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Interaction of tin(II) and arsenic(III) with DNA at the nanostructure film modified electrodes

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Abstract

Biosensors based on DNA and DNA-carbon nanotubes film immobilized at the surface of a screen-printed carbon electrode were used for simple *in vitro* tests of chemical toxicity. The damage to DNA caused by tin(II) and arsenic(III) compounds as components of specific reaction media was evaluated by means of an electrochemical DNA marker, $[Co(phen)_3]^{3+}$, as the portion of original dsDNA which survives an incubation of the biosensor in the cleavage medium. The results were confirmed by the electrically heated electrode and by the measurement of the DNA guanine moiety signal.

Keywords: Tin; Arsenic; DNA damage; DNA biosensor; Voltammetry

1. Introduction

Tin is common metal in the everyday environment. It is widely used in the food industry to conserve soft drinks as well as for metallic packing of food [1,2]. Inorganic tin, mainly tin chloride is also used as a reducing agent to label radiopharmaceuticals with technecium (^{99m}Tc) [3]. Organotin compounds are used in different fields, such as antifouling paints, agricultural chemicals, stabilizers in polymers [4]. Even the toxicity of inorganic tin is reported as low, several studies demonstrated that tin chloride induces DNA lesions and, thus, may be considered as potential genotoxic agent [5,6]. Dantas et al. [7] showed that $SnCl_2$ induced lesions could also be generated by Fenton-like reaction. The DNA lesions can produce injuries in the cell, like mutagenesis, inviabilizating the cell up to death. $SnCl_2$ was shown to: (i) inhibit the immune response in rodents, (ii) alter the gene expression and (iii) induce tumor generation in thyroid gland [8].

There is a large number of literature reports on arsenic, both a carcinogen and a chemotherapeutic agent (the paradox of arsenic). The risk behavior is attributed mainly to the inorganic arsenic(III) which is predominant in drinking water from deep anaerobic wells. As(III) is oxidized to As(V) under aerobic conditions and reduced back to As(III) in cells [9]. Arsenic effects are influenced by glutathione (GSH)—a reduction and complexation agent. Arsenic-induced apoptosis is related to an enhanced production of prooxidants and depletion of glutathion [10]. This is supported by the observation that arsenite is related to proteins which are induced by and protect against oxidative stress.

In recent years the DNA biosensors have become a widely used tool for the study of the DNA interaction with many compounds [11–15]. Double stranded DNA immobilized at the voltammetric electrode surface was shown to be a substrate for oxidative damage by (reactive oxygen species) ROS based on Fenton and Haber–Weiss reactions [16,17]. As_2O_3 association

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Table 1
Effect of $SnCl_2$ on DNA at 10 min incubation of DNA/SPE and MWNT–DNA/SPE sensors in various cleavage mixtures in 5×10^{-3} mol/L phosphate buffer solution pH 7.0 under stirring, aerobic conditions

Cleavage mixture			Relative marker signal (I/I_0)	
No.	Composition	Concentration [mol/L]	DNA/SPE	MWNT–DNA/SPE
I	$SnCl_2$	1×10^{-5}	0.91±0.05	–
II	$SnCl_2$	1×10^{-5}	0.85±0.02	–
	$CuSO_4$	2×10^{-4}		
III	$SnCl_2$	1×10^{-5}	0.45±0.09	0.35±0.08
	$CuSO_4$	2×10^{-4}		
	H_2O_2	5×10^{-2}		
IV	$SnCl_2$	1×10^{-5}		
	$CuSO_4$	2×10^{-4}	0.63±0.12	0.51±0.04
	Ascorbic acid	1×10^{-3}		
V	$SnCl_2$	1×10^{-5}		
	Ascorbic acid	1×10^{-3}	0.83±0.02	0.71±0.05
	H_2O_2	5×10^{-2}		

with DNA modifier was reported [18] together with a degradation of DNA by inorganic and organic arsenic compound (without glutathion) [19]. According to our knowledge DNA biosensor was not yet used for the detection of DNA damage with tin compounds.

In this paper, three types of the DNA biosensors, i.e. simple DNA modified screen-printed electrode, DNA-carbon nanotubes modified screen-printed electrode and electrically heated carbon paste electrode were used to study an effect of tin(II) and arsenic(III) compounds as the components of specific reaction media. The experiments were evaluated by means of $[Co(phen)_3]^{3+}$, which is a well known electrochemical DNA marker [20,21], as the portion of original dsDNA which survives an incubation of the biosensor in the cleavage medium and by the measurement of the DNA guanine moiety signal. Investigation of the reactivity of tin and arsenic in the presence of various co-reactants such as heavy metals, ascorbic acid for Sn and glutathion for As towards the surface attached dsDNA under *in vitro* conditions was the aim of the study.

2. Experimental

2.1. Apparatus and reagents

A computerized voltammetric analyzer ECA pol, model 110 (Istran, Bratislava, Slovakia), computerized voltammetric analyzer Eco-Tribo Polarograph (Polarosensors, Prague, Czech Republic) fitted with a screen-printed three-electrode assembly (FACH, Prešov, Slovakia) including a carbon working electrode (SPE, 25 mm^2 geometric surface area), a silver/silver chloride reference electrode (Ag/AgCl-SPE with the potential of 0.284 V vs. conventional Ag/AgCl/sat. KCl electrode) and a carbon counter electrode were used for voltammetric measurements. The working electrode without any electrochemical preconditioning was chemically modified *ex situ* by covering with 5 μl of the DNA stock solution (DNA/SPE) or with the mixture (1:1 volume ratio) of the carbon nanotube suspension in dimethylformamide (2 mg/mL) and DNA stock solution (0.1 mg/mL) [22] (DNA–MWNT/SPE) and leaving the electrode to dry overnight. For the measurements with heated carbon paste electrodes, Autolab PGSTAT10 electrochemical analyzer (Eco Chemie) controlled by GPES software was used. Heated carbon paste electrodes were modified with DNA by adsorption from the DNA solution at 0.5 V for 120 s as described elsewhere [23]. The measurements were carried out in a 10 mL glass one-compartment voltammetric cell at room temperature (22 °C).

Calf thymus dsDNA was obtained from Merck (1.24013.0100) and used as received. Its stock solution (0.1 mg/mL) was prepared in 1×10^{-2} mol/L Tris–HCl and 1×10^{-3} mol/L EDTA solution of pH 8.0 and stored at −4 °C. The absorbance values ratio for 260 and 280 nm equal to 1.82 confirmed the absence of proteins [24]. The complex compound $[Co(phen)_3](ClO_4)_3$ was synthesized in our laboratory according to [25] and checked by chemical analysis. Tin(II) chloride was obtained from Lachema, Czech Republic. Its solution was prepared in deionized water in concentration 1 mg/mL directly before each experiment. The standard solutions of the inorganic arsenic (0.05 mol/L) was from Merck. Multi-wall carbon nanotubes (MWNT) were from Aldrich, Germany. All other chemicals were from Lachema, Czech Republic. Deionized and double distilled water were used throughout.

2.2. Procedure

The modified procedure reported previously [26] was used for the measurement with the DNA redox marker. Briefly, the DNA (DNA–MWNT) sensor was pre-treated by immersing to 5×10^{-3} mol/L phosphate buffer pH 7.0 under stirring for 5 min, and then rinsed with water. The $[Co(phen)_3]^{3+}$ marker was accumulated from 5 mL of its 5×10^{-7} mol/L solution in 5×10^{-3} mol/L phosphate buffer pH 7.0 under stirring for 120 s at an open circuit. The differential pulse voltammogram (DPV) was recorded immediately from +300 to −500 mV at the pulse amplitude of 100 mV, 2 mV scan step and the scan rate of 10 mV/s. The marker peak current (I_0) was obtained using the evaluation against a base-line by standard software and the

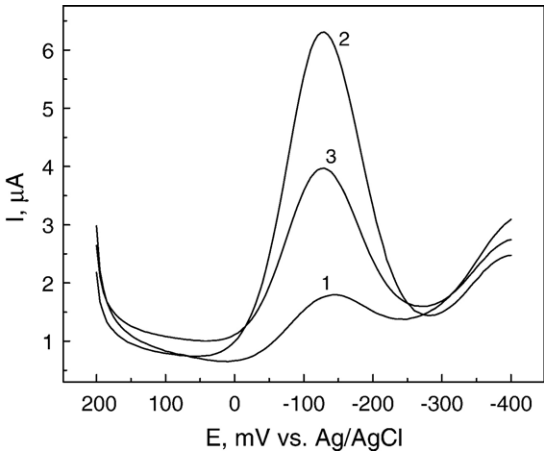

Fig. 1. DP voltammograms of $[Co(phen)_3]^{3+}$ obtained at unmodified SPE (1), DNA/SPE before (2) and after (3) 10 min incubation in the cleavage mixture contained 1×10^{-5} mol/L $SnCl_2$, 2×10^{-4} mol/L $CuSO_4$ and 5×10^{-2} mol/L H_2O_2 in 5×10^{-3} mol/L phosphate buffer pH 7.0.

Table 2
Effect of the concentration of $SnCl_2$ in cleavage mixture (and incubation time of DNA/SPE in the cleavage mixture (III)

Conditions	Relative marker signal (I/I_0)		
Concentration of $SnCl_2$ [mol/L]	1×10^{-6}	5×10^{-6}	1×10^{-5}
(10 min incubation time)	0.74±0.05	0.53±0.02	0.45±0.09
Incubation time [min]	2.5	5	10
(1×10^{-5} mol/L $SnCl_2$)	0.84±0.10	0.52±0.08	0.45±0.09

correction subtracting the mean marker peak current measured at the unmodified SPE ($n=10$) under the same conditions. Then, the DNA/SPE (DNA–MWNT/SPE) sensor was regenerated by a removal of the electrostatically accumulated $[Co(phen)_3]^{3+}$ ions from the DNA layer at treating in the buffer medium of higher ionic strength (1×10^{-1} mol/L phosphate buffer pH 7.0) under stirring during 60 s. A negligible marker signal was checked by the DPV record in blank. The peak current I_0 was obtained in triplicate.

To detect the damage in DNA, the same DNA (DNA–MWNT) sensor was incubated in a separate cell with the cleavage mixture in 1×10^{-2} mol/L phosphate buffer solution pH 7.0 for 10 min under stirring, and then rinsed with water. The marker peak current (I) was obtained again in duplicate using the DPV measurement/biosensor regeneration scheme and the normalized (relative) signal I/I_0 was calculated. The stability of the DNA/SPE and MWNT–DNA/SPE was checked by the same process in 5×10^{-3} mol/L phosphate buffer pH 7.0 under stirring and the decreasing of the marker signal was found as non-significant.

3. Results and discussion

3.1. Effect of tin

The interaction of inorganic tin(II) with the DNA was studied using the tin chloride as the component of DNA cleavage mixture. Table 1 summarizes the data of a relative DPV signal of the electrochemical DNA marker $[Co(phen)_3]^{3+}$ obtained at the DNA/SPE sensor after its 10 min incubation in the cleavage mixtures of different content.

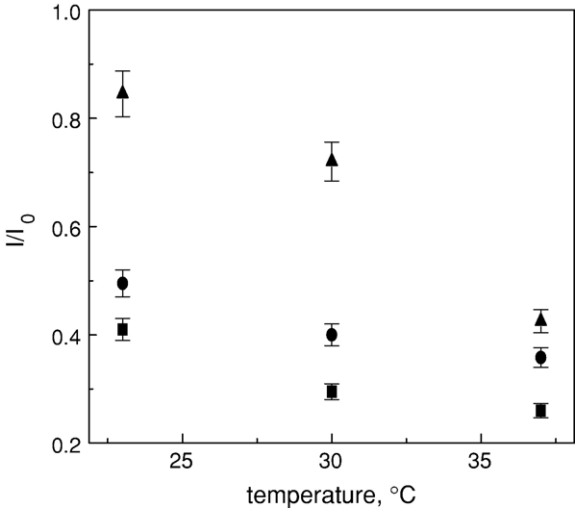

Fig. 2. Dependence of the relative marker signal (I/I_0) on temperature of the heated DNA/CPE at its incubation in cleavage mixture contained 1×10^{-5} mol/L $SnCl_2$, 2×10^{-4} mol/L $CuSO_4$ and 5×10^{-2} mol/L H_2O_2 in 5×10^{-3} mol/L phosphate buffer pH 7.0. Incubation time: ▲ 2.5 min, ● 5 min, ■10 min.

Table 3
Effect of arsenic(III) on DNA at 10 min incubation of DNA/SPE and MWNT–DNA/SPE sensors in various cleavage mixtures in 5×10^{-3} mol/L phosphate buffer solution pH 7.0 under stirring, aerobic conditions

Cleavage mixture		Relative marker signal (I/I_0)			
Composition	Concentration mol/L	Without As(III)		With As(III)	
		DNA/SPE	MWNT–DNA/SPE	DNA/SPE	MWNT–DNA/SPE
As(III)	1×10^{-3}				
Fe(II)	2×10^{-4}	0.88±0.05	–	0.92±0.03	–
H_2O_2	1.2×10^{-4}				
As(III)	1×10^{-3}				
Fe(II)	2×10^{-4}	0.83±0.12	0.78±0.11	0.63±0.06	0.38±0.10
H_2O_2	1.2×10^{-4}				
GSH	2×10^{-3}				

It can be seen that no significant DNA damage was found by applying cleavage mixtures (I), (II) and (IV). We can say that Sn (II) does not cause significant damage to DNA in aerobic conditions neither alone (I) nor together with copper ions as metal redox catalyst (II) and another reducing agent such as ascorbic acid (V). In case of the cleavage mixture (III), Sn was present in the medium of H_2O_2. Here we observed a degradation of DNA to about 60%. The effect of $CuSO_4$ and H_2O_2 only on DNA under the conditions used was negligible. This result is in agreement with the results obtained by Dantas et al. [7], who considered $SnCl_2$ in Fenton-like reaction. In the cleavage mixture (IV) Sn is present in a well known medium able to produce oxygen free radicals [16]. However, rather limited damage to DNA was observed. Sn(II) and ascorbic acid are probably mutualy inhibited [27]. The cleavage effect of the mixture (III) can be also seen in Fig. 1 which shows the typical DPV signals of DNA marker obtained at the DNA/SPE before and after its incubation in the cleavage mixture (III).

Both, DNA/SPE and DNA–MWNT/SPE possess similar results. The last biosensor shows higher sensitivity then simple DNA/SPE and, therefore, can be efficiently used for the detection of the damage to DNA. Since the cleavage mixture (III) caused the deepest DNA damage, we used it in further

Table 4
Effect of arsenic(III) on DNA guanine peak current measured after 10 min incubation of DNA/SPE ($E=+460$ mV) and MWNT–DNA/SPE ($E=+690$ mV) sensors in the cleavage mixtures in 5×10^{-3} mol/L phosphate buffer solution pH 7.0 under stirring

Cleavage mixture		Guanine signal, I [μA]			
Composition	Concentration mol/L	Without As		With As(III)	
		DNA/SPE	MWNT–DNA/SPE	DNA/SPE	MWNT–DNA/SPE
As	1×10^{-3}				
Fe(II)	2×10^{-4}	0.83±0.02	1.40±0.27	0.61±0.02	1.07±0.08
H_2O_2	1.2×10^{-4}				
GSH	2×10^{-3}				

studies. Various concentrations of $SnCl_2$ in cleavage mixture as well as various incubation time were investigated (Table 2). A dependence of the DNA damage on the concentration of tin was found. The highest concentration of $SnCl_2$ in the cleavage mixture was 1×10^{-5} mol/L with respect to a low solubility of $SnCl_2$ in aqueous solution. It is also possible to see a strong effect of incubation time of the DNA sensor.

Very similar results were also obtained using another sensor, i.e. an electrically heated carbon paste electrode (CPE) surface modified with DNA. Fig. 2 shows the dependence of the relative signal I/I_0 on the temperature of the biosensor surface during its incubation in cleavage mixture (III). It indicates a kinetics of the cleavage mixture behavior [23], particularly at a short incubation time.

3.2. Effect of inorganic arsenic

After the previous study of the arsenic effect in the absence of GSH [19], inorganic arsenic(III) was investigated as a component of the DNA cleavage mixture with glutathion. Values of the relative DPV signal of the DNA marker are summarized in Table 3. These data demonstrate no significant or very low DNA degradation by arsenic in the presence of Fe^{2+} ions as the redox catalysts. However, an addition of glutathion (GSH), which is known as a co-reactant of As under biological conditions [9,28], caused rather deep damage to DNA by As(III) indicated especially at DNA–MWNT/SPE. The DNA degradation was tested also by the measurement of a voltammetric signal of the DNA guanine moiety. Its anodic peak appeared at +460 mV and 690 mV vs. Ag/AgCl-SPE used [22]. The results presented in Table 4 show an evident decrease in the guanine at the electrode surface after its incubation and confirm again the cleavage effect of the As(III)–GSH couple. No other significant changes in the DNA anodic voltammogram were observed.

4. Conclusion

Using simple, however efficient, DNA biosensors, the damage to surface attached dsDNA was found by *in vitro* tests in the presence of inorganic tin(II) and arsenic(III) ions as the components of specific clevage reaction media. Tin(II) is effective particularly as a coreactant of copper (II) ions and hydrogen peroxide. Arsenic(III) is active in the presence of glutathion which is also known to reduce As (V) and to complex As under biological conditions.

The DNA modified voltammetric electrodes can serve as chemical toxicity sensors. The electrodes with nanostructured films formed by carbon nanotubes exhibited higher analytical sensitivity.

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References

[1] J.R.N. McLean, D.H. Blakey, G.R. Douglas, J.R. Kaplan, The effect of stannous and stannic (tin) chloride on DNA in Chinese hamster ovary cells, Mutat. Res. 119 (1983) 195–201.
[2] S. Blunden, T. Wallace, Tin in canned food: a review and understanding of occurrence and effect, Food Chem. Toxicol. 41 (2003) 1651–1662.
[3] M.V. Vidal, B. Gutfilen, L.M.B. Fonseca, M. Bernardo-Filho, Influence of tobacco on the labeling of red blood cells and plasma proteins with techcetium-99 m, J. Exp. Clin. Cancer Res. 17 (1998) 41–46.
[4] M. Hoch, Organotin compounds in the environment—an overview, Appl. Geochem. 16 (2001) 719–743.
[5] F.J.S. Dantas, M.O. Moraes, J.C.P. De Mattos, R.J.A.C. Bezerra, E.F. Carvalho, M. Bernardo-Filho, A. Caldeira-de-Araújo, Stannous chloride mediates single strand breaks in plasmid DNA through reactive oxygen species formation, Toxicol. Lett. 110 (1999) 129–136.
[6] J.C.P. De Mattos, F.J.S. Dantas, R.J.A.C. Bezerra, M. Bernardo-Filho, J.B. Cabral-Neto, C. Lage, A.C. Leitao, A. Caldeira-de-Araújo, Damage induced by stannous chloride in plasmid DNA, Toxicol. Lett. 116 (2000) 159–163.
[7] F.J.S. Dantas, M.O. Moraes, E.F. Carvalho, J.O. Valsa, M. Bernardo-Filho, A. Caldeira-de-Araújo, Lethality induced by stannous chloride on *Escherichia coli* AB 1157: Participation of reactive oxygen species, Food Chem. Toxicol. 34 (1996) 959–962.
[8] C.R. Silva, M.B.N. Oliveira, S.F. Melo, F.J.S. Dantas, J.C.P. De Mattos, R.J.A.C. Bezerra, A. Caldeira-de-Araujo, A. Duatti, M. Bernardo-Filho, Biological effects of stannous chloride, a substance that can produce stimulation or depression of the central nervous system, Brain Res. Bull. 59 (2002) 213–316.
[9] T.G. Rossman, Mechanism of arsenic carcinogenesis: an integrated approach, Mutat. Res. 533 (2003) 37–65.
[10] M.F. Hughes, Arsenic toxicity and potential mechanisms of action, Toxicol. Lett. 133 (2002) 1–16.
[11] A.M. Oliveira-Brett, V.C. Diculescu, Electrochemical study of quercetin–DNA interactions: Part I. Analysis in incubated solutions, Bioelectrochemistry 64 (2004) 133–141.
[12] A.M. Oliveira-Brett, V.C. Diculescu, Electrochemical study of quercetin–DNA interactions: Part II. In situ sensing with DNA biosensors, Bioelectrochemistry 64 (2004) 143–150.
[13] S. Hahn, S. Mergenthaler, B. Zimmermann, W. Holzgreve, Nucleic acid based biosensors: the desires of the user, Bioelectrochemistry 67 (2005) 151–154.
[14] M. Vaníčková, J. Lehotay, J. Čižmárik, J. Labuda, Kinetic study of the degradation of a potential local anesthetic drug in serum using the DNA-based electrochemical biosensor, Bioelectrochemistry 66 (2005) 125–127.
[15] A. Ferancová, M. Bucková, E. Korgová, O. Korbut, P. Gründler, I. Wärnmark, R. Štepán, J. Barek, J. Zima, J. Labuda, Association interaction and voltammetric determination of 1-aminopyrene and 1-hydroxypyrene at cyclodextrin and DNA based electrochemical sensors, Bioelectrochemistry 67 (2005) 191–197.
[16] J. Labuda, M. Bučková, M. Vaníčková, J. Mattusch, R. Wennrich, Voltammetric detection of the DNA interaction with copper complex compounds and damage to DNA, Electroanalysis 11 (1999) 101–107.
[17] M. Fojta, V. Staňková, E. Paleček, P. Koscielniak, J. Mitáš, A supercoiled DNA-modified mercury electrode-based biosensor for the detection of DNA strand cleaving agents, Talanta 46 (1998) 155–161.
[18] M. Ozsoz, A. Erdem, P. Kara, K. Kerman, D. Ozkan, Electrochemical biosensor for the detection of interaction between arsenic trioxide and DNA based on guanine signal, Electroanalysis 15 (2003) 613–619.
[19] J. Labuda, K. Bubničová, L. Koval'ová, M. Vaníčková, J. Mattusch, R. Wennrich, Voltammetric detection of damage to DNA by arsenic compounds at a DNA biosensor, Sensors 5 (2005) 411–423.

[20] M.T. Carter, A.J. Bard, Voltammetric studies of the interaction of tris(1,10-phenanthroline) cobalt(III) with DNA, J. Am. Chem. Soc. 109 (1987) 7528–7530.
[21] M.T. Carter, M. Rodriguez, A.J. Bard, Voltammetric studies of the interaction of metal chelates with DNA: 2. Tris-chelated complexes of cobalt(III) and Iron(II) with 1,10-phenanthroline and 2,2′-bipyridine, J. Am. Chem. Soc. 111 (1989) 8901–8911.
[22] A. Ferancová, R. Ovádeková, M. Vaníčková, A. Šatka, R. Viglaský, J. Zima, J. Barek, J. Labuda, DNA modified screen-printed electrodes with nanostructured films of multi-wall carbon nanotubes, hydroxyapatite and montmorillonite, Electroanalysis 18 (2006) 163–168.
[23] O. Korbut, M. Bučková, P. Tarapčík, J. Labuda, P. Gründler, Damage to DNA indicated by an electrically heated DNA modified carbon paste electrode, J. Electroanal. Chem. 506 (2001) 143–148.
[24] D.W. Pang, H.D. Abruña, Micromethod for the Investigation of the Interactions between DNA and redox-active molecules, Anal. Chem. 70 (1998) 3162–3169.
[25] L.S. Dollimore, R.D. Gillard, Optically active co-ordination compounds. Part XXXII. Potassium (+)tris-[L-cysteinesulphinato(2-)-*SN*]cobaltate(III): a versatile agent for resolution of 3+ species, J. Chem. Soc., Dalton Trans. 78 (1973) 933–940.
[26] J. Labuda, M. Bučková, L'. Heilerová, A. Čaniová-Žiaková, E. Brandšteterová, J. Mattusch, R. Wennrich, Electrochemical detection of an antioxidative activity of plant extracts at the DNA-modified screen-printed electrode, Sensors 2 (2002) 1–10.
[27] E.M. El-Demerdash, M.I. Yousef, M.A. Zoheir, Stannous chloride induces alterations in emzyme activities, lipid peroxidation and histopathology in male rabbit: Antioxidant role of vitamin C, Food Chem. Toxicol. 43 (2005) 1743–1752.
[28] M. Vahter, Mechanism of arsenic biotransformation, Toxicology 181–182 (2002) 211–217.