



Sensing benzo[a]pyrene-DNA adducts formation via decrease of hybridization reaction

V. Lanzone^a, S. Scarano^b, M. Del Carlo^a, F. Corrado^c, M. Esposito^c, M. Minunni^{b,**}, D. Compagnone^{a,*}

^a Department of Food Sciences, University of Teramo, via Lerici 1, 64023 Mosciano S.A., Teramo, Italy

^b Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy

^c Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055 Portici, Napoli, Italy

ARTICLE INFO

Article history:

Received 12 July 2012

Received in revised form 4 September 2012

Accepted 3 October 2012

Available online 12 October 2012

Keywords:

Benzo[a]pyrene

DNA sensors

Surface plasmon resonance

Electrochemical DNA sensor

PAHs

ABSTRACT

Toxicity of benzo[a]pyrene occurs because of the formation of covalent adducts with DNA guanines. In this work we report the attempt to detect this DNA-adduct using both an electrochemical assay based on gold nanoparticles and a surface plasmon resonance DNA sensor. Detection was achieved via inhibition of the hybridization reaction of oligonucleotide sequences after formation of the covalent adduct. Detecting oligonucleotide sequences have been designed to get high reactivity vs formation of the adduct and benzo[a]-pyrene 7,8-dihydrodiol 9,10-epoxide has been used to generate adducts. Formation of adducts in the nanomolar range was evaluated using the surface plasmon resonance sensor with both the oligonucleotide sequences containing guanines.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Benzo[a]pyrene (BP) is the most toxic compound belonging to polycyclic aromatic hydrocarbons (PAHs), the well known class of environmental pollutants generated mainly by incomplete combustion processes. The mutagenic biological activity of BP requires metabolic activation to reactive electrophiles and their subsequent covalent binding to DNA [1–3]. Metabolites meeting these requirements have been identified as the different stereoisomers of benzo[a]-pyrene 7,8-dihydrodiol 9,10-epoxide (BPDE) [4,5]. In particular, the biological activity of BP appears to be causally linked to the covalent binding of (+)-anti-BPDE to DNA and occurs almost exclusively at the exocyclic amino group of deoxyguanosine (dG) [6], via trans or cis addition to the benzylic C-10 position in the diol epoxide [7–10]. Previous studies on the binding of BPDE to DNA, polynucleotides, or oligonucleotides have demonstrated heterogeneous adduct distribution along the DNA sequence and distinguished two different types of complexes, denoted type I and type II [7]. The binding of BPDE occurs preferentially at certain DNA sites, eventually identified as “hot spots” in key genes, inducing transversal mutations. These mutations have been found in genes, such as p53, at exons 157, 248, and 273 mutated in tumours [11,12].

The binding of (+)-anti-BPDE to the oligonucleotides 5'-(CCTATCGXTATCC) and 5'-(CCTATm5CGXTATCC) (X being T, A, or C) in the single-stranded or duplexed form has been investigated [7] by thermal stability studies and ultraviolet spectroscopy (UV), nuclear magnetic resonance (NMR), circular dichroism (CD) to gain information about adopted adduct conformations. PAHs other than benzopyrene have been demonstrated to have the ability to form adducts with DNA [13–16].

DNA sensors detecting toxic compounds have been developed during the last 15 years with the basic general concept that any compound interacting with DNA is a potentially toxic agent. Using this approach, different electrochemical sensors based on immobilization of genomic DNA on carbon-based electrodes have been reported [17–22].

We have recently detected PAHs in food samples using single strand genomic DNA from salmon testis immobilized onto carbon based screen printed electrodes measuring the changes of the signal of guanine oxidation [21]. We have also demonstrated, using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method, that benzo[a]pyrene oxidized under mild UV radiation is able to form the same kind of adducts with guanine of the metabolically reactive BPDE [23]. These oxidation products form stable adducts and are able to reduce the hybridization reaction of an oligonucleotide sequence measured via an amplified enzymatic detection scheme [24]. On the basis of the latter data, the use of a selected oligonucleotide DNA sensor for the detection of potentially toxic compounds, forming stable adducts with DNA, seems feasible. In this paper we report a study of oligonucleotide sequences

* Corresponding author. Tel.: +39 0861266942; fax: +39 0861266915.

** Corresponding author.

E-mail addresses: minunni@unifi.it (M. Minunni), dcompagnone@unite.it (D. Compagnone).

designed as “detecting” for compounds forming adducts with guanines. We have used two sensing approaches for the detection of the hybridization reaction: an enzyme amplified electrochemical approach based on gold nanoparticles and a surface plasmon resonance biosensor (SPR). The model compound for the formation of the DNA-adducts used was BPDE. Direct detection via SPR was more sensitive than the electrochemical approach allowing the detection of BPDE in the nanomolar range.

2. Materials and methods

2.1. Reagents

Caution: BPDE is carcinogenic agent and should be handled with care, as outlined in National Cancer Institute guidelines.

Oligonucleotide “detecting” sequences (i.e. sequences incubated with BPDE for the formation of DNA adducts) used as thiolated or unmodified and the complementary biotinylated sequences were all from Life Technologies Corporation (Milan, Italy). Gold screen-printed electrodes (SPGE) and carbon screen-printed electrodes (SPCE) were obtained from EcoBioServices & Research (Florence, Italy). Gold nanoparticles (GNPs) have been prepared in our laboratory using the citrate method [25] and then functionalized by the selective immobilization of oligonucleotides on GNPs surface (affinity modules) [26]. SSC 2× buffer consisted in 300 mM NaCl, 30 mM C₆H₅Na₃O₇, pH 7.4. DEA buffer consisted in 0.1 M diethanolamine, 1 mM MgCl₂, 100 mM KCl, pH 9.6. THC 10× buffer was prepared with 100 mM Na₂HPO₄, 1 M NaCl, pH 7. Tris–HCl buffer consisted in 50 mM Tris–HCl, 150 mM KCl, 0.5 mM EDTA, pH 7.5. TWEEN® 20, albumin from bovine serum (BSA), 6-mercapto-1-hexanol (MCH), 1-naphthyl phosphate and the other chemicals were purchased from Sigma–Aldrich (Milan, Italy). BPDE was obtained by National Cancer Institute (NCI), Midwest Research Institute, (Kansas City, Missouri).

N-hydroxysuccinimide (NHS) was from Fluka (Milan, Italy); 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDAC) was from Merck–Calbiochem (Darmstadt, Germany); hydrochloric acid, ethanolamine hydrochloride (EA), and streptavidin from *Streptomyces avidinii* were all from Sigma–Aldrich (Milan, Italy). Other chemicals were purchased from standard commercial sources at analytical grade.

Hybridization buffer consisted in 20 mM Na₂HPO₄, 300 mM NaCl, 0.1 mM EDTA, pH 7. The immobilization buffer consisted in 20 mM Na₂HPO₄, 150 mM NaCl, 0.1 mM EDTA, pH 7.4. Streptavidin was immobilized on activated carboxylated dextran layer by using 10 mM acetate buffer pH 4.5. Running buffer used in Biacore XTM experiments was PBS buffer. All salts used for buffers were analytical grade and were purchased from Merck (Darmstadt, Germany). Deionized water (Millipore) was used throughout all the preparations and buffer solutions were finally filtered (0.22 µm) before use.

2.2. Electrochemical DNA biosensor

2.2.1. Oligonucleotide-modified screen-printed gold electrodes (SPGE)

The hybridization assay scheme was the same used by Carpini et al. [27] with minor modifications; the lyophilized detecting thiolated oligonucleotides were initially dissolved in 0.5 M phosphate buffer solution (pH 8.5) and then were placed onto the gold working electrode surface (6 µL/electrode). Chemisorption was allowed to proceed overnight (≈19 h). The immobilization step was followed by a post-treatment with MCH 1 mM (6 µL for 1 h at room temperature).

Modified electrodes were washed twice with 200 µL of SSC 2× buffer before the hybridization reaction and then exposed to 6 µL of the biotinylated complementary sequence solution for 20 min. After hybridization, the sensors were washed twice with 200 µL of DEA buffer and 0.1% TWEEN® 20. The biotinylated hybrid obtained at the electrode surface was reacted for 20 min with a 6 µL solution containing 1 U/mL of streptavidin–alkaline phosphatase conjugate and 8 mg/mL of BSA in DEA buffer. The sensors were washed twice with 200 µL of DEA buffer and 0.1% TWEEN® 20. The planar electrochemical cell was finally covered with 100 µL of a 1-naphthyl phosphate solution (1 mg/mL in DEA buffer). The oxidation signal of the enzymatically produced 1-naphthol was measured after 20 min by differential pulse voltammetry (modulation time = 0.05 s; interval time = 0.15 s; step potential = 5 mV; modulation amplitude = 70 mV; potential scan: from 0.0 to +0.6 V) using an AUTOLAB PGSTAT 12, digital potentiostat/galvanostat, software GPES (Eco Chemie BV, Utrecht, The Netherlands); peak heights was taken as the analytical signal. All the potentials were referred to the silver pseudo-reference electrode; the experiments were carried out at room temperature (25 °C).

2.2.2. Affinity modules based electrochemical DNA biosensor

Affinity modules were prepared as follows: 7.5 µL of a 500 µM solution of the thiol-modified detecting sequence was added to 100 µL of 18 nm diameter colloidal gold suspension. After mixing, the solution was left 16 h at room temperature in dark. 10 µL of 10× THC were then added and the affinity module were stored for further 48 h. The suspension was then washed three times with 1× THC (10 mM Na₂HPO₄, 0.1 M NaCl, pH 7) and stored for at least three months. The final colloidal gold concentration was 30 nM and the final oligonucleotide concentration was 3.7 µM [26].

The heterogeneous assay was carried out after deposition of the affinity modules onto a SPCE surface. The electrode surface was firstly kept at a constant potential of 1.7 V for 300 s in 100 µL of 0.1 M PBS buffer pH 7.0. Then, the affinity modules were drop-casted on the screen-printed carbon electrode surface (5 µL of affinity modules suspension diluted 1:2 in 1× THC on carbon working electrode surface). After drying in a thermostat and polarization at +0.5 V in 0.1 M KCl for 120 s, the complementary sequence was let to react for 20 min and the procedure followed the steps reported above for SPGE.

In the homogeneous assay all the steps of incubation and washing were carried out within a vial. The washing of excess reagents was carried out twice by the addition of 500 µL of DEA buffer and 0.1% TWEEN® 20 and elimination of the supernatant after centrifugation at 12,300 rpm for 10 min. Incubation temperatures were controlled through the use of a heat block. The hybridization reaction was carried out for 5 min at the annealing temperature to increase the selectivity of the base pairing in the duplex formation; all other steps were carried out at 30 °C. 1-Naphthol was detected on SPGE as previously reported.

2.3. Surface plasmon resonance measurements

SPR measurements were all carried out on Biacore XTM, by using carboxylated dextran CM5 biochips (General Electric Healthcare Bio-Sciences AB; Uppsala, Sweden).

The single stranded DNA sequences used as complementary to the detecting sequences in SPR assays carried a biotin residue to their 5' end and were immobilized on dextran via streptavidin/biotin binding as follows: at a constant flow rate of 5 µL/min, the dextran layer was modified with streptavidin (35 µL, 200 µg/mL in 10 mM acetate buffer, pH 4.5) after an activation step (35 µL) with 50 mM NHS and 200 mM EDAC. A blocking step by EA for 20 min to saturate unreacted sites was eventually carried out for both receptors. The biochip surface was then let under flow of the

proper running buffer until equilibration of the baseline. All experiments were performed at least three times at a temperature of 25.0 °C, to determine reproducibility and standard deviations. The flow rate was set at 5 $\mu\text{L}/\text{min}$ for all measurements and injection volumes were 35 μL for all detecting sequences. After each measurement cycle, the regeneration step was performed by injection of 10 mM HCl for 5 s at 5 $\mu\text{L}/\text{min}$. Each biochip can work on two flow cells (0.06 μL per cell), separately or in series. In this work, measurements were conducted on both cells working in serial mode, one of them was, in fact, used as reference cell. Sensorgrams were elaborated by BIA evaluation 3.1 software.

2.4. BPDE-oligonucleotide sequence adduct formation

For affinity modules based electrochemical DNA biosensor, 25 μL of affinity modules solution, diluted 1:2 in $1\times$ THC buffer, was incubated with the same volume of BPDE (0–0.1–1–10 μM) in Tris–HCl buffer, pH 7.4. The reaction was carried out in the dark for 24 h at 37 °C.

In the SPR-based assay, 5 μL of oligonucleotide sequence solution (100 μM) were incubated with 5 μL of BPDE (250–500–1000 μM in THF) in PBS hybridization buffer (final volume = 50 μL). Final BPDE/DNA molar ratios were 2.5:1, 5:1 and 10:1. The reaction was carried out in the dark for 24 h at 37 °C.

3. Results and discussion

3.1. Oligonucleotides sequence selection

A 24-mer oligonucleotide sequence was selected for the development of both the electrochemical and the SPR-based DNA hybridization sensors. As reported in Section 1, the formation of the adducts has been observed for mutated genes in tumours (p53); the most frequently mutated triplets have been identified as “hot spots”. Moreover, Margulis et al. [28] studied the effect of the base sequence on the formation of *trans* and *cis* adducts of diol epoxide and $\text{N}^2\text{-G}$. They concluded that G surrounded by two pyrimidines (C/T) is more reactive towards the diol epoxide than a G surrounded by a pyrimidine and a purine or just purines (A/G). In order to maximize the response to the toxic species we have then designed different following detecting sequences: a 24-mer 5'-TTTCGTCAAAGCGGTCTGTGATA-3' containing 6 guanines in 4 hot-spot triplets (underlined) and having 4 out of the 6 guanines one adjacent pyrimidine (named in the paper as HS detecting sequence). A second 24-mer 5'-CTCACTCAACACTCATTAGCGACA-3' (noHS detecting sequence) with theoretical lower reactivity for

BPDE because of the presence of only 2 guanines with no hot-spot configuration and only one adjacent pyrimidine. The latter was designed to compare reactivity of the detecting sequence. A third detecting sequence, 5'-TTTCTCTTCTCTCTCTCCCCCTC-3' (noGA detecting sequence) was selected as blank because it does not contain guanines and adenines (low reactivity for adenine was also reported in some papers) [29].

3.2. Electrochemical DNA biosensor

DNA biosensors were developed using the electrochemical amplified assay scheme developed in [24] and reported in Section 2. The thiolated HS detecting sequence was immobilized on gold by self-assembled monolayer formation, and let to react with the biotinylated complementary sequence. Detection of hybridization reaction was achieved using a streptavidin–alkaline phosphatase conjugate and the use of 1-naphtylphosphate as substrate of the enzymatic reaction. Gold screen-printed electrodes (SPGE) were initially used as substrate for DNA immobilization. In fact, preliminary data, obtained using SPGE, suggested the possibility to detect BPDE at micromolar level [30]. However, a relevant non-specific signal onto the electrode surface was observed leading to poor sensitivity (signal/background ratio 3.3) and to inter-electrode (inter-day) RSDs > 20%.

In order to improve sensitivity it was decided to exploit the properties of gold nanoparticles (GNPs) taking advantage of the high surface/volume ratio. After immobilization of the thiolated HS onto GNPs according to [26], DNA affinity modules were obtained and were placed onto the surface of a carbon based screen-printed electrode (SPCE). The same protocol was used for the detection of the hybridization reaction. Improvement of sensitivity was immediately clear and is reported in Fig. 1 for the current signal obtained comparing hybridization of the HS detecting sequence; the signal/background ratio using the affinity modules was 16.0.

Despite this improvement in sensitivity, intra- and inter-day reproducibility was still not satisfactory ($\text{CV} > 20\%$); thus, the assay was carried out homogeneously with all the incubation steps performed separately in vial and the final detection of 1-naphtol using SPGE. In this way, temperature control was possible and the hybridization reaction was carried out at the annealing temperature of the oligonucleotide sequences in order to increase selectivity. A scheme of this homogeneous assay is reported in Fig. 2.

Using this approach intra-day RSDs were 7–9% and inter-day RSDs were <15%. Dose response curves obtained using the

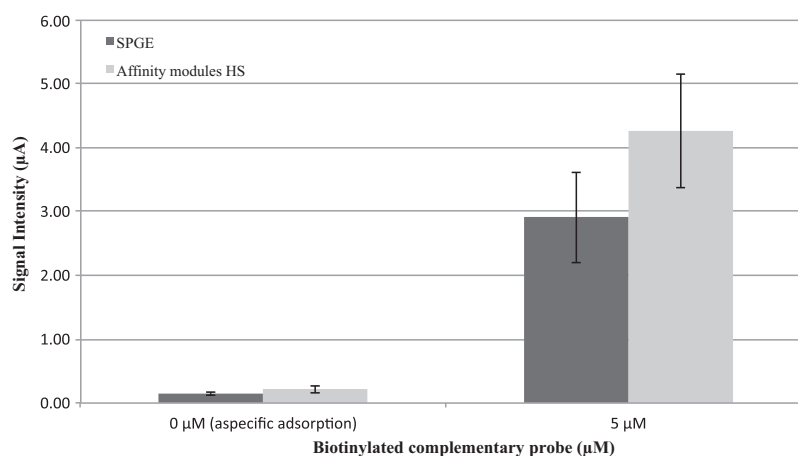


Fig. 1. Comparison of hybridization signal (complementary biotinylated sequence 5 μM) and aspecific adsorption (complementary biotinylated sequence 0 μM) obtained with SPGE and affinity modules.

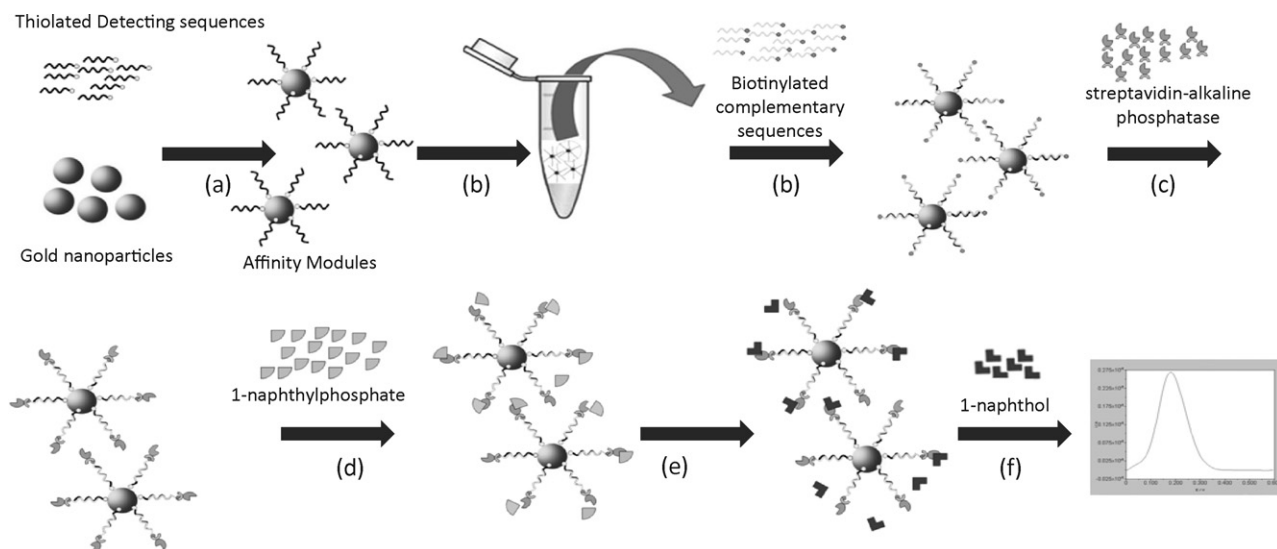


Fig. 2. Description of the electrochemical DNA biosensor based on the enzymatic amplification of the hybridization signal in the homogenous assay. (a) Affinity modules formation, (b) hybridization with the complementary biotinylated sequence, (c) coupling with the streptavidin–alkaline phosphatase conjugate, (d) incubation with the enzymatic substrate (1-naphthylphosphate), (e) enzymatic reaction and (f) electrochemical detection of enzymatic product with differential pulse voltammetry (DPV).

homogeneous assay and different concentrations of the complementary sequence are reported in Fig. 3 for the HS and the noGA detecting sequences and demonstrates clearly the ability of the developed method to detect down to micromolar concentrations of target.

3.3. SPR DNA sensors

SPR DNA sensors for the selected DNA detecting sequences were realized after the immobilization of the complementary sequence, on the sensor chip via streptavidin–biotin immobilization. Dose response curves were obtained after injections of the detecting sequence ($n=3$ for each concentration) within the concentration range of 3.9–1000 nM, followed by regeneration of the sensor chip. The SPR flow system allows direct detection of the hybridization reaction. The sensors dose–response curves, reported in Fig. 4, exhibited different dynamic ranges for the three detecting sequences tested.

The HS couple displayed the widest dynamic range, up to 1000 nM, whereas noHS and noGA showed smaller dynamic ranges for the relative detecting sequences up to around 125 nM. Excellent reproducibility was achieved with all the DNA sensors: average

RSDs were respectively 3% for HS, 7% for noHS, and 13% for noGA detecting sequences.

3.4. BPDE-DNA adduct formation and detection

The formation of the Guanine–BPDE adduct was obtained after incubation of the detecting sequences with the toxic compound following the procedure reported in Section 2.4. The yield of the reaction, obtained in different conditions and on different DNA sequences, has been reported to be in the 10–20% range [10]. Different enantiomeric products have been reported and characterized using UV, CD and NMR. The ability to decrease the signal of the hybridization reaction was then tested both for the electrochemical and SPR DNA sensors.

For electrochemical sensors, affinity modules modified with the HS detecting sequences, were diluted 1:2 (concentration corresponding to 15 nM) and incubated with different concentrations of BPDE up to 10 μ M; no variation in the hybridization reaction was observed. This data can be attributed either to the presence of an excess amount of DNA immobilized onto the gold nanoparticles or to the lower accessibility of the BPDE to guanines immobilized onto GNP that decreases the reaction rate of the toxic compound.

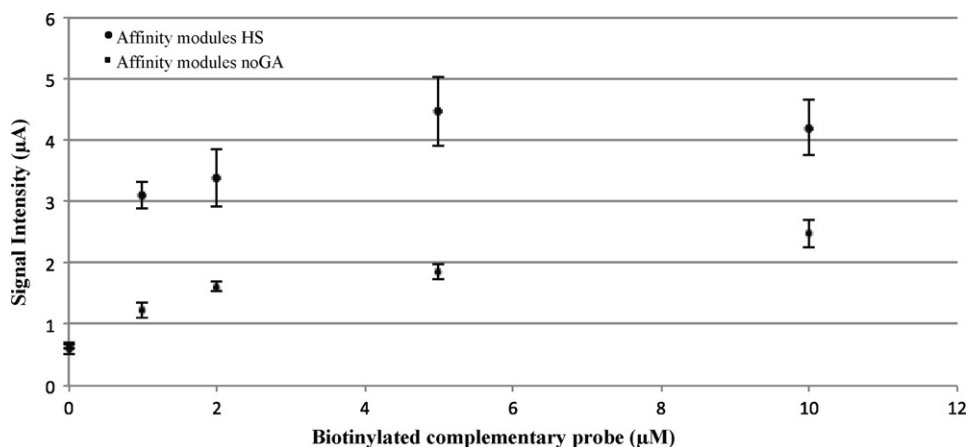


Fig. 3. Dose response curves of complementary biotinylated sequence for hot spot (HS), and the sequence without guanines and adenines (noGA) with affinity modules.

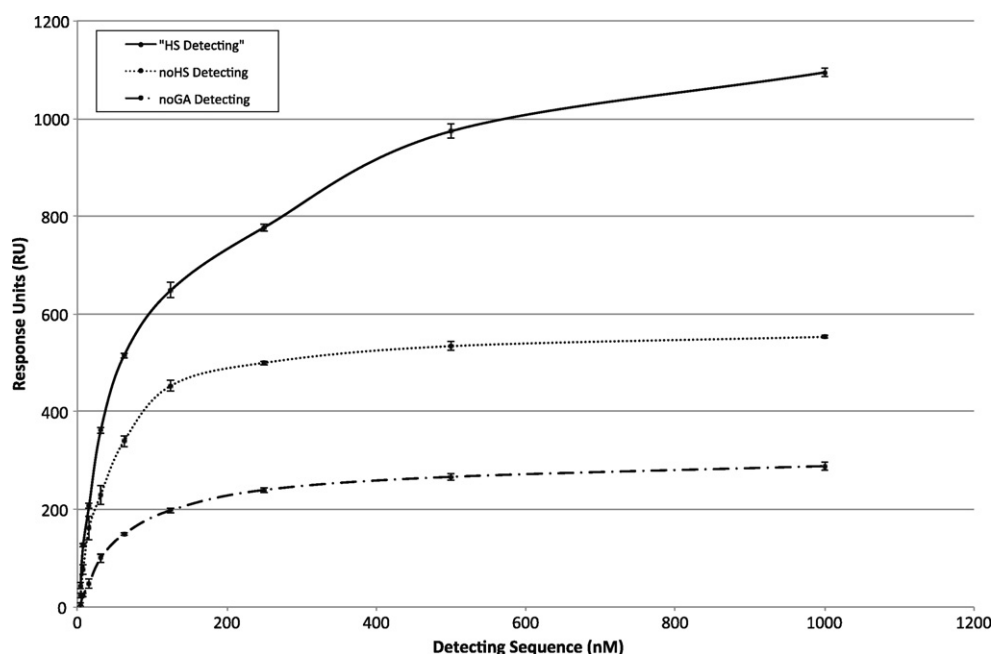


Fig. 4. Dose–response curves for detecting hot spot (HS), no hot spot (noHS) sequences, and the sequence without guanines and adenines (noGA) obtained by SPR. Binding responses were evaluated in triplicate in the concentration range of 3.9–1000 nM.

Analysis of the DNA adducts using SPR DNA sensors were carried out at the concentration of 62.5 nM of detecting DNA; the use of this concentration of detecting sequence was considered the best compromise to have a good analytical signal and maximization of the signal expected from the decreased hybridization signal (see Fig. 4 non-saturating conditions for the detecting sequence). A typical sensorgram comparing HS detecting sequence treated and untreated with BPDE is reported in Fig. 5.

A clear decrease of the hybridization reaction was obtained on the BPDE/HS adduct. Normalized response of the 3 detecting sequences after treatment with different DNA:BPDE ratios is reported in Fig. 6. All the BPDE/HS and BPDE/noHS adducts prepared at different BPDE/DNA ratios (10:1, 5:1, 2.5:1) displayed signals statistically lower than that obtained from untreated samples. In the range tested, our data did not evidence a dose-dependent effect by varying the BPDE/detecting sequence molar ratio.

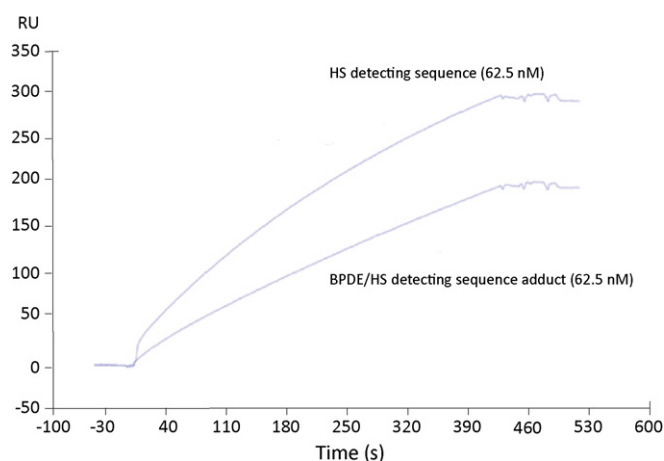


Fig. 5. Direct comparison between SPR sensorgrams of 10:1 BPDE/HS adduct and HS detecting sequence alone. Detecting hot spot (HS) concentration was 62.5 nM in both solutions.

The decrease in percentage, averaged over the different molar ratios, gave 22% of signal loss for the HS detecting sequence, and 26% for the noHS one. The noGA detecting sequence gave no detectable reduction of the hybridization signal. The effective reduction of the hybridization reactions resulted to be caused by the presence of the adduct on the detecting DNA with BPDE; in fact assays with DNA–BPDE reaction mixture immediately after mixing (no incubation) were also carried out; the signal was identical to the unreacted sequence. Moreover, BPDE alone was injected on the biosensor at 1 μ M, giving no variation of the SPR signal. The similar data obtained for HS and noHS detecting sequences, and the independence of the response on the ratios investigated are apparently in contrast with data reported in literature on higher reactivity for hot spot triplets.

In order to investigate these aspects we checked the yield of the reaction between HS detecting sequence and BPDE for all the tested ratios by using HPLC, following the procedure of Feng and Wang [31]. Yield was in the 20–30% range in all the cases, very similar to the reduction in the SPR signals. Moreover we took advantage of the real time analysis carried out using SPR that allows calculations of kinetic constants. Kinetic data were collected for the BPDE/HS and BPDE/noHS adducts and compared with the unmodified detecting sequence alone to evaluate differences. To this aim, pre-formed 10:1 BPDE/HS and BPDE/noHS adduct was diluted to different concentrations, ranging from 250 to 31.2 nM in binding buffer and injected on the HS and noHS biosensor. To estimate the dissociation rate constant (k_d), a delay time of 200 s was imposed before the washing step at the end of the sample injection. Affinity (K_A) and dissociation (K_D) constants were calculated using the BIA evaluation 3.1 software (Biacore). Results evidenced very similar values for both modified and unmodified samples, i.e. $K_A = 3.77 \times 10^8 \text{ M}^{-1}$ and $K_D = 2.66 \times 10^{-9} \text{ M}$ for the 10:1 BPDE/HS detecting adduct; $K_A = 3.36 \times 10^8 \text{ M}^{-1}$ and $K_D = 2.98 \times 10^{-9} \text{ M}$ for the HS detecting sequence alone. Data for BPDE/noHS detecting adduct were: $K_A = 9.92 \times 10^8 \text{ M}^{-1}$ and $K_D = 1.01 \times 10^{-9} \text{ M}$; for the noHS detecting sequence alone $K_A = 7.84 \times 10^8 \text{ M}^{-1}$ and $K_D = 1.28 \times 10^{-9} \text{ M}$. Results were confirmed on different biochips ($n = 3$).

Both the data on reaction yield and kinetic analysis confirm, in our opinion, that in the experimental conditions used in SPR

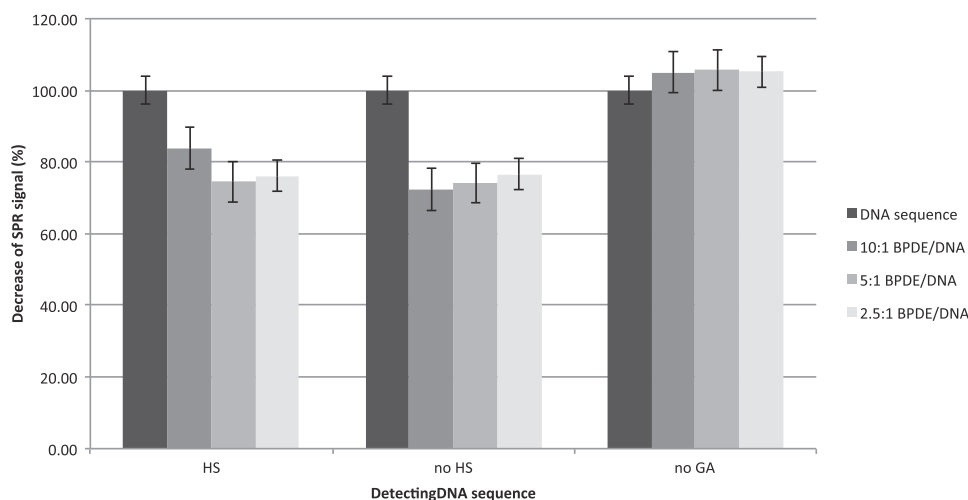


Fig. 6. Decrease, expressed as percentage of SPR signal with respect to detecting sequences alone, of the hybridization signal obtained after incubation of BPDE at different ratios with the detecting sequences.

measurements, only the DNA sequences that did not react with BPDE undergo hybridization with the complementary sequence. This hypothesis is also supported by the dramatic change in melting point obtained for the formation of a single adduct for a 13-mer oligonucleotide [10]. We can then conclude that reactivity with just one guanine it is enough to decrease the affinity of the DNA and avoid hybridization. Thus, no particular design in the oligonucleotide sequence is necessary for the detection of this kind of compounds forming adducts. In addition to these considerations, it should be emphasized that the lowest ratio tested is equivalent to a concentration of 156 nM of BPDE, that is fairly lower than the micromolar concentration range reported for the detection of contaminants using DNA sensors (mainly electrochemical).

4. Conclusions

Two different sensing approaches have been attempted to detect the formation of the DNA-adducts between BPDE and selected oligonucleotide sequences. The formation of the adduct was supposed to decrease the hybridization reaction. On the basis of preliminary data, a gold nanoparticles based amplified assay has been optimized as well as a SPR-based detection scheme. Detection at nanomolar level was achieved using the SPR sensor while no inhibition signal was obtained for the electrochemical assay. This was attributed either to the limited reactivity (accessibility) of BPDE for DNA-bound to gold nanoparticles or to an excess of oligonucleotides that does not allow sensitive detection of the adducts. A further optimization of the strategy for the electrochemical assay is then needed.

The presence of only 2 guanines in the 24-mer noHS detecting sequence was enough to allow no binding sequences containing adducts and, then, decrease of the hybridization reaction signal. Considering the detection obtained in the nanomolar range and the possibility to detect, in principle, any other compound forming adducts with DNA that can inhibit the hybridization reaction with the complementary sequence, the data reported in the paper appears promising for the realization of routine sensing of potentially toxic compounds via SPR DNA sensors.

Acknowledgement

Authors wish to thank the IZS ME 02/10 RC and the 280202 RF 2008 projects of the Italian Ministry of Health for financial support.

References

- [1] V.H. Gelboin, Benzo[a]pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed function oxidases and related enzymes, *Physiological Reviews* 60 (1980) 1107–1166.
- [2] C.S. Cooper, P.L. Grover, P. Sims, The metabolism and activation of benzo[a]pyrene, *Progress in Drug Metabolism* 7 (1983) 295–396.
- [3] P. Pradhan, A. Graslund, A. Seidel, B. Jernstrom, Implications of cytosine methylation on (+)-anti-benzo[a]pyrene 7,8-dihydrodiol 9,10-epoxide N₂-dG adduct formation in 5'-d(CGT), 5'-d(CGA), and 5'-d(CGC) sequence contexts of single- and double-stranded oligonucleotides, *Chemical Research in Toxicology* 12 (1999) 816–821.
- [4] D.R. Thakker, H. Yagi, W. Levin, A.W. Wood, A.H. Conney, D.M. Jerina, Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens, in: M.W. Anders (Ed.), *Bioactivation of Foreign Compounds*, Academic Press, New York, 1985, pp. 177–242.
- [5] R.G. Harvey, *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*, Cambridge University Press, Cambridge, UK, 1991, pp. 26–49.
- [6] A.M. Jeffrey, Polycyclic aromatic hydrocarbon-DNA adducts: formation, detection and characterization, in: R.G. Harvey (Ed.), *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Symposium Series, vol. 283, American Chemical Society, Washington, DC, 1985, pp. 187–208.
- [7] I. Ponten, A. Seidel, A. Graslund, B. Jernstrom, Synthesis and characterization of adducts derived from the syn-diastereomer of benzo[a]pyrene 7,8-dihydrodiol 9,10-epoxide and the 5'-d(CCTATAGATATCC) oligonucleotide, *Chemical Research in Toxicology* 9 (1996) 188–196.
- [8] B. Jernstrom, M. Funk, H. Frank, B. Mannervik, A. Seidel, Glutathione S-transferase A1-1 catalysed conjugation of bay and fjord region diol epoxides of polycyclic aromatic hydrocarbons with glutathione, *Carcinogenesis* 17 (1996) 1491–1498.
- [9] D.P. Michaud, S.C. Gupta, D.L. Whalen, J.M. Sayer, D.M. Jerina, Effects of pH and salt concentration on the hydrolysis of a benzo[a]pyrene 7,8-diol-9,10-epoxide catalyzed by DNA and polyadenylic acid, *Chemico-Biological Interactions* 44 (1983) 41–52.
- [10] I. Ponten, A. Seidel, A. Graslund, B. Norden, B. Jernstrom, Spectroscopic studies of the trans adducts derived from the (+)- and (–)-anti-benzo[a]pyrene 7,8-dihydrodiol 9,10-epoxide and the oligonucleotide 5'-d(CCTATAGATATCC), *Carcinogenesis* 15 (1994) 2007–2213.
- [11] M.F. Denissenko, A. Pao, M. Tang, G.P. Pfeifer, Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hot spots in p53, *Science* 274 (1996) 430–432.
- [12] S.N. Rodin, A.S. Rodin, On the origin of p53 G:C→T:A transversions in lung cancers, *Mutation Research* 508 (2002) 1–19.
- [13] T. Toyooka, Y. Iwaki, DNA damage induced by coexposure to PAHs and light, *Environmental Toxicology and Pharmacology* 23 (2007) 256–263.
- [14] J. Yang, L. Wang, P.P. Fu, H. Yu, Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list, *Mutation Research* 557 (2004) 99–108.
- [15] K.L. Platt, S. Aderhold, K. Kulpe, M. Fickler, Unexpected DNA damage caused by polycyclic aromatic hydrocarbons under standard laboratory conditions, *Mutation Research* 650 (2008) 96–103.
- [16] H. Shemer, K.G. Linden, Aqueous photodegradation and toxicity of the polycyclic aromatic hydrocarbons fluorene, dibenzofuran, and dibenzothiophene, *Water Research* 41 (2007) 853–861.
- [17] F. Lucarelli, A. Kicela, I. Palchetti, G. Marrazza, M. Mascini, Electrochemical DNA biosensor for analysis of wastewater samples, *Bioelectrochemistry* 58 (2002) 113–118.

- [18] G. Marrazza, I. Chianella, M. Mascini, Disposable DNA electrochemical biosensors for environmental monitoring, *Analytica Chimica Acta* 387 (1999) 297–307.
- [19] J. Wang, M. Chicarro, G. Rivas, X. Cai, N. Dontha, P.A.M. Farias, H. Shirashi, DNA biosensor for the detection of hydrazines, *Analytical Chemistry* 68 (1996) 2251–2254.
- [20] G. Chiti, G. Marrazza, M. Mascini, Electrochemical DNA biosensor for environmental monitoring, *Analytica Chimica Acta* 26 (2001) 155–164.
- [21] M. Del Carlo, M. Di Marcello, M. Perugini, V. Ponzilli, M. Sergi, M. Mascini, D. Compagnone, Electrochemical DNA biosensor for polycyclic aromatic hydrocarbon detection, *Microchimica Acta* 163 (2008) 163–169.
- [22] K. Kerman, B. Meric, D. Ozkan, P. Kara, A. Erdem, M. Ozsoz, Electrochemical DNA biosensor for the determination of benzo[a]pyrene-DNA adducts, *Analytica Chimica Acta* 450 (2001) 45–52.
- [23] D. Compagnone, R. Curini, G. D'Ascenzo, M. Del Carlo, C. Montesano, S. Napolitano, M. Sergi, Neutral loss and precursor ion scan tandem mass spectrometry for study of activated benzopyrene-DNA adducts, *Analytical and Bioanalytical Chemistry* 401 (2011) 1983–1991.
- [24] M. Del Carlo, M. Di Marcello, M. Giuliani, M. Sergi, A. Pepe, D. Compagnone, Detection of benzo(a)pyrene photodegradation products using DNA electrochemical sensors, *Biosensors and Bioelectronics* 31 (2012) 270–276.
- [25] G. Frens, Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions, *Nature Physical Science* 241 (1973) 20–22.
- [26] M. Moreno, E. Rincon, J.M. Pérez, V.M. González, A. Domingo, E. Dominguez, Selective immobilization of oligonucleotide-modified gold nanoparticles by electrodeposition on screen-printed electrodes, *Biosensors and Bioelectronics* 25 (2009) 778–783.
- [27] G. Carpinì, F. Lucarelli, G. Marrazza, M. Mascini, Oligonucleotide-modified screen-printed gold electrodes for enzyme-amplified sensing of nucleic acids, *Biosensors and Bioelectronics* 20 (2004) 167–175.
- [28] L.A. Margulis, V. Ibanez, N.E. Geacintov, Base-sequence dependence of covalent binding of benzo[a]pyrene diol epoxide to guanine in oligodeoxyribonucleotides, *Chemical Research in Toxicology* 6 (1993) 59–63.
- [29] M. Hall, P.L. Grover, Polycyclic aromatic hydrocarbons: metabolism, activation and tumour initiation, in: C.S. Cooper, P.L. Grover (Eds.), *Handbook of Experimental Pharmacology*, vol. 94, Springer-Verlag, Berlin, 1990, pp. 327–372.
- [30] M. Del Carlo, M. Sergi, M. Giuliani, D. Compagnone, A. Kiss, Electrochemical DNA sensors for the detection of benzo[a]pyrene toxicity, in: G. Neri, N. Donato, A. D'Amico, C. Di Natale (Eds.), *Lecture Notes in Electrical Engineering LNEE*, vol. 91, Springer, 2011, ISBN 978-940071323-9, pp. 351–354.
- [31] F. Feng, H. Wang, Simultaneous analysis of four stereoisomers of anti-benzo[a]pyrene diol epoxide–deoxyguanosine adducts in short oligodeoxynucleotides using reversed-phase high-performance liquid chromatography, *Journal of Chromatography A* 1162 (2007) 141–148.

Biographies

V. Lanzone is a Ph.D student in Food Science at the University of Teramo. She graduated in Chemical Science and Technologies at the University of L'Aquila, where she received also the Master Degree in Chemistry. Her research activity is on detection of contaminants in food matrices, particularly biosensors for detection and evaluation of the carcinogenic potential of polycyclic aromatic hydrocarbons (PAHs) and their

photo-oxidation products in food. She is also involved in the synthesis of biomimetic peptides for contaminants detection.

S. Scarano has a post-doctoral position at the Department of Chemistry, University of Florence (Italy). She took the Degree in Chemistry at Pisa University in 2002 and the PhD in Chemical Science in Florence University. Since 2007 she has been involved in biosensor development using different transduction principles, mainly optical (SPR, SPR imaging), piezoelectric (QCM), and latest electrochemical-based sensing. Her scientific interests cover the development of biosensors for applications to anti-doping controls and clinical diagnostics, coupled to the study of different bioreceptors such as aptamers, bio-mimetic peptides, antibodies, and nucleic probes.

M. Del Carlo holds a PhD in Analytical Chemistry (University of Florence, Italy) and a Master of Science in Biosensors (University of Newcastle upon Tyne, UK). He is Senior Researcher of Analytical Chemistry at the Department of Food Science, University of Teramo since 2002; his main research interests are in the field of electrochemical sensors and biosensors for food safety and quality application. In this area he has published more than 40 peer reviewed papers, 1 review and 1 chapter on book.

F. Corrado is Manager Biologist in the Department of Animal Health of Clinical Sciences "L. Sacco" of the Istituto Zooprofilattico Sperimentale del Mezzogiorno, Naples, Italy. She graduated in Biological Sciences at the University of Naples "Federico II". PhD in "Environmental Microbiology and Ecosystem skin" at the Second University of Naples 2001–2004. Her research interest is focussed on biochemistry of proteins, laboratory of Microbiology and Virology and extraction of genomic DNA from eukaryotic cells.

M. Esposito is Manager Chemist in the Department of Chemistry of the Istituto Zooprofilattico Sperimentale del Mezzogiorno, Naples, Italy. He graduated in "Chemistry and pharmaceutical technologies" at the University of Naples "Federico II". Ph.D. in "Chemistry and clinical biochemistry" at the University of Naples "Federico II". His main research interests are in the field of Persistent Organic Pollutants (PCDD/Fs, PCBs, PAHs) and focussed on the development of analytical methods for determination of these contaminants in food and tissues or biological fluids.

M. Minunni, Associate Professor, in Analytical Chemistry at University of Florence, Italy, leads the group of optical and piezoelectric-based sensing. PhD in Environmental Sciences in 1994. She has been visiting scientist and postdoctoral researcher at: Nestlé Research Centre, Lausanne, Switzerland, Pharmacia Biosensor AB, Uppsala, Sweden, UCC College Cork, Ireland, Technical University of Munich, Germany. She has been working in biosensor development since 1990, using various transduction principles. Her research interests have mainly covered developments of affinity sensors: immuno and nucleic acid-based sensing. Funded by different institutions both public and private: Ministry of Health, European Union, Regione Toscana, World Antidoping Agency.

D. Compagnone is Full Professor of Analytical Chemistry at the University of Teramo, Italy. He has been involved in the research area of electrochemical sensors and biosensors since 1988 at the Cranfield Institute of Technology (PhD in Biotechnology) and later working in different Institutions as Università de L'Aquila, Università G. D'Annunzio, University of New Orleans, University College Cork, Università Tor Vergata (researcher 1995–2001). His main area of interest is the development, application and validation in real samples of rapid methods of analysis mainly focussed on electrochemical sensing and biosensing for food quality and safety.