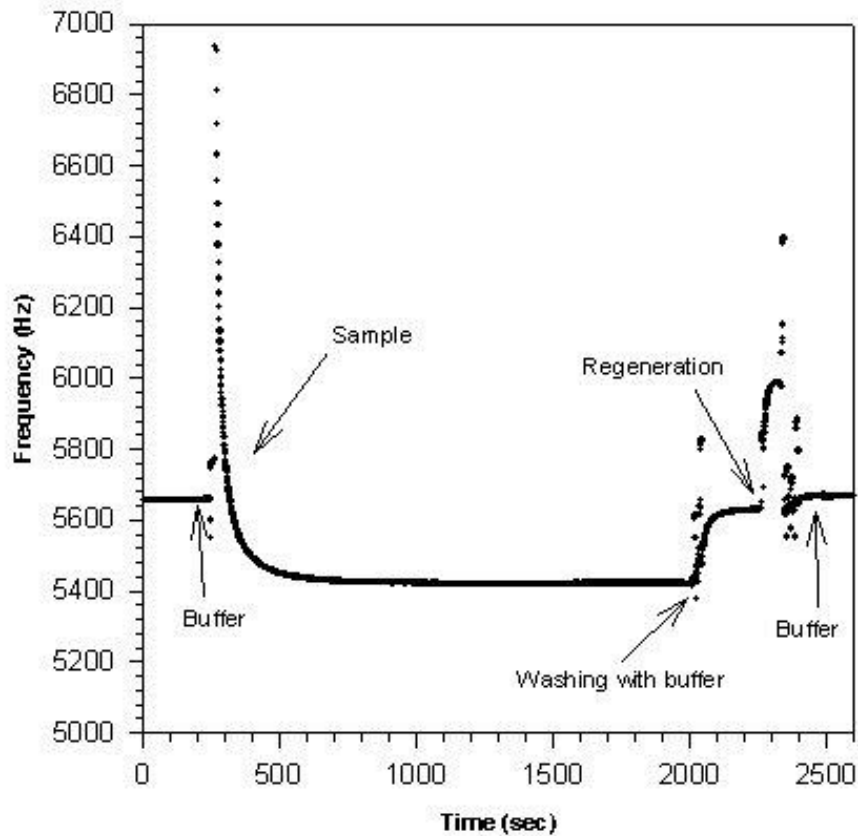


Figure 3: Frequency variations during a hybridization-regeneration cycle performed with a real sample.

The results obtained with the piezoelectric biosensor with these samples were compared with a reference method for routine analysis. The method was based on the restriction isotyping with Hha1 and polyacrilamide gel electrophoresis [4].

RESULTS

The coupling of PCR and the hybridization detection with the quartz crystal biosensor has been performed with several real samples

including all the six genotypes.

To verify the possibility to distinguish the complementary strand from the mismatch using the real samples of 244 bases, the procedure was tested using about 50 samples representing all the possible genotypes of apoE gene. All the samples were previously characterized using the reference method of electrophoresis.

To control the absence of adsorption or aspecific effects some "blank" samples were analyzed: these samples came from the PCR reaction as the others, but they did not contain in the solution for the amplification the genomic DNA.

When probe 1, which has the base adenine in the middle of the sequence, is immobilized, the 100% complementary sequence is the one containing the base thymine whereas the mismatch is the one with cytosine: distinguishing between these different sequences we could characterize the real samples identifying the genotypes, basing the differentiation on what reported in table 3.

The results are reported in table 4.

Using the procedure previously reported we could perform a typing of apolipoprotein E distinguishing between three different groups of genotypes. We point out that coupling the analysis with two crystals immobilizing on each crystal a probe presenting a sequence of bases characteristic of the region around codon 112 and 158, we could perform a complete discrimination between all the six genotypes.

The system could be applied to the detection of other polymorphisms choosing different probes that could be immobilized on the gold surface of the crystal.

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