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Voltammetric and electrochemical gravimetric selective detection of interactions between Tl(I) and guanine and the influence on activity of DNA drug-intercalators

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ABSTRACT

The interactions of Tl(I), a well known toxic species, with selected oligonucleotides were examined. The oligonucleotide sequences selected for the investigation were taken from gene *hOGG1* responsible for repairing of DNA damage. Cyclic voltammetry was particularly useful, since nitrogen N-7 in guanine can be electrooxidized while its binding with Tl(I) leads to the loss of electroactivity. So, this selected interaction could be quantitatively used in drawing Scatchard's plot and calculating the binding constants and the number of active sites in guanine molecules occupied by one metal ion. Further, we have shown that the presence of Tl(I) leads to a decrease in activity of doxorubicin (DOX), a popular anticancer drug, vs. DNA. The obtained circular dichroism (CD) spectra and the measurements with an electrochemical quartz crystal microbalance (EQCM) led to a conclusion that in the presence of monovalent thallium cations the DNA double helix was neither damaged/oxidized nor its conformation changed substantially.

1. Introduction

The literature data allow scientists to suppose that polymorphism in some genes might cause an increased risk of getting cancer; it is about the genes important for fundamental activities of the cells, such as proliferation, apotheosis and growth and repairing genes [1]. It is known that heavy metals generate polymorphism in genes [2]. An example of such metal is thallium. Thallium has been known as a toxic element for many years. Monovalent thallium has received much more attention than Tl(III), but Tl(III) is much more toxic. The monovalent thallium ion toxicity is related to the interference with the vital potassium-dependent processes. When Tl is present in soils it may be easily taken up by plants, primarily as a result of its tendency to substitute K in biogeochemical reactions/cycling [3]. In the vicinity of rivers, not only the dissolved fraction can be distributed, but also the mobile fraction of Tl can be moved downstream carried by the sediment particles [4].

The crystal structure of the Tl(I) complexes with nucleic bases was already reported in the literature. It appeared that the metal ion is bound mainly at N-3 and N-1 sites to pyrimidine bases, at N-1, N-7, and N-9 positions to purine bases [2,5–7]. It was also shown that thallium(I) binds to the phosphate groups [8], however, in high ionic-strength solutions this interaction should be negligible. The highest number of bonds between DNA and Tl(I) appears within the major groove; then circa 20–35% of the places are occupied. In the minor groove Tl(I) occupies only 10% of the active places [9].

Monovalent thallium is more toxic than Cu, Cd, Ni, Mn, Sn, As and Pb and is of similar toxicity to Hg(II) [10,11]. Since Tl(I) may cause polymorphism in some genes, in this paper we present a study of interactions between thallium(I) and DNA components. In our research we have employed a fragment of *hOGG1* gene sequence containing the cysteine codon. This gene is responsible for repairing the damaged/oxidized guanine in DNA [12]. The aim of our investigation was to check whether the presence of thallium(I) leads to conformational changes, electrochemical properties and interference in the binding of drugs with selected DNA sequences. Doxorubicin (DOX) was selected as example drug. Additionally, one of the sequences was modified by the human-telomere fragment to examine whether its presence can minimize the negative influence of the thallium ions. The electrochemical and spectroscopic methods have been used to determine the corresponding equilibrium constants and the percentage of guanine molecules engaged in the complex with Tl(I).

2. Experimental

2.1. Chemicals

All chemicals were of the highest quality available. NaOH (p.a., POCh, Poland), KH_2PO_4 (p.a., POCh, Poland), K_2HPO_4

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(p.a., POCh, Poland), NaCl (p.a., POCh, Poland), KCl (p.a., POCh, Poland) were used as provided by manufacturer. All working solutions of Tl(I) were prepared by a proper dilution of a stock 1 mg L^{-1} $TlNO_3$ solution (d=1.02 g mL^{-1}) (Merck, Suprapur). Doxorubicin was purchased from Sigma. The oligonucleotides were purchased from MWG-Biotech, Germany. The following sequences (parts of *hOGG1* gene) of the bases were used:

- telomere fragment: 5′-TTAGGG-3′
- probe-1 DNA: 5′-GCA ATG GGT TAC TGA TGG GTT ACG-3′
- probe-2 DNA: 5′-GCA ATG GGT TAC TGA TGG GTT ACG TTAGGG-3′
- complementary target DNA: 5′-CGT AAC CCA TCA GTA ACC CAT TGC-3′

hOGG1 protein recognizes and removes 8-oxoguanine (8-oxoG) from damaged DNA. 8-oxoG is the most common, highly mutagenic DNA damage that is caused by the action of a strong oxidant like the reactive oxygen species or the cations of toxic metals [13–15]. Telomere fragment is not a part of the gene; it was selected because it contains three guanines in row. We just wanted to check whether thallium will prefer to bind the guanines in one of the strands of dsDNA or just a telomere fragment attached to one of the strands in the helix. The chosen number of guanines in the row is not random; a group of three guanines is often the target for the intercalating drugs, e.g. for doxorubicin [16,17].

Before hybridization process, to make sure that the solutions contain only single-stranded DNA, the vials with the probe and complementary target DNA were heated for 15 min at the melting temperature determined by the producer. After this time the solutions were rapidly cooled in ice, diluted with water and mixed in equimolar ratio. Such prepared mixtures were left for 24 h at room temperature to reach the complete hybridization. In the electrochemical and spectroscopic measurements the concentration of dsDNA solutions was 2 μM.

2.2. Apparatus

2.2.1. Voltammetric measurements

Cyclic voltammetry (CV) was performed using an Autolab, EcoChemie potentiostat, controlled via producer's software, and a special cell with thermal circulation. The three-electrode system, consisting of a glassy carbon electrode, GCE, (ϕ=3 mm, BAS Instruments) used as the working electrode, an Ag/AgCl electrode (reference electrode) and a platinum wire used as the auxiliary electrode were employed. Each time before the use the working disk electrode was briefly polished with 0.05 and 0.3 μm Al_2O_3 powders on a wet pad. After polishing, to remove alumina oxide completely from the surface, the electrode was rinsed with a direct stream of ultrapure water. The electrode surface was inspected optically with an Olympus, model PME 3, inverted metallurgical microscope. The solutions before measurements were degassed with pure argon. Tl(I) solutions were prepared by diluting a 1.2×10^{-6} M $TlNO_3$ solution with water.

2.2.2. Electrochemical quartz crystal microbalance (EQCM) measurements

A microbalance, model Autolab–EQCM (Autolab) with 6 MHz Au/TiO_2 quartz crystal resonators, was used. The resonant frequency was measured as a function of the mass of the substance attached to the crystal interface. For a thin rigid film, the interfacial mass change, Δm, is related to the shift in resonance oscillation frequency Δf of the EQCM through the Sauerbrey equation [18–20]:

$$\Delta f = \frac{-2\Delta m n f_0^2}{A\sqrt{\mu_q \rho_q}} \quad (1)$$

where f_0 is the oscillation frequency in the fundamental mode, n is the overtone number, A is the piezoelectically active surface area, ρ_q is the density of quartz (ρ_q=2.648 g cm^{-3}), and μ_q is the shear modulus of quartz ($\mu_q=2.947\times10^{11}$ g cm^{-1} s^{-2}). The oscillator was tuned to the series resonance frequency of the working piezoelectrodes to minimize the effects due to energy dissipation in the protein films. All experimental variables influencing the resonant frequency [18] of the EQCM electrodes, such as temperature, pressure, viscosity and density of the solution, were kept constant during the measurements. The piezoelectrically active (geometrical) surface area of the working Au electrode was 0.3523 cm^2 and the real surface area A equaled 0.4286 cm^2 (roughness factor R=1.2). The real surface area of the Au–EQCM electrode was determined from the charge under the Pb underpotential-deposition voltammetric peak (0.01 M lead(II) perchlorate in 0.1 M perchloric acid). A generally accepted value of charge for the Pb monolayer on Au is Q_{Pb}=302 μC/cm^2 [21]. The Au–EQCM electrode was electrochemically pretreated by cycling: first, between 0 V to 1.8 V (with a 10-s scan stop at 1.8 V) in 0.5 M NaOH with scan rate 50 mV/s, and then between −0.3 and 1.5 V (vs. Ag/AgCl) in 0.1 M H_2SO_4 solution until a stable voltammogram, typical for a clean gold electrode, was observed [22]. Finally, before the deposition of DNA film, the gold-coated QCM crystals underwent a pre-treatment consisting of several potential scans between −0.65 V and 0.95 V at a high scan rate (>1 V/s) in 0.1 M perchloric acid solution. This cycling was continued until a stable cyclic voltammogram consistent with that of a polycrystalline gold electrode was obtained.

2.2.3. Circular dichroism (CD)

A J-815 CD spectrometer (Jasco), controlled by producer's software, was used. A quartz cuvette (1 cm length) was used as the optical window. CD parameters: scanning speed 100 nm/min, data pitch 0.5 nm, bandwidth 2 nm, accumulation (number of scans) 10. CD spectra of oligonucleotide solutions were obtained before and after (1 h) addition of Tl(I).

2.3. UV–Vis detection of DOX–DNA interactions

UV–Vis spectra of solutions containing alone DOX (50, 100, 200 and 1000 nM) and mixtures of DOX and DNA (4 μM) in the absence and the presence of 100 nM Tl(I) were obtained in the range 200–900 nm. The spectra were obtained 1 h after the preparation of the solutions. A Perkin-Elmer, type Lambda-25, spectrometer was used. The measurements were done three times for each of three independently prepared solutions of the same composition.

2.4. Immobilization of DNA strands

The accumulation of non-modified DNA on the electrode surface can be done in two ways: (1) by physical deposition and (2) by adsorption at a constant potential. For voltammetric experiments a droplet (7 μl) of the DNA solution (2 μM in 0.02 M PBS) was placed on the electrode surface and left to dry at room temperature. The droplet volume was sufficient to cover the electrode surface completely. For the EQCM measurements the adsorption of the appropriate DNA sequence at the Au–EQCM electrode surface was done at a constant potential in DNA-containing chloride-free phosphate buffer (0.02 M). The freshly cleaned Au–EQCM electrode was immersed in an appropriate

DNA solution (4 μM) for 15 min. Since the DNA molecules are negatively charged in 0.02 M phosphate buffer (pH 7.4), the immobilization of DNA on Au–EQCM surface was performed by holding the potential of the electrode at a constant positive value of +0.15 V for 15 min. After that time the frequency change stabilized.

To make sure that both applied ways of DNA immobilization led to the same amount of adsorbed molecules/cm^2, we calculated the total surface concentration of DNA according to the procedure described in the literature [23]. The total surface concentration of DNA adsorbed on either GC or Au–EQCM electrode surface was calculated to be 6.48×10^{-11} and 5.93×10^{-11} mol/cm^2 for physical and electrochemical accumulation, respectively. Additionally the obtained DNA layers were tightly covering the surface.

3. Results and discussions

Voltammetric experiments should be especially useful in examination of interactions between thallium(I) and guanine. For the detection of the interactions, solutions containing Tl(I) at different concentrations (1–100 nM) and various DNA sequences at constant concentration (2 μM) were examined. After 1 h of reacting, the voltammetric signals were obtained in the mixtures and compared with the signals for alone dsDNA in the solution. A waiting time of 1 h was sufficient to get the reaction completed. This time was selected basing on the data shown in Fig. 1A, where the oxidation peak current height of guanine vs. incubation time in the Tl(I) solution is plotted. Several voltammograms used to the construction of Fig. 1A are presented in Fig. 1B. The time of reaching the current plateau was taken at the reaction time, so Tl(I) required circa 1 h for the full binding. We have also made some kinetic measurements. To determine the reaction rate constant, k, we have plotted the ratio of the free and total (initial) mass of Tl(I) vs. time and obtained linear relationships (each point taken for the regression was the mean of five independent EQCM measurements). The linear equation for this pseudo first order reaction has the form:

$$\ln\frac{m_{\mathrm{Tl}(I),\ \mathrm{free}}(t)}{m_{\mathrm{Tl}(I),\ \mathrm{total}}} = -kt \qquad (2)$$

After fitting the experimental data obtained for the telomere fragment, dsDNA and dsDNA containing one strand modified with the telomere fragment, we have arrived at the following k values: $5.4\times10^{-4}\ \mathrm{s}^{-1}$, $2.8\times10^{-4}\ \mathrm{s}^{-1}$ and $4.9\times10^{-4}\ \mathrm{s}^{-1}$ for the telomere fragment, dsDNA and dsDNA containing one strand modified with the telomere fragment, respectively. The correlation coefficient for the linear dependences was higher than 0.995. These dependences were determined for the total mass of Tl(I) equal to 61 ng. Since the rate constant of the reaction of doxorubicin with dsDNA is of similar value (circa $7\times10^{-4}\ \mathrm{s}^{-1}$) the reaction of Tl(I) precedes in the same time regime and therefore the presence of Tl(I) must have an influence on the efficiency of doxorubicin. The rate constant for the reaction of doxorubicin with dsDNA was calculated identically; the spectroscopic and QCM data were used for this purpose. The value of $7\times10^{-4}\ \mathrm{s}^{-1}$ is the mean value for two numbers corresponding to the spectrophotometric and QCM data. The experiments reported below helped to estimate the inhibiting action of thallium(I) on the ability of dsDNA to react with doxorubicin.

3.1. Examination of interactions of Tl(I) with DNA

To make sure the interactions of DNA sequences with Tl(I) are characterized correctly we have used a variety of methods. The corresponding measurements are described below. All electrochemical experiments were carried out with DNA preconcentrated on

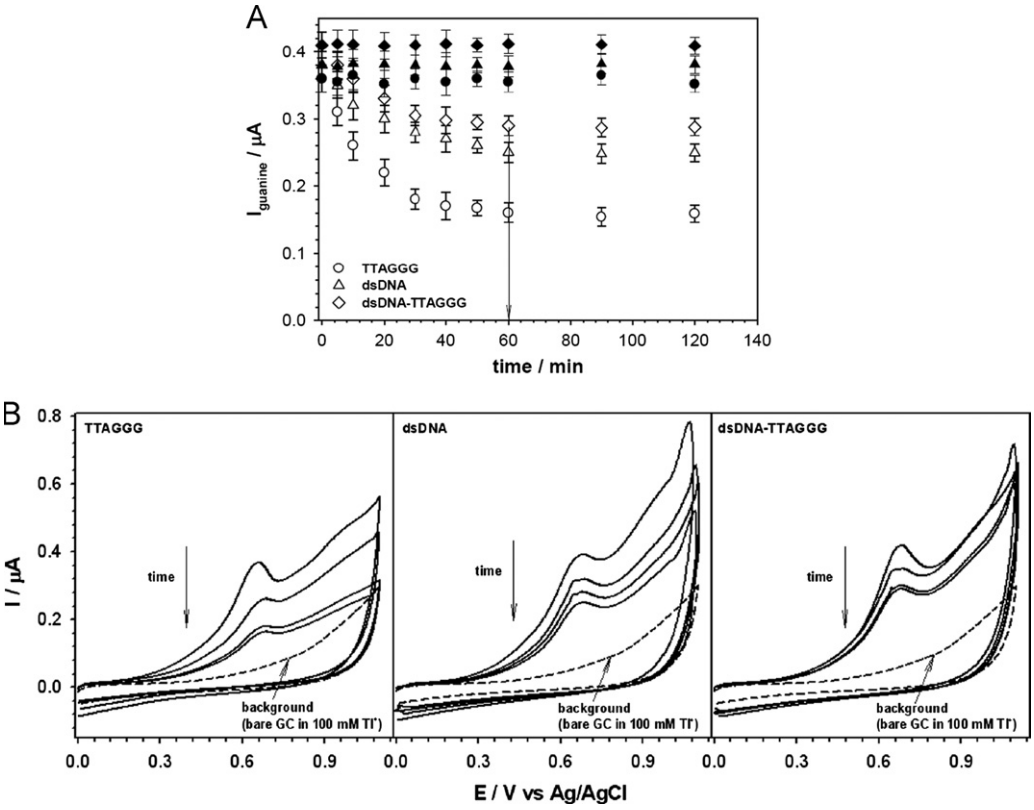

Fig. 1. (A) Voltammetric peak height of guanine plotted vs. incubation time in 100 nM Tl(I) solution (empty symbols). Filled symbols correspond to absence of thallium(I) in solution. (B) Example cyclic voltamograms obtained in 0.02 M PBS buffer (pH 7.4) at scan rate 20 mV/s for different time of incubation (0, 10, 30 and 60 min) of GC/DNA electrode in 100 nM Tl(I) solution. Diameter of disc GC electrode: 3 mm, T=22 °C.

the electrode surface. All measurements were repeated at least seven times. The highest relative standard deviation was 13%.

3.1.1. CD spectroscopy

The measurement of ellipticity (θ) is widely used to monitor the conformational changes of dsDNA caused by various factors [24–26]. It is used in this paper to detect possible changes in the conformations of the dsDNA molecules after the interactions with Tl^+. B-DNA is a typical conformation of a double-stranded helix in the cells. It is the most stable form, characterized by the central position of the hydrogen bonds along the helix axis and a small difference between the minor and major grooves sizes. Typical CD spectra obtained for each studied oligonucleotide sequences are presented in Fig. 2A and B. The shape of the CD spectra of DNA is determined by the interplay of three types of the interactions: the hydrogen bonding between the complementary bases (i), the vertical (stacking interactions) (ii) and the electrostatic repulsion of the negatively charged phosphate groups (iii). At all CD spectra the negative and positive bands can be seen. The negative band at circa 240 nm corresponds to the hydrogen bonding between the nucleic bases, while the positive band at 270 nm describes the base stacking interactions [27]. Since the negative and positive bands are located at the appropriate wavelength and have similar heights (Fig. 2A and B) it means that our DNA samples are of B conformation [28]. A small increase of θ in its positive band (only circa 11%) appeared as a result of the interaction with Tl^+, while the value of θ in the negative band did not change. It means that the binding of Tl(I) to DNA components neither damaged nor changed significantly the double-stranded helix. In fact, the B conformation was not affected.

The valence state of the thallium determines its toxicity, distribution and mobility. Tl(I) can be oxidized to Tl(III) by a strong oxidant, such as reactive oxygen species, bromic water and MnO_2, but also under conditions of photoirradiation and solar light [29]. It is known that Tl(I) in the human body can be easily oxidized by free radicals. Free radicals are formed in living cells during the metabolic processes and by exogenous sources, including carcinogenic compounds and ionizing radiation. It is estimated that 1–5% of oxygen metabolized in living organisms are univalently reduced to yield free oxygen radicals. A human, while resting, consumes circa 500 L of oxygen per twenty four hours and generates approximately 1 mol of free radicals, mainly $OH^{\bullet}$. Free radicals can oxidize all biological macromolecules including proteins, lipids and DNA. Also the UV radiation, with which the humans have contact in sunny days, is capable of generating ROS [30]. To check a possibility of oxidizing Tl(I) to a much more toxic form, Tl(III), a UV lamp of 8 W was employed in the experiments. The solutions containing 100 nM Tl(I) were exposed to the UV lamp for 2 h. Then the dsDNA was added and after 1 h the CD spectra were recorded. They are presented in Fig. 2A and B as green dashed lines. In such case significant changes in θ were observed for both bands compared to the pure DNA solution untreated and treated with UV radiation. It means that generated Tl(III) caused local lesions in the double helix. Additionally, the position of positive bands shifts towards more positive wavelength. This shift can be interpreted as a consequence of the presence of 8-oxoguanine (product of the reaction between Tl(III) and DNA). 8-oxoguanine is a ubiquitous oxidative base lesion. The presence of 8-oxoguanine is well documented in the voltammogram presented in Fig. 3; the peak seen at +0.31 V corresponds to the oxidation of 8-oxoguanine. This peaks appears only after the UV irradiation of the solution with Tl(I). Their presence in double-stranded helix caused the destabilization of the structure because it influences the thermodynamics of the molecule.

It is known that UV irradiation leads to a deformation of the sugar-phosphate backbone in DNA, which is a result of the formation of pyrimidine dimers (C–C, T–T, C–T) [31]. The T–T

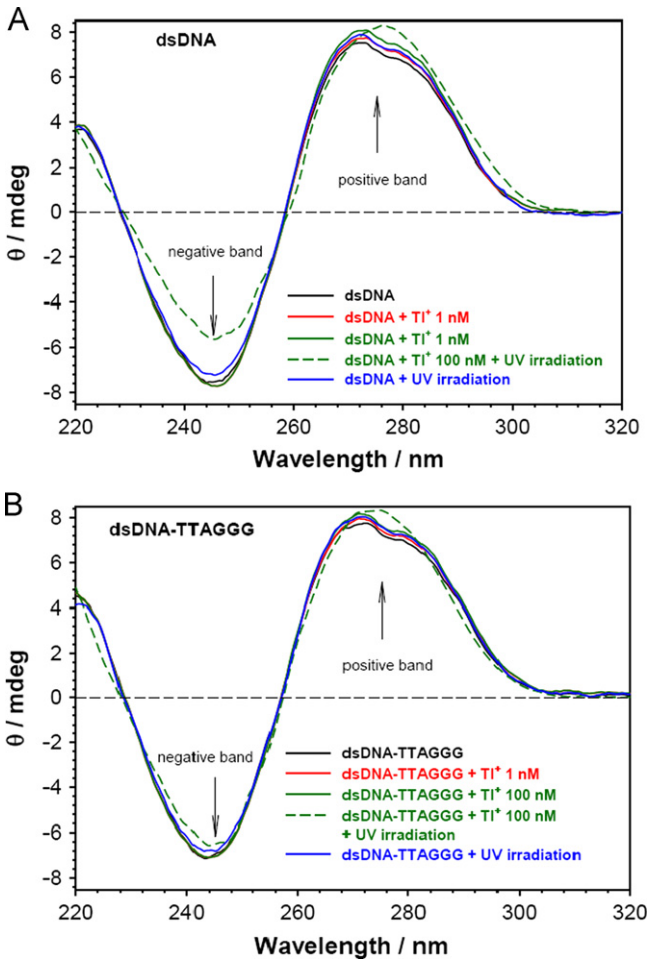

Fig. 2. CD spectra of DNA sequences (3 μM): (A) double-stranded DNA – dsDNA and (B) dsDNA containing one strand modified with the telomere fragment – dsDNA–TTAGGG, present in thallium(I) solutions of different concentration and under conditions of UV irradiation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

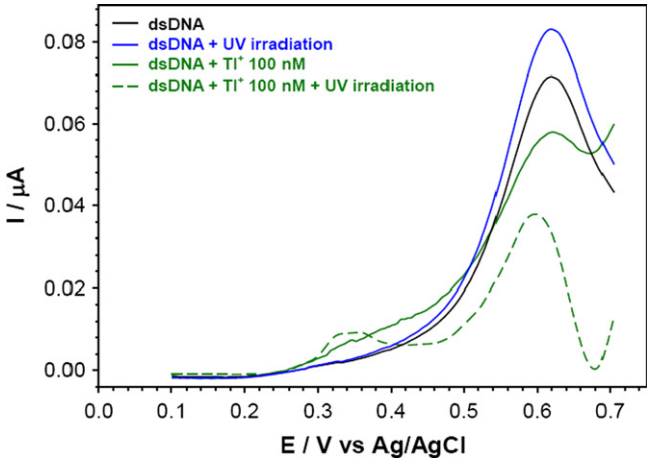

Fig. 3. Differential pulse voltammograms of dsDNA deposited at GC surface (black solid line) and soaked in Tl(I) solution either treated (green dashed line) or untreated (green solid line) with UV irradiation for one hour. Blue solid line describes the situation of exposure of pure dsDNA to UV irradiation. Experimental conditions: diameter of disc glassy carbon electrode 3 mm, 0.02 M PBS, pH 7.4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

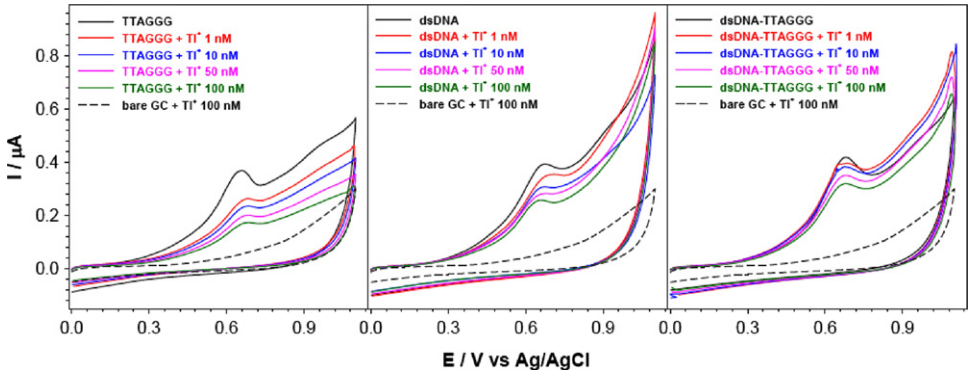

Fig. 4. Cyclic voltammograms of DNA deposited at GC surface and soaked in Tl(I) solution for one hour. Experimental conditions: disc glassy carbon electrode 3 mm diameter, 0.02 M PBS, pH 7.4, scan rate 20 mV/s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

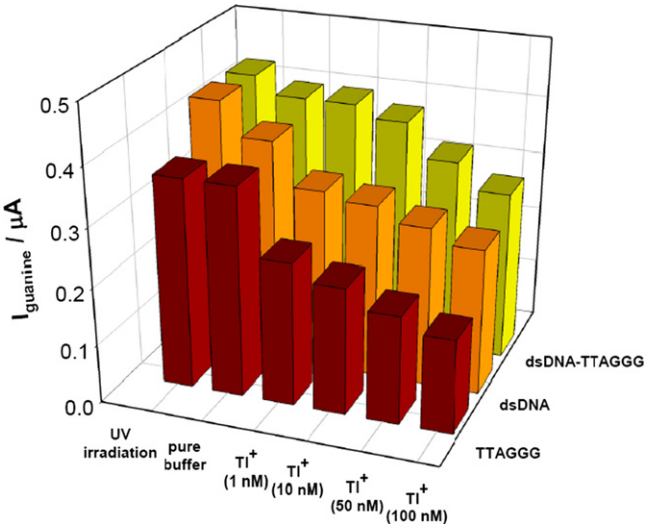

Fig. 5. Dependence of voltammetric peak height of oxidation current of guanine in DNA sequences vs. concentration of Tl(I) and added UV irradiation. Experimental conditions as in Fig. 4.

couple is the most frequently appearing dimer; it is formed by two neighbor thymine molecules (in the same chain) which are linked by two bonds between atoms C5 and C6 of the participating bases. Other connections are formed between thymine and cytosine. The dimers between two cytosine molecules are formed rarely. To be sure that observed decrease of the guanine oxidation peak height and the presence of 8-oxoguanine signal is the effect of the influence of Tl(III) the control experiment was made. The DNA damage, caused only by UV irradiation, to DNA preconcentrated on the GC electrode surface was examined. During the drying step, the microdrop was exposed to UV radiation. After two hours the electrode was rinsed with pure water and immersed in 0.02 M PBS buffer (pH 7.4). Differiential pulse voltammetry was quickly carried out to evaluate the guanine oxidation current. As it is seen in Fig. 3, the damage of the dsDNA film by UV irradiation was manifested by a circa 15% increase in the current signal. The formation of the dimers (a result of the UV treatment) may lead to the partial unwinding of the DNA double helix, then to a better exposition of the guanine redox centers and finally to easier electrooxidation of guanine. Therefore the current drop can be related to the presence of thallium only.

3.1.2. Electrochemical and gravimetric data

Typical cyclic voltammograms of studied DNA samples adhered to the GC electrode surface obtained before and after interactions with Tl^+ (for incubation time of 1 h) in 0.02 M PBS buffer (pH 7.4) are presented in Fig. 4. The voltammogram exhibits one peak located at 0.62 V which corresponds to the electrooxidation of guanine [32–34]. The oxidation current of guanine decreased by 50% after adding Tl(I) (100 nM) to the solution, see Fig. 5. Additionally we also observed a shift of the voltammetric peak towards more positive potential value. Before obtaining voltammograms the glassy carbon electrodes modified with the DNA film was immersed to the $TlNO_3$ solution of pH 5.7 for 1 h. It is known that a weakly acidic environment does not damage the double-stranded DNA but can make the film more tight what can lead to an increase in the overpotential and the corresponding shift of the peak [35].

The nitrogen atom at position 7 in the guanine molecule is very important because it takes place in the redox process [32]. During the oxidation process of guanine molecule the oxygen atom is substituted to the C8 atom. However, this substitution is possible only if the double bond between N7 and C8 is broken and the hydrogen atom is added to N7. So, the formation of the complex of Tl(I) with N-7 guanine probably makes the oxidation process more difficult. The complexes of Tl^+ with other nitrogen atoms cannot affect the voltammetric oxidation signal of guanine in DNA.

To get the complete quantitative information about Tl^+ and DNA interactions the gravimetric and spectroscopic experiments were done. The resonant frequency shifts ($-\Delta f$) corresponding to a 900-s DNA mass accumulation were 37 ± 7; 87 ± 6 and 108 ± 5 Hz, for the telomere fragment, dsDNA and dsDNA containing one strand modified with the telomere fragment, respectively, see Fig. 6. The modified electrode (Au–EQCM/DNA) was carefully washed with water and exposed to Tl(I) solution. The resonant frequency shifts corresponding to the binding of thallium ions by DNA residues were 44 ± 6; 56.7 ± 10 and 85 ± 7 Hz for the telomere fragment, dsDNA and dsDNA containing one strand modified with the telomere fragment, respectively, see Fig. 5. The increases in apparent mass ($m_{Tl(I)}=4.3\Delta f$), related to the observed experimental frequency shifts for bonding of Tl^+ to DNA, are: 189.2; 243.8 and 365.5 ng, respectively. These data gave us a possibility to determine the total number of Tl(I) ions bound to the DNA components. From the changes in guanine-oxidation peak height the number of Tl(I) ions bound selectively to guanine at position N-7 can be calculated. The total number of nucleotides and the total number of Tl(I) ions bound to the DNA film were calculated from the EQCM measurements (the molecular mass of the used oligonucleotides was provided by the vendor). The number of guanine species occupied by Tl(I) at N-7 were calculated from the drop in the voltammetric peak height. The obtained data are shown in Table 1. By comparing the number of Tl(I) ions bound to the nitrogen atoms at position 7 in the

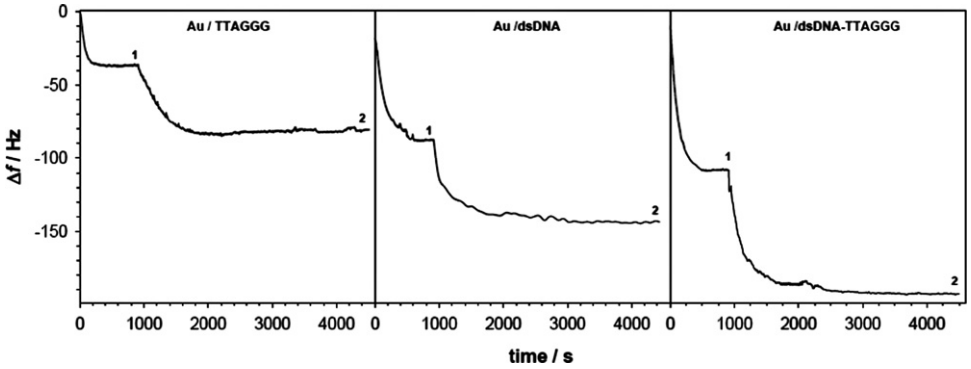

Fig. 6. Frequency shifts observed during immobilization of studied DNA sequences (1) and during exposure of Au–EQCM/DNA to monovalent thallium(I) solution in concentration 100 nM (2). Experimental conditions: 0.02 M phosphate buffer, pH 7.4.

Table 1
Parameters describing interactions of monovalent Tl cations with DNA sequences.

	(TTAGGG)	dsDNA	dsDNA-TTAGGG
Number of nucleotides	$(30.2 \pm 3.8) \times 10^{13}$	$(65.9 \pm 5.2) \times 10^{13}$	$(90.9 \pm 7.3) \times 10^{13}$
Number of guanine molecules in nucleotides	$(15.1 \pm 1.2) \times 10^{13}$	$(16.5 \pm 1.5) \times 10^{13}$	$(25.3 \pm 1.4) \times 10^{13}$
Total number of Tl(I) cations bound to DNA	$(56 \pm 4.1) \times 10^{13}$	$(72 \pm 5.4) \times 10^{13}$	$(108 \pm 7.0) \times 10^{13}$
Number of guanine species occupied by Tl(I) at N-7	$(2.60 \pm 0.08) \times 10^{13}$	$(1.65 \pm 0.14) \times 10^{13}$	$(4.3 \pm 0.21) \times 10^{13}$
Equilibrium constant for Tl(I)–$(N\text{-}7)_{guanine}$, $[M^{-1}]$	$(1.23 \pm 0.4) \times 10^{5}$	$(0.85 \pm 0.25) \times 10^{5}$	$(1.46 \pm 0.54) \times 10^{5}$
Binding-site size Tl(I)–$(N\text{-}7)_{guanine}$	6	10	6

guanine molecule and the total number of Tl(I) ions bound to all DNA components we concluded that the complex N-7–Tl^+ constituted really a small part of total bound thallium.

3.1.3. Quantitative description of the interactions between N-7 of guanine and Tl^+

The most important process within the interaction of Tl(I) with DNA is the binding of that cation with N-7 atom of guanine due to biochemical implications. Therefore we have limited our investigations to that process. The following formula describes the interactions between the dsDNA strand and the ligand [36]:

$$\frac{r}{C_f} = K(1-nr)\left[\frac{1-nr}{1-(n-1)r}\right]^{n-1} \quad (3)$$

where K is the selective binding constant for Tl(I)–$(N\text{-}7)_{guanine}$, n is the binding-site size, and $r = C_b/C_{matrix\ unit}$, where C_b is the concentration of the Tl(I) bound to N-7 of guanine molecule determined from the voltammetric curves, C_f is the concentration of the free ions of Tl(I) in the solution, and $C_{matrix\ unit}$ is the analytical concentration of the binding units (guanine nucleotides) in the DNA strand. The concentrations of free ligand was determined by subtracting the amount of ligand bound to DNA (EQCM data) from the amount of Tl(I) introduced to the solution. The amount of Tl(I) bound to N-7 of guanine can be selectively determined from the height of voltammetric peak of oxidation of guanine. The quantity of guanine sites is known, since the oligonucleotides were synthesized.

An analysis of Scatchard's plots shown in Fig. 7 allowed us to calculate K and n. The calculated selective binding constants (K) and the number of N-7 guanine atoms (n) required for 1 direct complex with Tl(I) were obtained by fitting the experimental data to formula (2) using the Sigma Plot program. The determined values are presented in Table 1. The values of selective binding constant for Tl(I)–$(N\text{-}7)_{guanine}$, (K) equaled $1.2 \cdot 10^5$, $0.85 \cdot 10^5$ and $1.46 \cdot 10^5\ M^{-1}$ for the telomere fragment, dsDNA and dsDNA containing one strand modified with the telomere fragment,

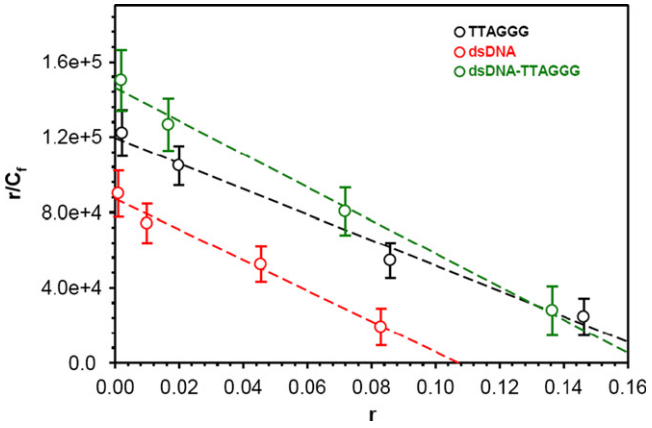

Fig. 7. Scatchard's plots obtained for interactions between Tl(I) and DNA sequences, constructed from voltammetric data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

respectively. Somewhat surprising is the fact of rather big numbers of binding sites assigned to one Tl^+ cation. The size of thallium cation is small and therefore the expected number should be 1.We speculate that this might be a result of nonequivalence of N-7 atoms in guanine. The percentage of occupied N-7 atoms in guanine is 16, 10 and 17, respectively, for the oligonucleotides examined. We can also say that in the case of dsDNA containing one strand modified with the telomere fragment the main role in the interaction is played by the guanine molecules in the selected telomere fragment. The standard Gibbs free energy change ($\Delta G^0 = -RT \ln K$) equals –12.6 kJ/mol at 21 °C, which indicates the spontaneity of the binding process of Tl^+ with guanine N-7. Only 168 guanine nucleotides per 1000, in the case of telomere fragment and dsDNA containing one strand modified with the telomere fragment, are occupied, while in the dsDNA fragment this number decreased to 99.

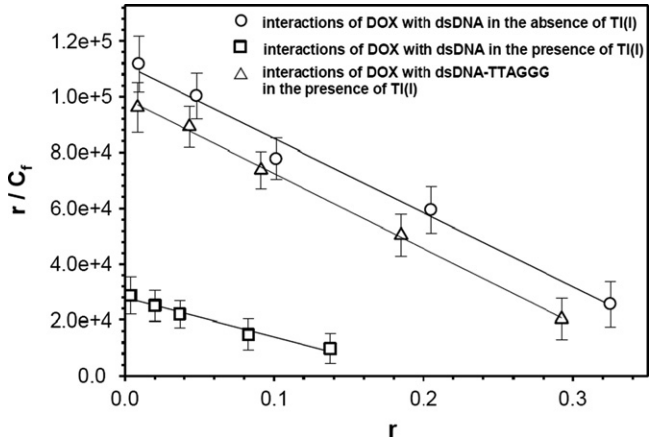

Fig. 8. Scatchard's plots obtained for interactions between DOX and dsDNA and dsDNA-TTAGGG in absence and presence of Tl(I).

3.2. Influence of presence of Tl(I) on DOX activity

We expected that the interactions of Tl(I) with dsDNA can influence the intercalation of DOX into the double-stranded DNA. To verify this hypothesis the Scatchard plots were done, see Fig. 8. The data for these plots were taken from the UV–Vis experiments. Interestingly, both: the equilibrium constant and the number of active sites in the oligonucleotiodes occupied by one doxorubicin molecule changed. The equilibrium constant dropped from 1.15×10^5 to $0.3 \times 10^5\ M^{-1}$ and the number of active sites changed from 2.3 to 4.7. This indicates a significant change in the intercalation process of doxorubicin. If the telomere fragment is bound to dsDNA the influence of presence of Tl(I) on the activity of doxorubicin is limited. There is no substantial drop in the value of the equilibrium constant ($1.0 \times 10^5\ M^{-1}$), which means that the telomere fragment protects dsDNA against Tl(I).

4. Conclusions

The use of voltammetry allowed the examination of selected interaction of Tl(I) with N-7 atoms of guanine in DNA chain. This is thanks to the fact that N-7 in the guanine molecule is electroactive and after binding to Tl(I) loses its electroactivity. On the other hand the experiments with an electrochemical quartz crystal microbalance provided the data that allowed the determination of the total number of monovalent Tl cations bound in variety of ways to DNA strands. The CD measurements indicate that the interactions with Tl(I) do not lead to a change in the DNA conformation. The addition of a telomere fragment with three guanines in row to one of the strands in the helix effectively limits the interactions of thallium(I) with dsDNA.

The quantitative description of interactions of Tl^+ with DNA, especially with the guanine molecules, may help in the study of e.g. guanine rich telomere or CpG islands in promoter regions of enzymes in the cancer cells. We want to stress here that Tl(I) under the condition of UV irradiation and the presence of molecular oxygen can be oxidized to Tl(III) and this species can oxidize guanine to 8-oxoguanine which base is mutagenic.

The investigation of Tl(I) make a base for further examination of DNA activity of Tl(III), as it is known that Tl(III) as a unstable ion always exists in the presence of Tl(I).

Finally, despite the fact that Tl(I) is not as poisonous as Tl(III), it was found that the interactions of doxorubicin with dsDNA are considerably weaker in the presence of Tl(I).

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