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Novel Electrochemical DNA Biosensors as Tools for Investigation and Detection of DNA Damage

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Abstract Supramolecular interactions of various organic xenobiotic compounds with deoxyribonucleic acid (DNA) are among the most important aspects of biological studies in clinical analysis, drug discovery, and pharmaceutical development processes. In recent years, there has been a growing interest in the electrochemical investigation of interactions between a studied analyte and DNA. Observing the pre- and post-electrochemical signals of DNA or monitoring its interaction with xenobiotics provides good evidence for the interaction mechanism to be elucidated. Such interaction can also be used for sensitive determination of these compounds. This short review summarizes our results obtained during the last 5 years in the field of novel electrochemical DNA biosensors utilizing carbon-based transducers as substrates for immobilization of DNA. It should provide evidence that the electrochemical approach (employing simple, fast, sensitive, and inexpensive DNA biosensors as tools for investigation and detection of DNA damage) brings new insight into human health protection or rational drug design and leads to further understanding of the interaction mechanism between xenobiotic compounds and DNA.

Keywords Carbon electrodes • Chemical carcinogens • DNA biosensors • DNA damage detection • Drugs • Oxidative stress • Review • Supramolecular chemistry

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Abbreviations

AA	2-Aminoanthracene
Ag/AgCl	Silver/silver chloride reference electrode
CFE	Microcrystalline natural graphite–polystyrene composite film-modified electrode
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
dsDNA	Double-stranded deoxyribonucleic acid
EIS	Electrochemical impedance spectroscopy
GCE	Glassy carbon electrode
IARC	International Agency for Research on Cancer
NTMA	4-Nitro-3-(trifluoromethyl)aniline
PB	Phosphate buffer
phen	1,10-Phenanthroline
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSS	Reactive sulfur species
SPCE	Screen-printed carbon electrode
SWCNT	Single-walled carbon nanotube
SWV	Square-wave voltammetry

1 Introduction

In the last decade, increasing attention has been paid to the binding of small organic molecules to nucleic acids. Such in vitro studies have a key importance for a detailed understanding to these supramolecular interactions, especially in terms of damage to deoxyribonucleic acid (DNA) caused by various xenobiotic compounds [1]. A variety of small molecules are known to interact reversibly with double-stranded DNA (dsDNA) through one of the following three modes: (1) electrostatic interactions with the negatively charged nucleic sugar–phosphate structure, (2) groove binding interactions, or (3) intercalations between the stacked base pairs of dsDNA [2–4].

Analysis of the interfacial biomolecular interaction between DNA-targeted drugs and immobilized DNA probes has a particular role in the rational design of novel DNA-binding drugs and to the drug screening. Interactions of anticancer drugs with nucleic acids have been studied by numerous physical and biochemical techniques. UV–vis absorption spectrophotometry, fluorescence spectroscopy, vibrational spectroscopy (Fourier transform infrared spectroscopy and Raman spectroscopy), polarized light spectroscopies (linear and circular dichroism), fluorescence anisotropy or resonance, surface plasmon resonance, nuclear magnetic resonance, viscometry, and gel or capillary electrophoresis have been applied to provide insight into binding modes, DNA affinity, and base pair selectivity of DNA-binding drugs [5]. However, these techniques mostly address the issues of the binding mechanisms and structural analysis (e.g., DNA base sequence selectivity, correlation of structure–activity relationships, linkages between the geometry and thermodynamic properties, or influences of substituent modifications on the physical, chemical, and biological properties of the drug–DNA complex formed) [6].

Nucleic acid layers combined with electrochemical transducers have produced a new kind of affinity biosensor capable of rapidly recognizing and monitoring DNA-binding organic compounds [1]. Electrochemical biosensors have been successfully used for a number of applications including monitoring DNA damage, studies of the interactions of DNA with various genotoxic agents (carcinogens, mutagens, toxins, drugs, etc.), and also for the detection of specific mutations in DNA sequences [7]. Thus, they potentially offer fast and inexpensive alternative to traditional methods of measuring analyte–DNA interactions [8–10].

Recently, various reviews of electrochemical DNA biosensors have been reported [1, 11–20]. The present review will focus on the most widely used strategies in the technology of electrochemical DNA biosensors, with the special emphasis placed on their construction and application in the field of DNA damage detection and investigation of supramolecular interactions between xenobiotic compounds and DNA. Our results, obtained during the last 5 years in the field of novel electrochemical DNA biosensors utilizing carbon-based transducers as substrates for immobilization of DNA, will serve as illustrative examples.

2 Electrochemical DNA Biosensors for Detection of DNA Damage

DNA belongs to main biological macromolecules that undergo serious structural changes such as oxidation of the DNA bases and sugar moieties and release of the bases, as well as strand breaks caused by chemical systems generating so-called reactive oxygen (ROS), reactive nitrogen (RNS), or reactive sulfur (RSS) species [21, 22] or caused by other classes of genotoxic substances [23]. Thus, one of the main application areas for DNA biosensors is the detection of damage to DNA.

ROS are formed either endogenously (during normal aerobic metabolism and under various pathological conditions) or exogenously (e.g., upon the exposure to UV light, ionizing radiation, or environmental mutagens and carcinogens). About ten thousands to millions of DNA damage events occur to a cell per day [23]. Accumulation of oxidative DNA lesions is associated with aging and with a variety of human diseases including cancer and neurodegeneration.

Altered chemical, physicochemical, and structural properties of damaged DNA are reflected in its redox behavior which is utilized in numerous techniques of DNA damage detection. Electrochemical DNA biosensors have been used not only to detect but also to induce and control DNA damage at the electrode surface via electrochemical generation of the damaging (usually radical) species [24]. In this way, chemical carcinogens and drugs (e.g., nitro derivatives of polycyclic aromatic hydrocarbons [25], adriamycin [26], niclosamide [27], or nitrofurazone [28]) have been investigated.

2.1 Construction of Biosensors

DNA biosensors are integrated receptor–transducer devices that use DNA as a biomolecular recognition element to measure specific binding processes with DNA, usually by electrical, thermal, or optical signal transduction [12]. Compared with other transducers, electrochemical ones received particular interest due to a rapid detection and great sensitivity. Among the electrochemical transducers, carbon-based electrodes (e.g., glassy carbon electrode (GCE), pyrolytic graphite electrode, pencil lead electrode, carbon composite electrodes, carbon paste electrodes, carbon film electrodes, screen-printed carbon electrodes (SPCEs), boron-doped diamond electrodes [29, 30], chemically modified carbon electrodes, or carbon nanoparticle-modified electrodes) exhibit several unique properties [12]. The wide electrochemical potential window in the positive direction allows sensitive electrochemical detection of oxidative damage caused to DNA by monitoring the appearance of oxidation peaks of DNA bases [31].

In the last 5 years, our attention has been paid to the development of novel electrochemical DNA biosensors based on carbon-based transducers as substrates for immobilization of DNA. Among others, following four electrodes exhibited the best properties for these purposes: GCE (supplied by Metrohm, Herisau, Switzerland), microcrystalline natural graphite–polystyrene composite film-modified electrode (CFE), SPCE (supplied by Food Research Institute, Bratislava, Slovakia), and SPCE modified with single-walled carbon nanotubes (SWCNTs).

The newly developed CFE [32] (Fig. 1a), formed by covering a classical solid working electrode with a conductive carbon film, represents a very promising alternative to electrode surfaces modified by several carbon nanoparticles with profitable electrocatalytic properties (nanotubes, graphene, etc.). The smallest particles of micronized natural graphite (type CR 2 995, Graphite Týn, Týn nad Vltavou, Czech Republic) reach the size around 1,000 nm [33], which is very close

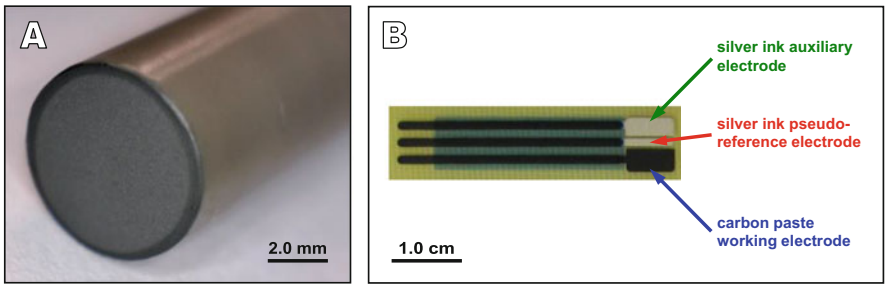

Fig. 1 Detailed pictures of the CFE (**a**) and the SPCE (**b**)

to dimensions of carbon nanoparticles commonly used in modern electroanalytical applications. However, the price of this electrode material is incomparably lower (about 1 euro cent per 1 g) than that of commercially available carbon nanoparticles. Therefore, it is more applicable for electroanalytical practice. Moreover, the CFE represents a suitable alternative to the commercially available disposable SPCEs [34]. Its simple, fast, and inexpensive preparation (the surface of a classical solid working electrode in a plastic electrode body is covered with a carbon ink suspension and left to evaporate to dryness), simple mechanical renewal of the electrode surface (by wiping off the old film with filter paper and forming a new one), good reproducibility of measurements, elimination of problems connected with "electrode history", and simple chemical modification are the main advantages.

The SPCE assembly (Fig. 1b) [25] consisted of a carbon paste working electrode, a silver ink pseudo-reference electrode, and a silver ink auxiliary electrode and was fabricated using a typical screen-printing equipment. The SPCE modified with carboxylated SWCNTs (SWCNTs/SPCE) was prepared as described in [35].

Adsorption is the simplest method to immobilize DNA on an electrode surface. It does not require reagents or special modifications in the DNA structure. There are many reports on DNA immobilization using a potential applied to GCEs, carbon paste electrodes, or SPCEs [1, 36–38]. The polished surface of the carbon electrode is usually pretreated by applying a positive potential (ca. 1.5–1.8 V vs. silver/silver chloride reference electrode (Ag/AgCl)) for a certain time. This pretreatment of the carbon surface increases its roughness and hydrophilicity [39, 40]. Afterward, the electrochemical adsorption of DNA is realized using a stirred solution at a potential of 0.5 V vs. Ag/AgCl for a preset time that depends on DNA concentration. This potential enhances the stability of the immobilized DNA through the electrostatic attraction between the positively charged carbon surface and the negatively charged hydrophilic sugar–phosphate backbone [24].

We used this immobilization approach in the development of a novel electrochemical DNA biosensor utilizing low molecular weight dsDNA isolated from salmon sperm as a biorecognition layer immobilized onto a GCE surface [41]. Such a biosensor was used for the high-throughput detection of dsDNA damage caused by various organic xenobiotic compounds [41–43]. The whole

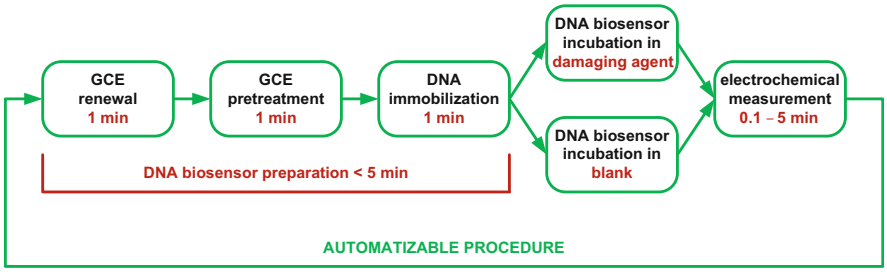

Fig. 2 Working procedure diagram for the automatization of measurement using the DNA-modified glassy carbon electrode (GCE)

preparation of this biosensor (involving removal of the previous dsDNA layer, pretreatment of the regenerated electrode surface, and deposition of the new dsDNA layer) takes no more than 5 min. This represents a significant shortening of the preparation time in comparison to procedures employing the air-drying of a DNA solution on the electrode surface [42]. Moreover, it allows an automatization of measurement and processing of experimental data (Fig. 2) if the electrochemical removal (desorption in a stirred solution at a potential of −0.5 V vs. Ag/AgCl) of the previous dsDNA layer is used.

Another way to immobilize DNA by adsorption on an electrochemical transducer has been described [44, 45]. In this case, the DNA biosensor was prepared by dipping a GCE in a DNA solution and leaving the electrode to dry. This sensor was then used to preconcentrate nitroimidazole [44] or mitoxantrone [45] on the surface and to study the interaction mechanism of these drugs with DNA by means of cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square-wave voltammetry (SWV).

A different approach for immobilization of DNA is based on evaporation of a small volume of DNA solution on the GCE surface [46]. We used this approach during the preparation of the DNA-modified CFE [32], SPCEs [25, 47], or SWCNTs/SPCEs [35]. Similarly, an electrochemical DNA biosensor has been developed [48], based on DNA adsorbed on a polished basal plane pyrolytic graphite electrode. An adsorptive method to immobilize DNA on the gold electrode has also been reported [49, 50]. The gold electrode was modified by dropping a small volume of DNA on its surface, followed by air-drying overnight and rinsing to remove unabsorbed DNA.

On the other hand, DNA-modified mercury electrodes can be prepared easily by immersing a hanging mercury drop electrode or a mercury film electrode into a drop of the DNA solution. This approach requires less amount of DNA for analysis [51–53]. DNA bases and nucleosides are strongly adsorbed at mercury electrodes. Nucleosides possess an extraordinary ability of self-association (two-dimensional condensation) at the surface of mercury electrodes and can form monomolecular compact films. At high positive potentials, all DNA bases can react with mercury electrodes, forming sparingly soluble compounds.

Nanostructured interfaces between the bare electrode and DNA, formed by various nanomaterials such as gold nanoparticles and carbon nanomaterials (e.g., SWCNTs, multi-walled carbon nanotubes, carbon nanofibers, graphene, and graphene oxide nanosheets) [54–63], represent another approach to the enhancement of the biosensor response due to inherent electroactivity, effective electrode surface area, etc. [35, 64]. Nanometer scale complex films of DNA, enzymes, polyions, and redox mediators were suggested for tests of genotoxic activity of various chemicals [65].

2.2 Detection Techniques

Voltammetric (especially CV, DPV, and SWV (Fig. 3a)) and chronopotentiometric detection modes are most frequently used [31]. Together with them, electrochemical impedance spectroscopy (EIS) (Fig. 3b) becomes to be very popular in the field of DNA-based biosensors [67]. According to electrochemically active species,

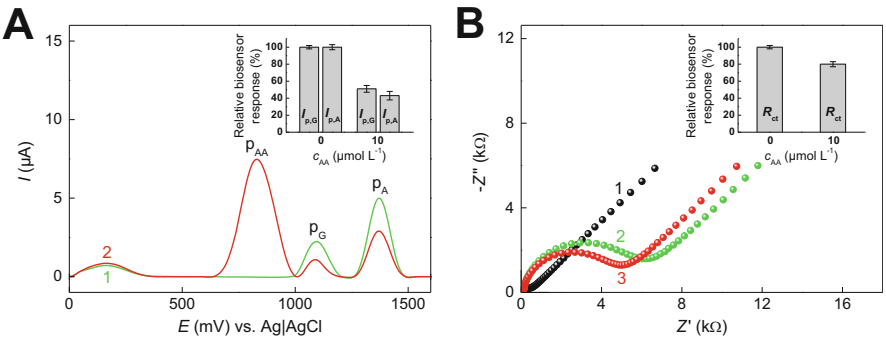

Fig. 3 (**a**) Baseline-corrected square-wave voltammograms recorded in 0.1 mol L^{-1} acetate buffer of pH 4.8. Legend: (*1*) measured at a DNA-modified GCE after 5 min incubation of the biosensor in 0.1 mol L^{-1} phosphate buffer (PB) of pH 7.0 and (*2*) measured at a DNA-modified GCE after 5 min incubation of the biosensor in 0.1 mol L^{-1} PB of pH 7.0 containing 1×10^{-5} mol L^{-1} 2-aminoanthracene (AA); p_G peak of a guanine moiety, p_A peak of an adenine moiety, p_{AA} peak of intercalated AA. Experimental conditions: polarization rate 3 V s^{-1}, pulse amplitude 0.04 V, frequency 200 Hz, potential step 0.015 V. *Inset*: the relative biosensor responses to DNA damage caused by AA, evaluated from the changes in the height of the guanine ($I_{p,G}$) and adenine ($I_{p,A}$) moiety peaks; the *error bars* are constructed for the significance level of 0.05 ($n = 3$) [66]. (**b**) Nyquist plots in the presence of 1×10^{-3} mol L^{-1} $[Fe(CN)_6]^{4-/3-}$ in 0.1 mol L^{-1} PB of pH 7.0. Legend: (*1*) measured at a bare GCE, (*2*) measured at a DNA-modified GCE after 5 min incubation of the biosensor in 0.1 mol L^{-1} PB of pH 7.0, and (*3*) measured at a DNA-modified GCE after 5 min incubation of the biosensor in 0.1 mol L^{-1} PB of pH 7.0 containing 1×10^{-5} mol L^{-1} AA. Experimental conditions: polarization potential 0.21 V vs. Ag/AgCl, potential amplitude 0.01 V, frequency range 0.1–5,000 Hz (51 frequency steps). *Inset*: the relative biosensor responses to DNA damage caused by AA, evaluated from the changes in the charge transfer resistance (R_{ct}) values; the *error bars* are constructed for the significance level of 0.05 ($n = 3$) [66]

which responses are evaluated at the detection of damage to DNA, the experimental techniques can be classified as follows [12]:

(a) Label-free and often reagent-less techniques which represent the work with no additional chemical reagents (redox indicators, mediators, enzyme substrates, etc.) needed to generate measured response
(b) Techniques which employ redox indicators either non-covalently bound to DNA (groove binders, intercalators, anionic or cationic species interacting with DNA electrostatically) or present in the solution phase (e.g., hexacyanoferrate anions ($[Fe(CN)_6]^{4-/3-}$))
(c) Techniques which employ electrochemically active labels (nanomaterials, enzymes, etc.) covalently bound to DNA (not frequently used in fundamental investigations of DNA damage)

Combination of these principles allows obtaining more complex information on DNA changes and damaging supramolecular interactions, as well [35, 68].

The first group of techniques utilizes surface activity or redox activity of DNA itself [69]. The electrochemical activity is based on the presence of redox active sites at nucleobases and sugar residues. Only DNA bases can undergo redox processes at carbon and mercury electrodes. Deoxyribose and phosphate groups are not electroactive. Electrochemical oxidation on carbon-based electrodes [70–72] showed that all bases (guanine, adenine, thymine, and cytosine) can be oxidized, following a pH-dependent mechanism. Electrochemical preconditioning of the GCE enabled a better peak separation and an enhancement of the current of the oxidation peaks for all four DNA bases in phosphate buffer (PB) of pH 7.4 (value close to physiological pH) used as the supporting electrolyte [71].

Electrochemical reduction of natural and biosynthetic nucleic acids at a dropping mercury electrode [1, 3, 73] showed that adenine and cytosine residues, as well as guanine residues in a polynucleotide chain, are reducible. The CV of DNA at a hanging mercury drop electrode showed a cathodic peak due to irreversible reduction of cytosine and adenine moieties. The reduction of the guanine moiety occurs at very negative potentials, but a peak due to the oxidation of the reduction product of the guanine moiety (7,8-dihydroguanine moiety) could be detected in the reverse scan [3].

As both the electrochemical reduction and oxidation of DNA bases are irreversible, measurements cannot be performed repeatedly. Initial increase in the anodic guanine moiety response after short-time incubation of the biosensor in damaging agents can indicate opening of the original dsDNA structure, while decrease in this response (Fig. 3a) is an evidence for the deep DNA degradation [68]. Decrease of the anodic guanine moiety peak height or area relative to that yielded by intact DNA was suggested as a measure representing degree of damage to this nucleobase and proposed as a screening test for environmental pollutants present in water or wastewater samples [9]. Some products of the DNA damage exhibit characteristic electrochemical signals (e.g., anodic peaks of 8-oxo-7,8-dihydroguanine [25, 74] and 2,8-dihydroxyadenine [75] moieties) which can be evaluated with better sensitivity than the change in the original guanine moiety response.

The second group of techniques employs electroactive compounds added to the measured system and interacting with DNA non-covalently as its indicators (cationic indicators, intercalators, and groove binders). Decrease in the intercalator or groove binder response indicates strand breaks and helix destruction. The redox indicators may be also used as diffusionally free species present in the solution phase. For instance, the $[Fe(CN)_6]^{4-/3-}$ anions indicate the presence of DNA layer on the electrode surface on the basis of electrostatic repulsion between the indicator anion and the negatively charged DNA backbone (Fig. 3b) [76, 77].

Moreover, the investigated xenobiotic compound itself can serve as a redox indicator. While its peak potential is shifted in the positive direction when the analyte binds to DNA by intercalation between the stacked base pairs of dsDNA, the peak potential is shifted in the negative direction when the interaction with DNA occurs by electrostatic attraction (interaction with the negatively charged nucleic sugar–phosphate structure) [78]. Such approach was used in our recent study [41] where the interaction between genotoxic 2-aminofluoren-9-one and dsDNA was investigated by DPV (performed at the bare GCE when both dsDNA and 2-aminofluoren-9-one were present in the measured solution). The intercalation of 2-aminofluoren-9-one between the dsDNA base pairs was the predominant supramolecular interaction observed.

2.3 Investigated Xenobiotic Compounds

There are thousands of organic compounds that bind and interact with DNA and can cause serious human diseases. The factors that determine affinity and selectivity in binding molecules to DNA need to be explained, because a quantitative understanding of the reasons that determine selection of DNA reaction sites is useful in designing sequence-specific DNA-binding molecules for application in chemotherapy and in explaining the mechanism of action of genotoxic compounds [31].

DNA damage induced by environmental pollutants (a lot of them are marked as chemical carcinogens) (Table 1) is a major endogenous toxicity pathway in biological system [84]. Most of organic pollutants may not directly cause DNA damage but their metabolized products by enzyme reactions are genotoxic and may cause DNA lesions [25, 85]. Electrochemical DNA biosensors enabling detection of such DNA damage could serve as a basis for in vitro genotoxicity screening for new organic chemicals at an early stage of their commercial development. For example, styrene is one of the most widely used industrial chemicals and itself shows little genotoxicity [86]. However, after being metabolized by liver cytochrome P450 enzymes, its oxidized product styrene oxide can induce DNA damage by formation of DNA adducts [87–89]; styrene oxide is classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen (group 2A) [90].

In our paper [25], an electrochemical DNA biosensor based on an SPCE with an immobilized layer of calf thymus dsDNA was used for in vitro investigation of the

Table 1 A survey of compounds investigated in the UNESCO Laboratory of Environmental Electrochemistry using various electrochemical DNA biosensors in connection with DNA damage

Xenobiotic compound	Detection technique	Transducer	Type of damage	References
Chemical carcinogens				
2,7-Diaminofluorene	DPV, SWV	GCE	Intercalation	[79]
	SWV	SPCE	Intercalation	[80]
2,7-Dinitrofluorene	CV, DPV, SWV	SPCE	Intercalation	[25]
2-Acetylaminofluorene	DPV, SWV	GCE	Intercalation	[79]
2-Aminoanthracene	SWV, EIS	GCE	Intercalation	[42]
2-Aminofluorene	DPV, SWV	GCE	Intercalation	[79]
	CV, SWV	CFE	Intercalation	[32]
	CV, EIS	CFE	Intercalation	[81]
	SWV	SPCE	Intercalation	[80]
2-Aminofluoren-9-one	DPV, SWV	GCE	Intercalation	[41]
2-Nitrofluorene	CV, EIS	GCE	Intercalation	[43]
	CV, DPV, SWV	SPCE	Intercalation	[25]
Anthracene	SWV, EIS	GCE	Intercalation	[42]
Fluorene	CV, EIS	CFE	Intercalation	[81]
Fluoren-9-one	SWV	SPCE	Intercalation	[80]
Drugs				
Ellipticine	CV	CFE	Intercalation	[82]
Flutamide	SWV, EIS	GCE	No damage detected	[42]
NTMA	SWV, EIS	GCE	No damage detected	[42]
Thioridazine	CV, SWV, EIS	SWCNTs/SPCE	Intercalation	[35]
Reactive radical species				
ROS (hydroxyl radicals)	CV, SWV, EIS	CFE	Oxidative damage	[83]
	CV, SWV, EIS	SWCNTs/SPCE	Oxidative damage	[35]
RNS (nitro radical anions)	SWV	SPCE	Oxidative damage	[25]

interaction between genotoxic nitro derivatives of fluorene (namely, 2-nitrofluorene and 2,7-dinitrofluorene) and DNA. Two types of DNA damage were detected at the DNA-modified SPCE: (1) caused by direct association of the nitrofluorenes, for which an intercalation association was found using the known dsDNA intercalators $[Cu(phen)_2]^{2+}$ and $[Co(phen)_3]^{3+}$ (phen stands for 1,10-phenanthroline) as competing agents, and (2) caused by short-lived radicals generated by electrochemical reduction of the nitro group (observable under specific conditions only).

Similar investigation was performed in our study [32] where a novel voltammetric DNA biosensor based on the CFE in the role of a transducer was used for investigation of the interaction between model carcinogenic substance

2-aminofluorene (one of the most extensively studied examples of the aromatic amine class of carcinogens) and calf thymus dsDNA. The layer of dsDNA immobilized at the electrode surface was utilized as a biocomponent responsive interface. The biosensor was characterized regarding the detection of DNA damage (induced by direct interaction with 2-aminofluorene) using SWV responses of the guanine and adenine moieties and CV responses of the anionic redox indicator $[Fe(CN)_6]^{4-/3-}$ present in solution. The obtained results confirmed that the interaction of dsDNA with 2-aminofluorene caused dsDNA damage, leading to the formation of strand breaks and desorption of DNA fragments from the electrode surface.

A number of aromatic compounds induce oxidative DNA damage through the generation of ROS. ROS produced in vivo react with DNA and its precursors modifying them thus giving rise to the so-called oxidative stress. It is thought that the modification of DNA (DNA lesions) leads to the formation of incorrect base pairs (changes in the genetic information), which induces mutagenesis and carcinogenesis. Therefore, there is a deep interest in identifying free radical scavengers or antioxidants that inhibit oxidative DNA damage. Owing to their polyphenolic nature, flavonoids (compounds found in rich abundance in all land plants) often exhibit strong antioxidant properties [91–95]. Initially, flavonoids were investigated as potential chemopreventive agents against certain carcinogens. Previous intake of a large quantity of flavonoid inhibited the incidence of ROS produced damages to DNA. In sharp contrast with their commonly accepted role, there is also considerable evidence that flavonoids themselves are mutagenic and have DNA-damaging ability [31, 92, 93].

In agriculture, farmers use numerous pesticides to protect crops and seeds before and after harvesting. Pesticide residues may enter into the food chain through air, water, and soil. They affect ecosystems and cause several health problems to animals and humans. Pesticides can be carcinogenic and cytotoxic. They can produce bone marrow and nerve disorders, infertility, and immunological and respiratory diseases [96]. Recently, an electrochemical DNA biosensor was developed to study DNA damage caused by several pesticides such as atrazine, 2,4-dichlorophenoxyacetic acid, glufosinate ammonium, carbofuran, paraoxon-ethyl, and difluorobenzuron [97]. A biotinylated DNA probe was immobilized on a streptavidin-modified electrode surface. This DNA probe was hybridized with biotinylated complementary DNA target analyte. Streptavidin labeled with ferrocene was further attached to the hybridized biotinylated DNA. The close proximity of ferrocene to the electrode surface induced a current signal. The presence of pesticides caused an unwinding of the DNA and thus a decrease of the ferrocene oxidation current observed in voltammetric experiments. Paraoxon-ethyl and atrazine caused the fastest and most severe damage to DNA [97].

The interaction of DNA with drugs (Table 1) is among the important aspects of biological studies in drug discovery and pharmaceutical development processes [98]. There are several types of interactions associated with drugs that bind to DNA. These include intercalation, non-covalent groove binding, covalent binding (formation of DNA adducts), DNA cleaving, or nucleoside analog incorporation. Consequences of these binding interactions involve changes to both the DNA and

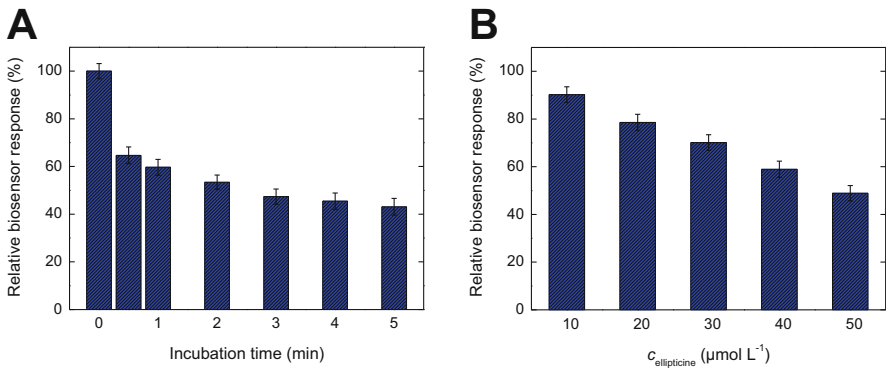

Fig. 4 (**a**) The relative biosensor responses to DNA damage caused by ellipticine, evaluated from the changes in the height of the anodic CV peak of 1×10^{-3} mol L^{-1} $[Fe(CN)_6]^{4-/3-}$ in 0.1 mol L^{-1} PB of pH 7.2 before and after incubation of the DNA-modified CFE in 0.1 mol L^{-1} PB of pH 7.2 containing 5×10^{-5} mol L^{-1} ellipticine for various times. (**b**) The relative biosensor responses to DNA damage caused by ellipticine, evaluated from the changes in the height of the anodic CV peak of 1×10^{-3} mol L^{-1} $[Fe(CN)_6]^{4-/3-}$ in 0.1 mol L^{-1} PB of pH 7.2 before and after 5 min incubation of the DNA-modified CFE in 0.1 mol L^{-1} PB of pH 7.2 containing various concentrations of ellipticine. For both (**a**) and (**b**), the *error bars* are constructed for the significance level of 0.05 ($n = 3$) [82]

drug molecules to accommodate complex formation. In many cases, changes to the structure of the DNA duplex result in altered thermodynamic stability and are manifested as changes in the functional properties of DNA [99]. Our study can serve as an example [82] where the damaging effect of an anticancer drug ellipticine was studied using CV performed at the DNA biosensor based on the CFE in the role of a transducer. The observed extent of dsDNA damage increased with the time of incubation of the biosensor in the solution containing ellipticine, as well as with the concentration of ellipticine present in the incubation solution (Fig. 4).

On the other hand, the investigated drug itself can successfully serve as a redox indicator in the detection of DNA damage. In our paper [35], simple electrochemical DNA biosensors composed of the SPCE and low molecular weight dsDNA recognition layer were reported and applied to the detection of damage to DNA by UV-C radiation and ROS produced by the Fenton-type reaction in model water samples, as well as in mineral water samples with additives. Complex DNA biosensor response was based on (1) SWV intrinsic signals of the guanine moiety, as well as that of the intercalative indicator thioridazine (an antipsychotic drug belonging to the phenothiazine drug group), (2) CV responses of the $[Fe(CN)_6]^{4-/3-}$ indicator in solution, and (3) EIS responses of the same redox indicator. For the last two types of measurements, the biosensor was also used with an interface between the SPCE and dsDNA formed by a composite of carboxylated SWCNTs and chitosan to enhance the transducer conductivity. Individual electrochemical/electrical signals

depended on the time of the biosensor incubation in a cleavage medium and their profiles characterized process of deep dsDNA degradation.

Also specific fraction of organic dyes (acridine, anthraquinone, etc.) belongs to the group of DNA intercalators (compounds able to interact with DNA through insertion of molecules with planar aromatic ring systems between DNA base pairs). For instance, acridine dyes have demonstrated to present mutagenic, carcinogenic, antibacterial, and antiviral properties [100]. Their similarity to several antibiotics, such as daunomycin or actinomycin, makes them interesting model systems for studying a variety of biophysicochemical problems [101]. Acridine derivatives initially bind (prior their intercalation between base pairs) to the minor groove of dsDNA through counterion displacement [102, 103].

Electrochemical DNA biosensors can be successfully used not only for the investigation of mutual interactions between xenobiotic compounds and DNA but also for the development of highly sensitive analytical methods utilizing a spontaneous accumulation of the analyte into the DNA structure (intercalation between the dsDNA base pairs) to increase the sensitivity of the determination [104]. Such a method was developed also in our laboratory for the DPV determination of genotoxic anthracene [105]: the limit of quantification of anthracene at the bare GCE was 2.2 μmol L^{-1}, while the limit of quantification of 0.15 μmol L^{-1} was reached at the DNA-modified GCE. The applicability of the method was furthermore successfully verified on model samples of gravel and sand, with the added/found recoveries of 98% and 96%, respectively.

3 Conclusions

It has been shown in this chapter that the DNA-modified electrodes (electrochemical DNA biosensors) already represent very effective and, at the same time, simple, fast, inexpensive, miniaturized, and mass-producible analytical devices for evaluation and classification of modes of genotoxic effects of individual xenobiotic compounds (e.g., chemical carcinogens, pesticides, drugs, dyes, or reactive radical species), as well as for prescreening of new drugs and newly synthesized chemicals. Moreover, the evaluation of DNA protection capacity of various natural and synthetic chemical substances (antioxidants) is also possible using the detection of DNA damage caused by prooxidants.

It can be expected that, in a near future, complex biorecognition layers utilizing various supramolecular interactions will be suggested to detect potentially risky compounds and to improve further abilities of biosensors to detect damage to DNA. The advanced level of medical and clinical diagnosis will be largely dependent on the successful development and implementation of new materials and technologies envisaging the fabrication of state-of-the-art biosensors. Attractive properties of electrochemical devices are thus extremely promising for improving the efficiency of environmental screening, diagnostic testing, and therapy monitoring.

For instance, one of the most important directions in the prospective development of DNA biosensors successfully applicable in practice can be seen in the investigation of protective membranes, which prevent the biosensor surface from unwanted fouling and interferences. One such an example is shown in our paper [47] where novel electrochemical DNA-based biosensors with outer-sphere Nafion and chitosan protective membranes were prepared for the evaluation of antioxidant properties of beverages (beer, coffee, and black tea) against prooxidant hydroxyl radicals.

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