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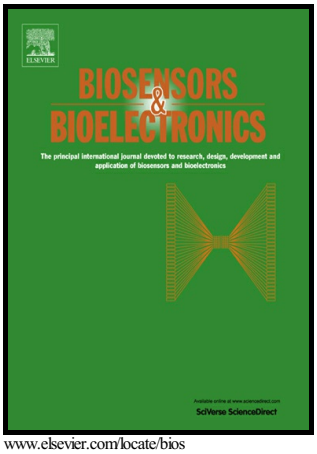

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A Novel Quantitative Electrochemical Method to Monitor DNA Double-Strand Breaks Caused by a DNA Cleavage Agent at a DNA Sensor

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Abstract

To date, DNA cleavage, caused by cleavage agents, has been monitored mainly by gel and capillary electrophoresis. However, these techniques are time-consuming, non-quantitative and require gel stains. In this work, a novel, simple and, importantly, a quantitative method for monitoring the DNA nuclease activity of potential anti-cancer drugs, at a DNA electrochemical sensor, is presented. The DNA sensors were prepared using thiol-modified oligonucleotides that self-assembled to create a DNA monolayer at gold electrode surfaces. The quantification of DNA double-strand breaks is based on calculating the DNA surface coverage, before and after exposure to a DNA cleavage agent. The nuclease properties of a model DNA cleavage agent, copper bis-phenanthroline ($[Cu^{II}(phen)_2]^{2+}$), that can cleave DNA in a Fenton-type reaction, were quantified electrochemically. The DNA surface coverage decreased on average by 21 % after subjecting the DNA sensor to a nuclease assay containing $[Cu^{II}(phen)_2]^{2+}$, a reductant and an oxidant. This percentage indicates that 6 base pairs were cleaved in the nuclease assay from the immobilised 30 base pair strands. The DNA cleavage can be also induced electrochemically in the absence of a chemical reductant. $[Cu^{II}(phen)_2]^{2+}$ intercalates between DNA base pairs and, on application of a suitable potential, can be reduced to $[Cu^{I}(phen)_2]^{+}$, with dissolved oxygen acting as the required oxidant. This reduction process is facilitated through DNA strands via long-range electron transfer, resulting in DNA cleavage of 23 %. The control measurements for both chemically and electrochemically induced cleavage revealed that DNA strand breaks did not occur under experimental conditions in the absence of $[Cu^{II}(phen)_2]^{2+}$.

Keywords: DNA cleavage, DNA biosensor, DNA cleavage agent, DNA interaction, copper bis-phenanthroline, DNA quantitation.

1. Introduction

Many compounds have been reported to interact with DNA and cause significant DNA damage that can lead to the inhibition of DNA replication, eventually promoting cell death (Gibson, 2002; Patrick, 2013). Compounds that promote cell death can be used in anticancer therapies to kill cancer cells. Understanding the interactions between DNA and bioinorganic compounds is crucial for drug discovery and development.

Interactions between DNA and compounds can be investigated using electrochemical DNA sensors. DNA strands immobilised on the electrode surface can facilitate the electron transfer

between the electrode surface and redox active molecules that interact with DNA. Electron transfer in DNA can be observed over distances as great as 200 Å and is termed long-range electron transfer (Liu and Barton, 2005; Treadway et al., 2002).

Electrochemical DNA sensors have been widely used to investigate the interactions between DNA and a myriad of potential and current drugs (Abreu et al., 2002; Brabec, 2000; Congur et al., 2015; Diculescu et al., 2006; Erdem and Congur, 2013; Erdem and Ozsoz, 2001; Fojta et al., 2000a; Janiszek et al, 2016; Jelen et al., 2002; La-Scalea et al., 2002; Marin et al., 1998; Oliveira-Brett et al., 1996, 1998, 2002; Perez et al., 1999; Wang et al., 1998). These studies are based on changes in the electrochemical signal of DNA and the compound before and after the interaction (Diculescu et al., 2016; Erdem and Ozsoz, 2002; Fojta, 2002a; Fojta et al., 2016; Palecek and Bartosik, 2012).

DNA cleavage, caused through interaction with DNA cleavage agents and proteins has, to date, been monitored mainly by electrophoresis (Molphy et al., 2014, 2015; Prisecaru et al., 2012, 2013; Reddy et al., 1999; Rodriquez et al., 1990). Typically, DNA is mixed with the chosen compound and, after a given time, the cleaved DNA fragments are analysed using gel or capillary electrophoresis. This method can be time-consuming, is non-quantitative and requires gel stains. The electrochemical monitoring of DNA cleavage is also possible. The main advantages of the electrochemical approach are its simplicity, low cost, high sensitivity, minimal power requirements and suitability for automation.

Labuda et al. (1999) detected DNA damage caused by reactive oxygen species at glassy carbon electrodes using $[Co(phen)_3]^{3+}$ as a redox marker. Double-stranded DNA (dsDNA) was treated with a mixture known to generate radicals - Cu(II) complex, a reducing agent and oxygen from aerated solution. The DNA was then adsorbed onto the GCE surface and changes in the DNA structure were monitored, using cyclic voltammetry, through changes in the electrochemical response of the redox marker, $[Co(phen)_3]^{3+}$. Reactive oxygen species can damage DNA base pairs that lead to the creation of DNA base pair derivatives, such as electroactive 8-oxo-7,8-dihydroguanine (8-oxoG). Oliveira-Brett et al. (2002) together with Diculescu (2004) and Oliveira (2010) monitored DNA oxidative damage through the appearance of an 8-oxoG signal at a glassy carbon electrode using differential pulse voltammetry. Lloyd et al. (1998) reported that the formation of 8-oxoG in DNA is correlated with formation of double-strand breaks in the strands. The 8-oxoG oxidation peak at the electrode can then be, in theory, used to detect the DNA cleavage caused by DNA cleavage agents. Similarly, Fojta et al. (1997a, 1998, 1999, 2000b, 2002b) observed DNA cleavage caused by reactive oxygen species using supercoiled DNA-modified mercury electrodes. After immersing the DNA sensor in the nuclease assays, additional signals associated with open circular and linear forms of DNA were observed using AC voltammetry. Hence, DNA cleavage can be monitored using different electrochemical methods, different types of DNA and using different electrode types. However, to our knowledge, none of these methods facilitate quantitative studies of DNA cleavage.

In this paper, we introduce a novel, simple and quantitative method to monitor the nuclease activity of DNA cleavage agents using a DNA electrochemical sensor. This method is based on calculating the DNA surface coverage before and after exposure to a DNA cleavage agent. The DNA surface coverage was calculated using a method developed by a Tarlov group (Steel et al., 1998) which utilises the redox probe $[Ru^{III}(NH_3)_6]^{3+}$. In this work, $[Cu^{II}(phen)_2]^{2+}$ was used as a model DNA cleavage agent. $[Cu^{II}(phen)_2]^{2+}$ can intercalate unselectively between the DNA base pairs through the phenanthroline rings and can also be attracted electrostatically by the negatively charged phosphate groups of the DNA backbone. The nuclease activity of $[Cu^{II}(phen)_2]^{2+}$ was discovered in 1979 (Faggiani et al., 1980; Marshall et al., 1981; Que et al., 1980; Sigman et al., 1979; Veal et al., 1991), and this compound displays DNA cleavage properties under specific conditions. In the presence of an

oxidant and reductant, $[Cu^{II}(phen)_2]^{2+}$ can cause double-strand breaks in DNA (Que et al., 1980). It is thought that the role of the reductant is to reduce $[Cu^{II}(phen)_2]^{2+}$ to $[Cu^{I}(phen)_2]^{+}$ and the oxidant is then thought to interact with the reduced form of copper bis-phenanthroline in a Fenton – type reaction (Molphy et al., 2014; Prisecaru et al., 2012, 2013).

$$[Cu^{II}(phen)_2]^{2+} + \text{reductant} \longrightarrow [Cu^{I}(phen)_2]^{+}$$
$$[Cu^{I}(phen)_2]^{+} + H_2O_2 \longrightarrow [Cu^{II}(phen)_2]^{2+} + HO^{\bullet} + HO^{-}$$

In this paper, DNA cleavage was induced both chemically, using ascorbic acid and hydrogen peroxide, and electrochemically, through the application of a cathodic potential to DNA sensors immersed in $[Cu^{II}(phen)_2]^{2+}$ solutions.

2. Experimental

2.1 Materials

The 30 base pair DNA oligonucleotides (Oligo DNA) were purchased from Sigma-Aldrich. Three different DNA oligonucleotides were used, having the following sequences:
Thiol-modified sequence: SH-$(CH_2)_6$- 5'AGTACAGTCATCGCTTAATTATCGTACGTA3'
Complementary sequence: 5'TCATGTCAGTAGCGAATTAATAGCATGCAT3'
$[Cu^{II}(phen)_2]^{2+}(NO_3)_2$ was synthesised according to procedure, S-1, supporting information. Ruthenium (III) hexammine trichloride and p-toluenethiol were purchased from Fisher. All other chemicals were purchased from Sigma-Aldrich. Argon gas was purchased from Air Products. Nuclease-free water was used to prepare 1xTE buffer which consisted of 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA, pH 8.0, that served in the dilution of Oligo DNA and in the hybridisation of the Oligo DNA strands. All other solutions were prepared using ultrapure water purified with a Milli-Q® system.

2.2 Equipment

The electrochemical measurements were performed with a CH Instruments Potentiostat, model 620A. A Solartron 1285 Potentiostat was used to apply a constant potential in some experiments. Gold disc electrodes (2 mm diameter), modified with an immobilised DNA self-assemble monolayers (SAM), were used as the working electrodes, with roughness factors of 2.70 +/- 0.04 (S-2, SI). Saturated calomel and silver/silver chloride were used as reference electrodes, and a platinum wire as the counter electrode. The supporting electrolyte was deoxygenated with argon for 15 min before experiments, and a blanket of argon was maintained above the solutions during measurements. DNA nuclease assays were performed in aerated solutions at 37°C to simulate biological conditions while all electrochemical quantitative measurements were performed at room temperature.

2.3 Preparation of DNA Sensors

In brief, the freshly cleaned electrodes were immersed in 0.5 M phosphate buffer, pH 7.0, containing 0.4 µM double-stranded Oligo DNA and left overnight. This resulted in the creation of a DNA SAM gold electrode (Pividori et al., 2000). The orientation of DNA strands can be manipulated somewhat by application of a suitable potential (Erts et al., 2003; Kelley et al., 1998; Zhang et al., 2002). Strands at the electrode surface were then, in theory, aligned to a more perpendicular position by applying a slight cathodic potential (-0.6 V vs. SCE) at the DNA sensor for 30 s. The electrodes were then immersed in 1 mM p-toluenethiol solution for 1 hour to backfill any 'pin holes' remaining between Oligo DNA strands after immobilisation. This avoids any non-specific interactions between analytes and the bare gold electrode surface. Moreover, thiols are believed to remove DNA strands that adsorb onto the electrode surface non-specifically, resulting in modified electrodes with DNA strands bound

only through the sulfur atom (Herne and Tarlov, 1997; Steel et al., 2000). Aromatic thiols, such as p-toluenethiol, were recently reported to be more effective back-filling agents than alkanethiols, such as 6-mercapto-1-hexanol (Moura-Melo et al., 2015).
The beakers used during the immobilisation step were silanised prior to use as Oligo DNA is known to adsorb onto glass surfaces (Ausubel et al., 1989) (S-3, SI).

2.4 Quantification of DNA Strands on the Electrode Surface

An electrochemical method to quantitatively determine the DNA surface coverage was first introduced by the Tarlov group in 1998 (Steel et al., 1998). This method is described in detail (S-5, SI). In summary, negatively charged phosphate groups on DNA are charge compensated by cations present in solution. In low ionic strength electrolyte (10 mM Tris-HCl), multivalent cations, such as the redox probe ruthenium (III) hexammine trichloride (RuHex), can replace cations present within the DNA, such as Na^+ or K^+. RuHex stays within the DNA layer due to electrostatic attraction to the anionic phosphate residues of DNA. The amount of adsorbed RuHex molecules can be determined using chronocoulometry (CC). The number of adsorbed RuHex can then be translated into the number of DNA molecules adsorbed at the electrode surface if the number of nucleotides and, thus, the number of phosphate groups are known. In theory, one RuHex ion is ion-paired to three phosphate groups (Steel et al., 1998).

2.5 Electrochemical Conditions

Cyclic voltammetry was carried out over the potential range +0.1 V to -0.35 V vs. SCE at a scan rate of 0.1 $V \cdot s^{-1}$. Chronocoulometry was carried out over the potential range +0.1 V to -0.6 V vs. SCE, pulse width 0.5 s.

3. Results and Discussion

3.1 Interactions between DNA and $[Cu^{II}(phen)_2]^{2+}$ at the DNA Sensor

$[Cu^{II}(phen)_2]^{2+}$ is a known intercalator. Moreover, it has been reported that $[Cu^{II}(phen)_2]^{2+}$, after interaction with a reductant and oxidant, can generate hydroxyl radicals in close proximity to DNA strands, resulting in DNA double-strand breaks (Prisecaru et al., 2012).
First, the DNA sensors were used to establish the interactions between DNA and the model nuclease compound, $[Cu^{II}(phen)_2]^{2+}$. The DNA sensor was immersed in deaerated 0.1 M phosphate buffer (PB), pH 7.0, containing 20 µM $[Cu^{II}(phen)_2]^{2+}$. The obtained results were compared to CV profiles registered at the bare gold electrode in the same solution (Figure 1).

Here Figure 1

The $[Cu^{II}(phen)_2]^{2+}$ oxidation and reduction waves were broad and ill-defined at the bare gold electrode, while clearly visible and defined at the DNA sensor. At the bare electrode, an anodic wave (Cu^I/Cu^{II}) is observed at -0.084 V and a coupled cathodic wave (Cu^{II}/Cu^I) at -0.200 V vs. SCE. The separation of the anodic and cathodic peak potentials, ΔE_p, was 116 mV which indicates a quasi-reversible reaction for a $1e^-$ redox process occurs at the bare gold electrode. The formal potential vs. SCE, $E^{o'}$, estimated as the average of peak potentials of both waves, was -142 mV at the bare gold electrode. At the DNA sensor, an anodic wave was observed at -0.079 V and the coupled cathodic wave at -0.113 V vs. SCE, giving a ΔE_p value of 34 mV. A ΔE_p value of 34 mV is less than the 59 mV value predicted for a diffusion-controlled reversible $1e^-$ redox process. This result indicates that $[Cu^{II}(phen)_2]^{2+}$ interacts (adsorbed) with DNA and can be oxidised and reduced through long-range electron transfer between the compound and the electrode. This electron transfer is facilitated through the DNA base pairs. The formal potential for Cu^{II}/Cu^I redox reaction at the DNA sensor was

approximated to be -96 mV. Carter et al. (1989) reported that if the formal potential shifts to more positive values in the presence of DNA, the complex interacts with DNA predominantly through intercalation, as opposed to electrostatic attraction. In this case, a positive shift in the formal potential of 46 mV was observed. Moreover, the oxidation and reduction waves disappeared after washing the sensor in 0.1 M PB, pH 7.0, for 20 minutes (Figure 1 – squared trace). Both, results indicate that in 0.1 M PB, ionic strength 0.178 M, $[Cu^{II}(phen)_2]^{2+}$ interacts with the Oligo DNA SAM through non-covalent interactions, indicative of intercalation and electrostatic attraction. Labuda et al. (1999) reported the transition of the interaction from predominantly electrostatic, in the low ionic strength buffers (ionic strength 0.009 M), to predominantly intercalative in high ionic strength buffers (ionic strength 0.089 M) for $[Cu^{II}(phen)_2]^{2+}$.

The $[Cu^{II}(phen)_2]^{2+}$ redox peak currents obtained at the DNA sensor were recorded as a function of scan rate (Fig. S1, SI) and the resulting linear relationship indicates that electron transfer between $[Cu^{II}(phen)_2]^{2+}$ and the DNA sensor is under adsorption control. However, at the bare gold electrode, a linear relationship between the reduction peak current of $[Cu^{II}(phen)_2]^{2+}$ and the square root of scan rate was observed, indicating that the redox reaction occurring at the bare gold electrode is diffusion-controlled (Fig. S2, SI). During the course of these experiments, $[Cu^{II}(phen)_2]^{2+}$ interacts with DNA at the electrode surface and remains within the DNA layer.

3.2 Chemically Induced DNA Cleavage

To monitor the double-strand breaks at the DNA sensor, the DNA surface coverage was measured before and after immersing the sensor in the $[Cu^{II}(phen)_2]^{2+}$ nuclease assay. The experiments were performed in 10 mM Tris-HCl, pH 7.4 and, subsequently, DNA surface coverage was analysed using the aforementioned Tarlov method. The nuclease assays consisted of 10, 20 or 50 μM $[Cu^{II}(phen)_2]^{2+}$, 1 mM ascorbic acid (AA) as the reductant and 1 mM H_2O_2 as the oxidant in 0.1 M PB, pH 7.0.

The DNA sensor was first immersed in 0.1 M PB, pH 7.0, at 37^{o}C for 2 hours. After cooling and washing the DNA sensor for 10 min, the DNA surface coverage was measured. This measurement yields the number of DNA molecules on the electrode surface after treating the sensor with the given conditions and before contact with the cleavage assay. Next, the DNA sensor was immersed in the nuclease assay at 37^{o}C for 2 hours and, after cooling and washing, the DNA surface coverage was measured again. The washing step between cleavage and DNA surface coverage measurements is necessary to ensure that $[Cu^{II}(phen)_2]^{2+}$ is removed from the DNA layer and does not affect the interactions between DNA and RuHex. Typical CC data obtained at the DNA sensor is presented in figure 2. Two step chronocoulometry was used to reduce and then re-oxidize RuHex ions trapped in the DNA layer. The DNA sensors were allowed 2 minutes equilibration time in the RuHex solutions before each CC measurement.

Here Figure 2

The plot of charge versus the square root of time can be constructed for each RuHex concentration and the intercepts determined (Figure 3).

Here Figure 3

The difference between the intercept in the absence and in the presence of RuHex is equal to Q_{ads}. A plot of Q_{ads} values versus RuHex concentration reveals the point when the DNA SAM is saturated with RuHex (Figure 4a). When the Q_{ads} value becomes constant, the DNA SAM

is saturated with RuHex. The plot C/Q_{ads} versus C (where C is the concentration of RuHex) returns a linear plot according to Langmuir theory (Figure 4b). This plot can be used to calculate the reduction charge of RuHex obtained for the saturated DNA layer (Q_{sat}) required to determine the binding constant between DNA and RuHex and further calaculations (S-5, SI).

Here Figure 4a and 4b

The DNA surface coverage registered before an interaction in the nuclease assay corresponds to a layer of 30 base-paired double-stranded DNA. After the interaction, some part of the DNA strands are cleaved and the DNA surface coverage measured. The number of cleaved base pairs was calculated as follows:

$$\text{number of cleaved base pairs} = 30\ \text{bp} - [(30\ \text{bp} \cdot \Gamma_{\text{DNA After}}) / \Gamma_{\text{DNA Before}}]$$

where Γ_{DNA} is the DNA surface coverage before ($\Gamma_{\text{DNA Before}}$) and after ($\Gamma_{\text{DNA After}}$) immersing the electrode in the nuclease assay.
The DNA surface coverage, after immersing the DNA sensor in the nuclease assay at a temperature of 37°C, decreased significantly (Table 1). The decrease depends on the concentration of $[Cu^{II}(phen)_2]^{2+}$. The DNA cleavage, with an average of 21 %, was observed after immersing the DNA sensor in a solution containing 20 μM $[Cu^{II}(phen)_2]^{2+}$. Thus, on average, 6 base pairs were cleaved from each immobilised 30 base pair DNA strand. After immersing the DNA sensor in the nuclease assay containing only 10 μM $[Cu^{II}(phen)_2]^{2+}$, the DNA surface coverage decreased by 13 %. This lower DNA cleavage efficiency is due to smaller number of radicals generated in the solution containing lower $[Cu^{II}(phen)_2]^{2+}$ concentration. Moreover, after an exposure of the DNA sensor to a solution containing the higher $[Cu^{II}(phen)_2]^{2+}$ concentration, 50 μM, the DNA surface coverage decreased by 23 %, i.e. 7 base pairs were cleaved from each DNA strand. The percentage cleavage observed in solutions containing 20 μM and 50 μM $[Cu^{II}(phen)_2]^{2+}$ is very similar, despite the significant difference in the concentration. This result indicates that the DNA layer is saturated at 20 μM $[Cu^{II}(phen)_2]^{2+}$ and this lower concentration can be used to estimate the nuclease activity of the compound.

A number of conditions were checked to make sure that the observed cleavage is associated only with the interaction between DNA and $[Cu^{II}(phen)_2]^{2+}$ in the presence of the exogenous reductant and oxidant (Table S4, SI). The heating of the DNA sensor at 37°C for 2 hours did not cause significant changes in the DNA surface coverage. DNA cleavage was not observed when the experiment was performed in 0.1 M PB, pH 7.0, containing only $[Cu^{II}(phen)_2]^{2+}$. Moreover, significant DNA cleavage was not registered when experiments were performed in the absence of $[Cu^{II}(phen)_2]^{2+}$, i.e. containing only a reductant or only an oxidant or a mixture of both reductant and oxidant. Similarly, significant DNA cleavage was not observed when the DNA sensor was immersed in the buffer containing only $[Cu^{II}(phen)_2]^{2+}$ and the oxidant (H_2O_2). However, the DNA surface coverage decreased by approximately 15 % in the solution containing only $[Cu^{II}(phen)_2]^{2+}$ and the reductant (ascorbic acid). In this experiment solution oxygen provided the required oxidant. Thus, the $[Cu^{II}(phen)_2]^{2+}$ exhibits DNA nuclease properties only under specific conditions, i.e. in the presence of a reductant and an oxidant.

3.3 Electrochemically Induced Cleavage

It was reported by Fojta et al. (2002b) that transition metal ion complexes that interact with DNA can be electrochemically reduced within a DNA layer and, in the presence of oxygen, can lead to DNA cleavage. Hence, the electrochemical reduction of $[Cu^{II}(phen)_2]^{2+}$, intercalated within the DNA base pairs in an aerated solution, should promote cleavage of DNA in the absence of an external reductant and oxidant. In this experiment, the DNA sensor, together with a Pt wire counter electrode and Ag/AgCl reference electrode, were immersed in 0.1 M PB, pH 7.0, containing 20 µM $[Cu^{II}(phen)_2]^{2+}$ and heated at 37°C for 20 min. During the next five minutes of heating, a potential of -0.205 V vs. Ag/AgCl was applied to the DNA sensor. After cooling and washing the DNA sensor for 10 minutes, the DNA surface coverage was measured. The chosen potential of -0.205 V vs. Ag/AgCl, based on SWV experiments (Fig. S3, SI), ensures that $[Cu^{II}(phen)_2]^{2+}$ is reduced in aerated solution at the DNA sensor. A 23 % decrease in the DNA surface coverage was observed after applying the cathodic potential at DNA sensor in a solution containing $[Cu^{II}(phen)_2]^{2+}$ (Table 2), indicating a DNA cleavage event.

The mechanism of electrochemically induced DNA cleavage by cleavage agents was proposed by Yang et al. (2002):

$$[Cu^{II}(phen)_2]^{2+} + e^- \longrightarrow [Cu^{I}(phen)_2]^{+}$$
$$2[Cu^{I}(phen)_2]^{+} + 2H^+ + O_2 \longrightarrow 2[Cu^{II}(phen)_2]^{2+} + H_2O_2$$
$$[Cu^{I}(phen)_2]^{+} + H_2O_2 \longrightarrow [Cu^{II}(phen)_2]^{2+} + HO^{\bullet} + HO^-$$

The heating of the DNA sensor at 37°C for 20 minutes, and the application of the reduction potential, did not cause change in the DNA surface coverage in the absence of the $[Cu^{II}(phen)_2]^{2+}$ (Table S5, SI). DNA cleavage was not observed when the DNA sensor was immersed in $[Cu^{II}(phen)_2]^{2+}$ solution without electrochemical reduction (Table S5,SI). Hence, the reduction of $[Cu^{II}(phen)_2]^{2+}$ to $[Cu^{I}(phen)_2]^{+}$ in aerated solution is necessary to cleave the DNA strands.

Additionally, methylene blue (MB^+) was used as a control compound. Methylene Blue is also a known intercalator; however, unlike $[Cu^{II}(phen)_2]^{2+}$, this compound does not promote a Fenton-like reaction and, thus, is not expected to cleave the DNA strands. MB can be reduced at the DNA sensor via long-range electron transfer to leucomethylene blue (LMB).

$$MB^+ + e^- + H^+ \longrightarrow LMB$$

The DNA sensor was immersed in 0.1 M PB, pH 7.0, containing 20 µM MB^+ for 20 min and a potential of -0.300 V vs. Ag/AgCl was applied for 5 minutes. The chosen potential was based on a cyclic voltammetry measurement made in MB^+ solution at 37°C (data not shown). The DNA surface coverage did not change after interaction with MB^+ and application of a potential (Table S5, SI). These results indicate that method presented in this paper can identify DNA cleavage agents from compounds that do not cause DNA cleavage, such as non-radical producing intercalators.

The chemically and electrochemically induced cleavage was also performed at DNA sensors that consisted of guanine-cytosine (G-C) or adenine-thymine (A-T) rich regions DNA strands. The DNA surface coverage decreased by 19 % (6 bp) for both A-T and G-C rich region strands after chemically induced cleavage, while after electrochemically induced cleavage the DNA surface coverage decreased by 20 % (6 bp) and 24 % (7 bp) for A-T and G-C rich region strands, respectively. Cleavage efficacy percentages recorded indicate that $[Cu^{II}(phen)_2]^{2+}$ has no selectivity towards G-C or A-T only regions (Table S6, SI).

Interestingly, the rate of DNA cleavage can be affected by immobilization of DNA onto the electrode surface. The current work did not monitor DNA cleavage rates and, thus, cannot be compared to reported analogous solution phase cleavage rates. However, Fojta et al. (1997b) reported that the cleavage rate of supercoiled DNA in the nuclease assay containing $[Fe(EDTA)]^{2-}$, sodium ascorbate and H_2O_2 at the HMDE is comparable to cleavage performed in solution in the initial stages of the reaction. After initial rates, the cleavage of the DNA immobilized at the electrode surface slows while the rate of DNA cleavage in the solution is only slightly diminished. It is possible that DNA located close to the surface is not fully accessible for interactions with hydroxyl radicals. It is known that binding DNA to some surfaces preserves it from chemically or enzymatically induced cleavage at the bonded sites (Rhodes and Klug 1980). Fojta et al. (1999) also showed that the interactions between enzyme DNaseI and supercoiled DNA immobilized at the HMDE are hindered in comparison to the interactions occurring in the solution. Interestingly, changing the potential of the electrode can affect the position of the enzyme towards the electrode surface causing increase or decrease in the rate of the interactions.

4. Conclusions

The $[Cu^{II}(phen)_2]^{2+}$ redox waves observed at the DNA sensor indicate that this compound does interact with DNA and is accumulated in the DNA SAM.

The DNA surface coverage decreased by approximately 21 % after exposing the DNA sensor to a nuclease assay containing 20 μM $[Cu^{II}(phen)_2]^{2+}$, a reductant, and an oxidant at 37°C for 2 hours. The $[Cu^{II}(phen)_2]^{2+}$ was shown to intercalate between DNA base pairs. When $[Cu^{II}(phen)_2]^{2+}$ is reduced electrochemically from an aerated solution at 37°C DNA cleavage is also observed (23 %). The electrochemical reduction $[Cu^{II}(phen)_2]^{2+}$ excludes the necessity for the addition of exogenous reductants and oxidants, and the nuclease assay can be performed within 30 minutes.

The experiments prove that the presented method – calculating the DNA surface coverage before and after exposure to the DNA cleavage agent – can provide the quantitative information about the nuclease efficacy of potential anti-cancer drugs.

We believe that these results represent an important step in the development of DNA damage sensors. Future work will be focused on quantifying the nuclease activity of a range of DNA bioinorganic DNA nuclease complexes and further validation will be obtained using bioinorganic complexes that do not exhibit DNA nuclease activity. An array of DNA sensors will be fabricated for the simultaneous quantitation DNA nuclease efficacy of a range of bioinorganic complexes. Miniaturisation of these DNA sensor arrays will then be carried out. This reported method, due to its simplicity and rapid response, has the potential to be utilised in drug development research prior to the expensive cancer cell line assays.

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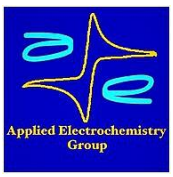

Declaration of interest: none.

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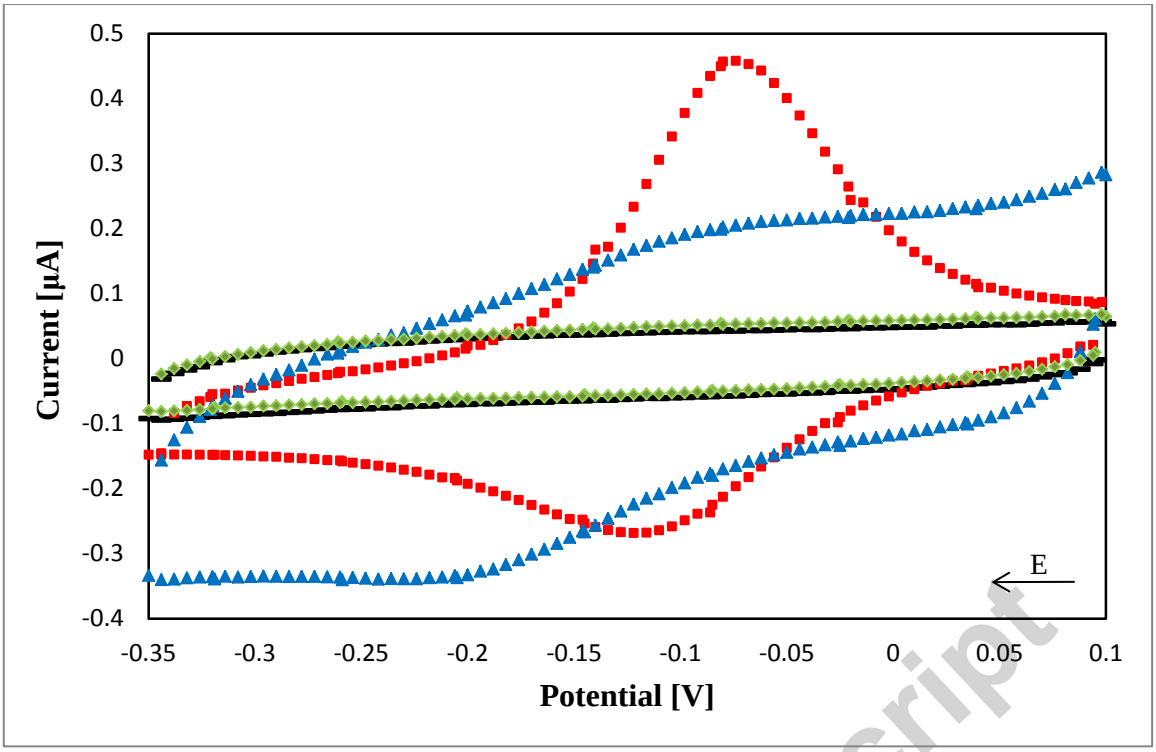


Figure 1. Typical CV voltammograms registered in 0.1 M PB, pH 7.0, at a DNA sensor (solid black trace), and in 0.1 M PB, pH 7.0, containing 20 µM $[Cu^{II}(phen)_2]^{2+}$ at a bare gold electrode (triangular blue trace) and at the DNA sensor (squared red trace), and in pure buffer again after washing the DNA sensor for 20 minutes (rhombic green trace); scan rate: 0.1 $V \cdot s^{-1}$. The PB was deoxygenated in all measurements. The arrow shows the initial direction and starting potential of the sweep.

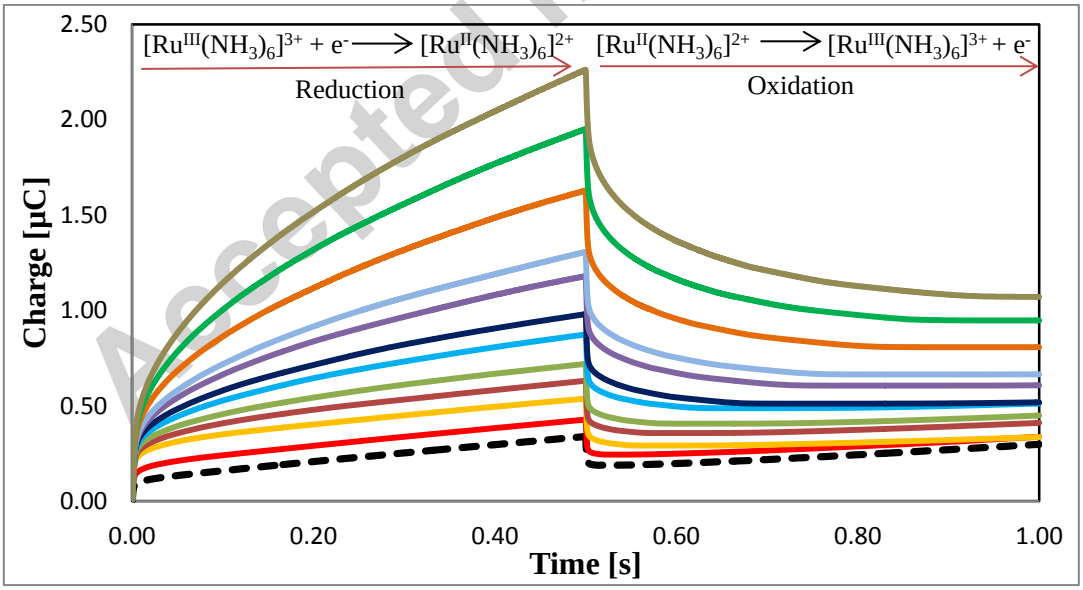


Figure 2. Typical Q versus t plots obtained at a DNA sensor in 10 mM Tris-HCl, pH 7.4. The dashed trace was registered in the absence of RuHex, the other traces in varied concentrations of RuHex (1 µM – 250 µM).

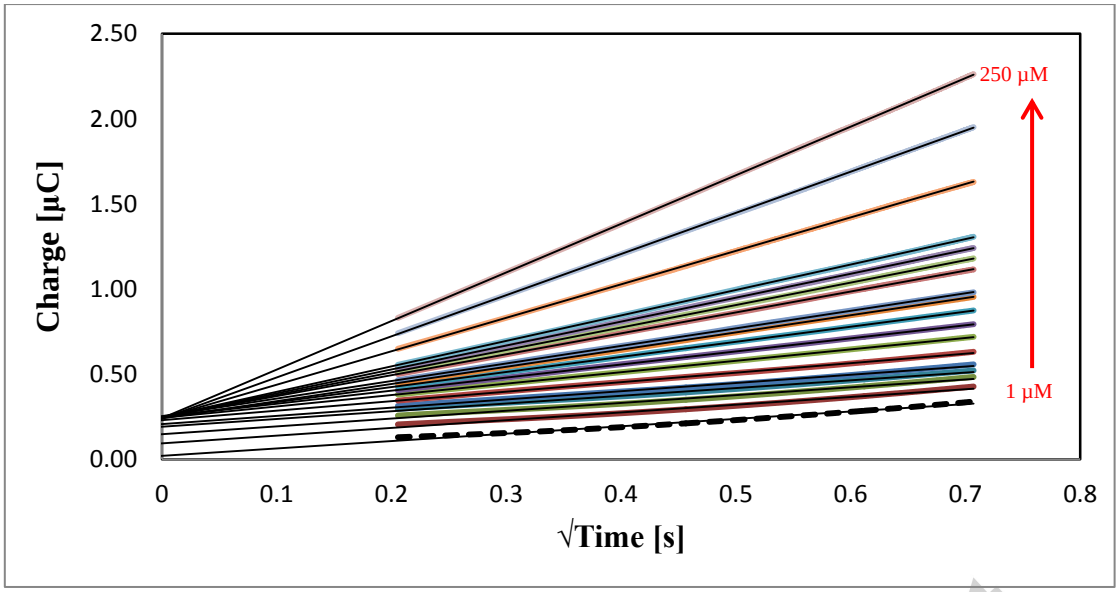
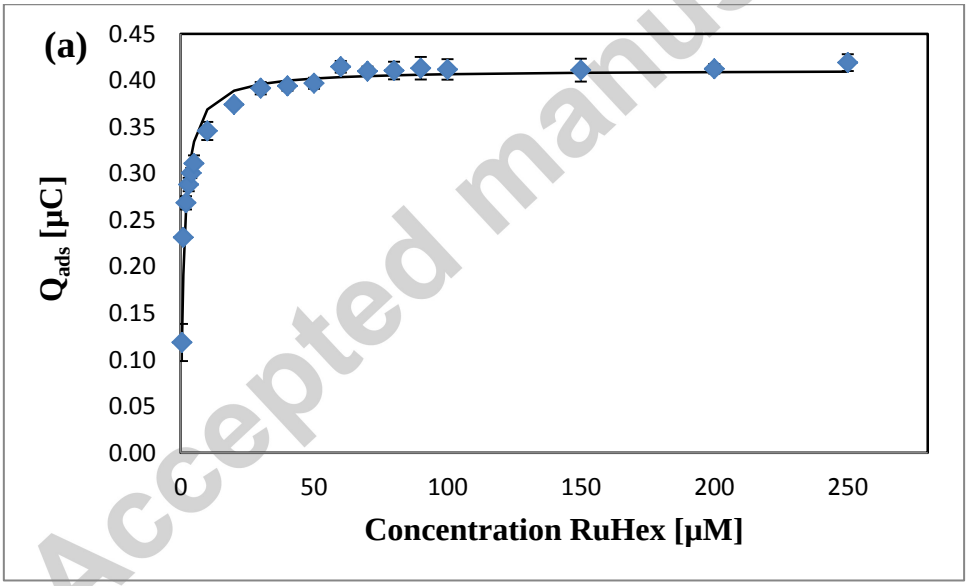


Figure 3. Typical Q versus √t plots obtained at a DNA sensor in 10 mM Tris-HCl, pH 7.4. The dashed trace was registered in the absence of RuHex, the other traces in varied concentrations of RuHex (1 µM – 250 µM). The intercepts in the presence of RuHex minus the intercept in the absence of RuHex is equal to Q_{ads}.



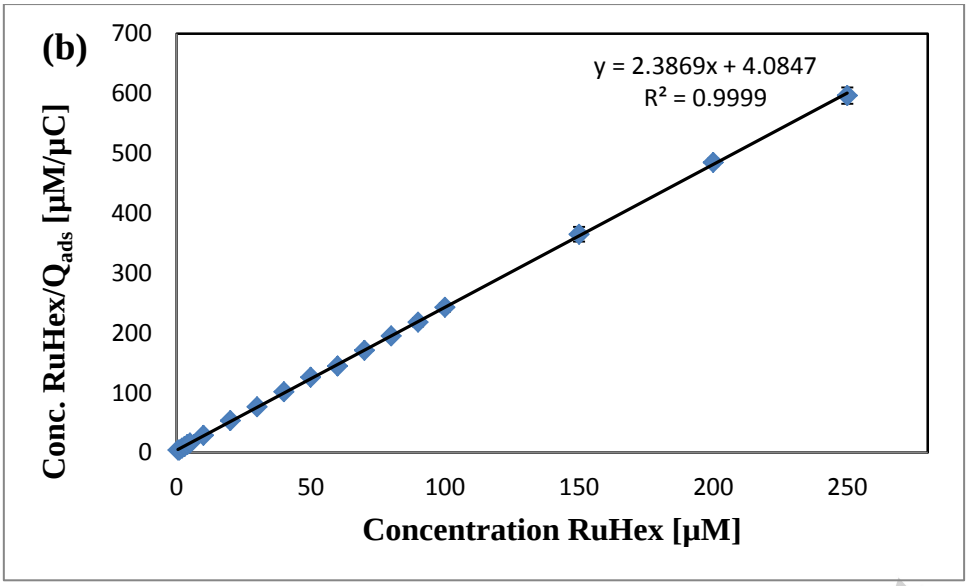


Figure 4. (a) Typical Q_{ads} versus RuHex concentration plot obtained at a DNA sensor in 10 mM Tris-HCl, pH 7.4, the concentration of RuHex was varied from 1 µM to 250 µM. (b) These data were plotted according to Langmuir theory. Typical Q_{dl}, Q_{ads} values, used to plot graph 4a and 4b, together with standard error and %RSD for these values, are presented in Table S1, S2 and S3 in supporting information S-6.

Table 1. The DNA surface coverage before and after immersing a DNA sensor in the nuclease assay for 2 hours at 37°C and the percentage amount of cleaved DNA together with the number of cleaved bases (30 bp = 100 %). % RSD values are given in brackets.

Experimental conditions	DNA surface coverage x10^{12} [molecules·cm^{-2}]		Cleavage effectiveness [%]	Average cleavage effectiveness [%] (% RSD)	Number of cleaved bases	Average number of cleaved bases
	before interaction	after interaction				
Results in 10 µM	5.66	4.90	13.43	**13.12**	4.03	3.94

$[Cu^{II}(phen)_2]^{2+}$, 1 mM AA and 1 mM H_2O_2	4.44	3.86	13.06	(2.20)	3.92	(2.19)
	4.51	3.93	12.86		3.86	
Results in 20 µM $[Cu^{II}(phen)_2]^{2+}$, 1 mM AA and 1 mM H_2O_2	6.30	5.05	19.81	**20.86** (6.15)	5.94	6.26 (6.21)
	4.77	3.79	20.48		6.14	
	4.16	3.23	22.29		6.69	
Results in 50 µM $[Cu^{II}(phen)_2]^{2+}$, 1 mM AA and 1 mM H_2O_2	4.86	3.82	21.40	**22.76** (5.18)	6.4	6.80 (5.09)
	5.30	4.06	23.40		7.0	
	5.11	3.91	23.48		7.0	

Table 2. The DNA surface coverage before and after immersing a DNA sensor in 20 µM $[Cu^{II}(phen)_2]^{2+}$ for 25 minutes at 37°C and applying a potential of -0.205 V vs. Ag/AgCl for 5 min, and the percentage amount of cleaved DNA together with the number of cleaved bases (30 bp = 100 %). % RSD values are given in brackets.

Experimental conditions	**DNA surface coverage x10^{12} [molecules·cm^{-2}]**		**Cleavage effectiveness [%]**	**Average cleavage effectiveness [%] (%RSD)**	**Number of cleaved bases**	**Average number of cleaved bases**
	before interaction	**after interaction**				
20 µM $[Cu^{II}(phen)_2]^{2+}$, after applying potential	2.51	1.93	23.10	**23.22** (3.86)	6.93	6.97 (3.83)
	4.22	3.20	24.17		7.25	
	3.26	2.53	22.39		6.72	
Without $[Cu^{II}(phen)_2]^{2+}$, after applying potential	No cleavage observed					

20 µM $[Cu^{II}(phen)_2]^{2+}$ without applying potential and external oxidant or reductant	No cleavage observed

Highlights:

- $[Cu^{II}(phen)_2]^{2+}$ intercalates within DNA immobilized at the electrode surface
- $[Cu^{I}(phen)_2]^{+}$ binds five times stronger to chosen DNA than $[Cu^{II}(phen)_2]^{2+}$
- A mixture of $[Cu^{II}(phen)_2]^{2+}$, a reductant and an oxidant cleaves 21% of chosen DNA
- An electrochemically reduced $[Cu^{II}(phen)_2]^{2+}$ in aerated solution cleaves 23% of DNA
- DNA is not cleaved under experimental conditions in the absence of $[Cu^{II}(phen)_2]^{2+}$