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Detection of benzo(a)pyrene photodegradation products using DNA electrochemical sensors

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ABSTRACT

The reactivity of photodegradation products of benzo(a)pyrene vs. DNA has been assessed using both genomic and oligonucleotide based DNA electrochemical sensors. The kinetic of a photooxidation reaction of benzo(a)pyrene (BaP) carried out in controlled conditions using a 6 W UV lamp peaked at 365 nm has been studied using LC with fluorimetric detection. Degradation of benzo(a)pyrene by both UV and UV/H_2O_2 exhibited pseudo-first-order reaction kinetics with half-lives ranging from 3.0 to 9.8 h depending on the pH and on the amount of H_2O_2. The oxidation products of benzo(a)pyrene obtained in different conditions were tested on genomic ssDNA electrochemical sensors obtained via immobilisation of salmon testis ss-DNA on graphite screen-printed electrodes. Guanines oxidation signals obtained using chronopotentiometry were used to detect the interaction of the products with DNA. The dose–response curve obtained with benzo(a)pyrene incubated 24 h at pH 4.7 was different from that of the parent compound indicating a different type of interaction with DNA. A DNA hybridisation sensor was also assembled using a thiolated/biotynilated 24-mer oligonucleotide immobilised on a gold screen-printed electrode and avidin–alkaline phosphatase conjugate. A voltammetric detection of naphtol was used to detect the hybridisation reaction. A net inhibition of the hybridisation reaction was observed after incubation with benzo(a)pyrene oxidation products that was attributed to the formation of stable adducts with the guanines of the biotinylated strand. LC–MS–MS studies of the oxidation products confirmed the presence of chemical species potentially forming adducts with DNA. The data reported demonstrate that DNA electrochemical sensors have the potential to be used to monitor remediation processes and to assess the potential toxicity vs. DNA of chemicals forming stable DNA adducts.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a large group of organic contaminants widely diffused in different ecosystems. They are composed by carbon and hydrogen atoms, forming at least two condensed aromatic rings. PAHs are generally produced as the result of incomplete combustion of organic material, improper storage and disposal of fuels and oils, and wood treatment processes (Shemer and Linden, 2007). Light PAHs (2–3 rings) easily spread in air while heavy PAHs associate with particulate in the environment (Billet et al., 2008). Because of their hydrophobic nature, PAHs can easily cross cell membranes and tend to bioaccumulate in lipid tissues. The mutagenic/genotoxic effects of different PAHs have been proved and these substances have been classified as potentially carcinogenic by the International Agency for Research on Cancer (IARC) (IARC Monographs, 1987). This effect is associated to the number of aromatic rings, and to the well known metabolic activation to reactive diol-epoxide intermediates that are able to covalently bind DNA. The N^2 amino-group of guanine and the N^6 of adenine have been reported to be the target sites for the attachment of the diol-epoxide intermediates (Schoket, 1999). Particular attention has also been paid to the levels of PAHs in food as reported recently by the European Food Safety Authority (EFSA) (EFSA, 2008). Contamination, in this case, can arise also from food processing (smoking, heat treatment) and cooking (e.g. grilled meat). For non-smoking humans, the major routes of exposure to PAH have been found to be from food and to some extent from inhaled air. The best studied among the PAHs is benzo[*a*]pyrene (BaP) and IARC concluded in 1987 that it is a probable human carcinogen. Maximum admissible levels of benzo[*a*]pyrene have been specified in Regulation (EC) No. 1881/2006 for different foods and varies from 1 (babyfood) to 10 μg/kg (bivalve mollusc). Of the almost 10,000 food samples examined in the study commisioned by EFSA (EFSA, 2008) 7% samples were found above the maximum admissible level for BaP; moreover, detectable levels of BaP were found in 72 of the 95 Codex food categories and in 47% of the samples tested.

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Toxicological concern regarding BaP has been mainly focussed on the metabolic activated form as benzo[a]pyrene diol-epoxide (Xue and Warshawsky, 2005; Penning et al., 1999; Scicchitano, 2005; Balu et al., 2006). The most known metabolic pathway for the "activation" of BaP involves the P-450 cytochrome pathway and produces the final carcinogen 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-BaP that is able to form adducts with DNA. Other metabolic pathways have been reported to form dione-BaP compounds that are able to form adducts as well.

Because of their very low solubility in water, PAHs do not show extremely high, acute toxicity to aquatic life; however, certain PAHs may pose a great hazard to aquatic organisms due to their potential to cause photo-induced toxicity when exposed to UV light (Lyons et al., 2002). Different studies have been carried out in order to investigate the phototoxic effect of PAHs (Hatch and Burton, 1999; Ibuki and Goto, 2002). Despite the well known phototoxic effect of PAHs and the well documented correlation between BaP and cancer, studies on its direct photo-toxicity are few (Cody et al., 1984; Kagan et al., 1989; Toyooka and Ibuki, 2007). Phototoxicity usually occurs when UV-rays excite the electrons in PAHs resulting in the formation of toxic singlet oxygen and other reactive oxygen species (ROS) by-products that cause oxidative damage in biological systems (Zhang et al., 2004). Moreover, the photo-activation may result into the formation of PAHs photooxidised products, which potentially have stronger bioactivity than the parent compound does (Kagan et al., 1989). Some of these reactive species, are expected to be able to form covalently bound adducts with proteins, DNA and RNA that are intermediates in the induction of PAH-induced cancer. PAHs also undergo photo and chemical oxidation processes, as UV-hydrogen peroxide assisted degradation that are used for their removal from contaminated water and soil. For these reasons much more attention should be paid to the photo-toxicity phenomena. Phototoxic PAHs are found to have the absorption peak in the UV-A range (320–400 nm) (Ibuki and Goto, 2002). Generally, toxicity evaluation of PAHs is carried out using aquatic organisms, such as fish larvae, protozoa and algae. These kinds of bioassays require long exposure times and large sample volume. Faster toxicity tests are based on microorganisms like luminescent bacteria (Lee et al., 2003). Due to the interaction ability of PAHs with nucleic acids, DNA electrochemical sensors seems to be interesting candidates for the development of analytical tools able to give a potential indication of toxicity. With respect to other techniques, these devices take the advantages of a rapid time of analysis and very small amount of sample required. Some studies have already demonstrated the possibility to use the variation of the electrochemical signal of guanine oxidation peak that occurs as a consequence of the interaction between the analyte and a genomic DNA, in order to assess the presence of investigated contaminants (Lucarelli et al., 2002; Marrazza et al., 1999; Wang et al., 1996; Chiti et al., 2001). Particularly, the use of a genomic DNA sensor for the detection of PAHs (Del Carlo et al., 2008) and for benzo[*a*]pyrene–DNA adducts has been already reported (Kerman et al., 2001). We have found that the detection of PAHs using a genomic DNA electrochemical sensor was correlated to the extent of contamination in mussels (Del Carlo et al., 2008); moreover, the signal was increased after UV exposure of contaminated and spiked samples. The aim of this work is to assess the interaction ability of photooxidised BaP products with DNA. For this purpose the degradation kinetics of the target compound was studied and a genomic electrochemical DNA biosensor was used to detect the interaction. The photooxidised products of BaP (BaP_{oxs}) were also able to inhibit the hybridisation of an oligonucleotide based DNA sensor, via formation of stable adducts with guanines. The data reported demonstrate that electrochemical DNA biosensors may be used as screening devices for monitoring both the photoremediation processes and the potential toxicity of compounds forming stable adducts with DNA.

2. Materials and methods

2.1. HPLC determination of BaP

BaP solutions were analysed using a HPLC chromatograph series 200, Perkin Elmer (Monza, Italy) equipped with a Grace Vydac C-18 column, ID 3 mm × 250 mm (Grace Vydac, Deerfield, IL, USA) and a LC 240 fluorimeter, Perkin Elmer (Monza, Italy) using an excitation wavelength of 288 nm and an emission wavelength of 406 nm. Isocratic elution was used; the mobile phase was 90:10 acetonitrile/water mixture, and both solvents were HPLC grade (Sigma, Milan, Italy). A flow rate of 0.5 mL/min was used during all the experiments. BaP retention time was 11.2 min. A calibration curve for BaP was obtained in the concentration range 1–1000 nM.

2.2. Benzo[a]pyrene photolysis experimental setup

Photolysis of BaP solutions was carried out with low (LP) Hg vapor UV lamps emitting at 365 nm using a 6 W Spectroline UV lamp model ENF-260C/FE (Spectronics Corporation, Westbury, NY, USA). An appropriate volume of BaP acetone stock solution (purity 99.9%; Supelco, Bellefonte, PA, USA) was dried under a nitrogen flow in a glass vessel and then diluted in 0.2 M sodium acetate buffer (sodium acetate, Sigma–Aldrich, Milan, Italy). 10% of methanol was also added to enhance the solubility of the analyte. Evaluation of the pH effect on the photodegradation reaction was achieved using two acetate buffer solutions at pH 4.7 and 7.0 respectively. Dark controls were carried out, to ensure that no loss of the BaP occurred via reactions other than photolysis (i.e. hydrolysis, evaporation, and adsorption to the walls of the reaction vessel).

Degradation kinetics were determined assaying 20 μL of BaP solutions via HPLC. BaP degradation kinetics (k'_d, $1/t$) were calculated using the following equation:

$$\ln\left(\frac{[\mathrm{BaP}]_0}{[\mathrm{BaP}]_t}\right) = k_t$$

2.3. Genomic DNA electrochemical biosensor preparation and assay

Electrochemical measurements were performed using an AUTOLAB PGSTAT 12 Electrochemical Analysis System with GPES 4.5 Software Package (Ecochemie, Utrecht, The Netherlands). Screen-printed electrodes were obtained from EcoBioServices and Research (Florence, Italy). The electrochemical measurements were carried at 30 °C using a block heater SBH 13-OD (Stuart, Staffordshire, UK). The screen-printed electrochemical cell (3 cm × 1.5 cm) consists of a graphite working electrode, a graphite counter electrode and a silver pseudo-reference electrode.

The genomic DNA biosensors were prepared using salmon testis single strand DNA (ST ss-DNA) obtained from Sigma–Aldrich (Milan, Italy) according to an already optimised procedure (Del Carlo et al., 2008). In brief, a DNA solution was prepared by dissolving DNA pellet in distilled water to a final concentration of 100 μM. 20 μL aliquots were stored at −20 °C until use. Water, plastic tips and vials were autoclaved prior to use in order to avoid DNAase contamination.

The working electrode surface was initially pre-treated at a potential of +1.6 V for 120 s and +1.8 V for 60 s in 0.2 M acetate buffer, pH 4.7. The electrochemical strip was then washed with 1 mL of 0.2 M acetate buffer pH 4.7 containing 0.01 M KCl and immersed in the DNA solution at fixed potential of +0.5 V vs. Ag/AgCl pseudo-reference electrode for 120 s under stirring. A cleaning step was