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Electrochemical genosensing of the interaction between the potential chemotherapeutic agent, *cis*-bis(3-aminoflavone)dichloroplatinum(II) and DNA in comparison with *cis*-DDP

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Abstract

The interaction of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) and the potential novel chemotherapeutic agent, *cis*-bis(3-aminoflavone)dichloroplatinum(II) (*cis*-BAFDP) was studied electrochemically with calf thymus double-stranded DNA (dsDNA) by using differential pulse voltammetry (DPV) with disposable pencil graphite electrode (PGE) at the surface. These studies were prompted by beneficial biological properties of *cis*-BAFDP in comparison with *cis*-DDP, which were proven in vitro both in human normal and cancer cells and in vivo. The changes in the experimental parameters such as the concentration of *cis*-DDP and *cis*-BAFDP were studied by using DPV; in addition, the reproducibility of this genosensor and the detection limit for each compound were determined. After the interaction of *cis*-DDP with dsDNA, the DPV signal of guanine and adenine was found to be decreasing. In comparison with *cis*-DDP, a dramatic decrease at adenine signal was also obtained after the interaction of *cis*-BAFDP and dsDNA. Similar results were also found in solution phase after the latter compound interacts with poly[A]. The features of the proposed electrochemical method for the detection of *cis*-BAFDP with DNA in comparison with *cis*-DDP are discussed and compared with those methods previously reported for the other type of DNA-targeted agents in the literature.

Keywords: DNA–drug interactions; Electrochemical genosensor; *cis*-Diamminedichloroplatinum(II); *cis*-Bis(3-aminoflavone)dichloroplatinum(II)

1. Introduction

The pharmaceutical industry is under ever-increasing pressure to increase its success rate by bringing drugs into the market. Enormous advances in genomics have resulted in a large increase in the number of potential therapeutic targets that are available for investigation. This growth in potential targets has increased the demand for reliable target validation, as well as technologies [1].

Studies of small molecules which react at specific sites along a DNA strand as reactive models for protein–nucleic acid interactions provide routes toward rational drug design as well as means to develop sensitive chemical probes for DNA [2]. A recent active area of research is to explore the nature and dynamics of binding small molecules to biomacromolecules. The design of site- and conformation-specific reagents provides new studies for rational drug design [3,4]. Small molecules are stabilized on binding to DNA through a

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series of weak interactions such as π-stacking associated with the intercalation of aromatic heterocyclic groups between the base pairs and hydrogen bonding and van der Waals interactions of functional units bound along the groove of the DNA helix [2].

The interaction of DNA with other molecules is an important fundamental issue in life sciences. The investigation based on DNA interactions has great importance in the understanding of action mechanisms of some anti-tumor and anti-viral drugs and some π-carcinogenic molecules, designing of new DNA-targeted drugs and screening of these drugs in vitro. The interactions of some anticancer drugs with DNA have been studied by a variety of techniques [5–8].

Electrochemistry offers great advantages over the existing devices based on optical schemes because electrochemical ones provide rapid, simple and low-cost point-of-care detection of specific nucleic acid sequences [9–12]. Electrochemical genosensors play an important role for pharmaceutical, clinical, environmental, and forensic applications. In recent years, there is a growing interest for design of biosensors that exploit interactions between surface-confined DNA and target drugs for their rapid screening [13–28].

Currently, *cis*-DDP (shown in Fig. 1A) is widely used in chemotherapy of many types of cancer [29]. However, besides effectiveness it gives many side effects which limit the clinical application of this compound [30,31].

Therefore, studies are focused on searching for novel analogs of *cis*-DDP, at least equally effective in chemotherapy but less toxic [32–34].

cis-Bis(3-aminoflavone)dichloroplatinum(II) (*cis*-BAFDP) (shown in Fig. 1B) was synthesized by modification of the *cis*-DDP molecule involving the introduction of a 3-aminoflavone unit in place of one of the hydrogen atoms of the amine group. Additionally, Nijveldt et al. [33] reported that flavonoids exhibit beneficial properties, e.g. anti-tumor and antioxidative, and they are more toxic in cancer cells than in normal ones.

It is believed that *cis*-DDP shows its anti-tumor properties by binding to DNA. Ca. 90% of total platinum–DNA adducts comprise 1,2-intra-strand cross-links including adjacent bases (65% 1,2-d(GpG) between N7 atoms, 25% 1,2-d(ApG) with the remaining part consisting of intra-strand cross-links (1,3-d(Gp-NpG)), inter-strand cross-links with DNA as well as binding to proteins and formation of monoadducts [32,35,36].

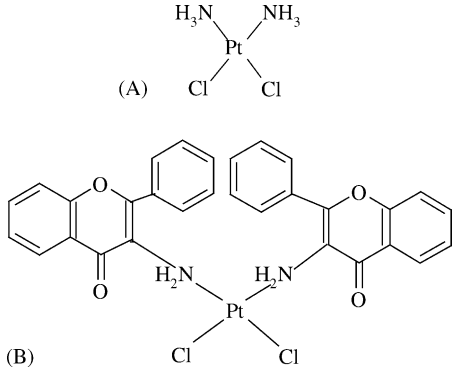

Fig. 1. Chemical structures of the tested compounds: (A) *cis*-DDP; (B) *cis*-BAFDP.

cis-BAFDP belongs to a class of compounds extensively investigated. Introduction of flavone ligand could change the DNA-binding properties of the compound as compared to *cis*-DDP. It was reported that *cis*-BAFDP exhibited significant anti-tumor activity against the development of leukemia after intraperitoneal implantation of L1210 cells into mice [37]. Stronger DNA degradation was also observed in L1210 cells in vitro after this compound application but not in the cells incubated with *cis*-DDP [38].

It is worth noting that *cis*-BAFDP was also less genotoxic, cytostatic and induced apoptosis and necrosis to a smaller degree in normal human lymphocytes in comparison with *cis*-DDP but was a more effective apoptosis inducer in human non-small cancer lung line A549 [39–41]. Pietras et al. [42] noticed that addition of antioxidant molecule (e.g. 3-aminoflavone) to *cis*-DDP prevented deoxyribose degradation by *cis*-BAFDP. *cis*-BAFDP and *cis*-DDP were also applied in the comet assay performed in human non-small cell lung cancer A549 cell line and in normal human lymphocytes. Different patterns of DNA damage obtained after their application can suggest another mechanism of action of each tested compound [43,44]. However, electrochemical methods have not been used for studying properties of *cis*-BAFDP in comparison with *cis*-DDP previously. Therefore in the study performed by Oliveria Brett et al. [15], the electrochemical determination of interaction in the solution phase between anticancer drug, carboplatin and DNA was studied by using glassy carbon electrode (GCE). These experiments were done as carboplatin was added to the solution containing the single-stranded DNA (ssDNA). In conclusion, there was a decrease observed in the oxidation current of adenine on increasing the concentration of carboplatin in solution while the guanine oxidation currents decreased only slightly. These electrochemical results clearly demonstrated that for low concentrations, carboplatin interacts preferentially with adenine rather than guanine groups in the DNA. They reported that since its binding to DNA occurred covalently and consequently, it could be possible to develop an indirect analytical method to determine platinum compounds with anti-tumor activity by measuring this interaction.

The interaction between some other platinum complexes, potent anticancer agents and DNA was studied by using DPV at wax-impregnated graphite electrode [17]. Brabec reported that the paraffin wax-impregnated spectroscopic graphite electrode (PWISGE) transducer displays an analytically useful response for submicromolar levels of $[Pt\text{-}(dien)(H_2O)]^{2+}$ at short accumulation times as 2–10 min based on diminution of the guanine oxidation signal.

There are no data concerning evaluation of electrochemical genosensing of the interaction between novel potential chemotherapeutic agent, *cis*-BAFDP and DNA in com-

parison to the interaction between *cis*-DDP and DNA. In this study, we demonstrated the electrochemical detection of the interaction between *cis*-BAFDP and DNA (or polynucleotides) in comparison to *cis*-DDP by using DPV in combination with the disposable genosensor, PGE, based on the differences in the adenine oxidation signals using *cis*-BAFDP and *cis*-DDP concentrations obtained by MTT assay.

2. Experimental

2.1. Apparatus

The oxidation signals of guanine and/or adenine were investigated by using differential pulse voltammetry (DPV) with an AUTOLAB-PGSTAT 30 electrochemical analysis system and GPES 4.8 software package (Eco Chemie, The Netherlands). The raw data were also treated in all DPV measurements by using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a "peak width" of 0.01. The three-electrode system consisted of the Pencil graphite working electrode, an Ag/AgCl reference electrode (Model RE-1, BAS, W. Lafayette, USA) and a platinum wire as the auxiliary electrode.

2.2. Chemicals

The calf-thymus DNA (as lyophilized powder) and polyadenylic acid (5′) poly[A] were obtained from Sigma (Germany). dsDNA stock solution (100 mg/L) and synthetic polynucleotide stock solution (100 mg/L) were prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More dilute solutions of DNA were prepared with ultrapure distilled water. Other chemicals were of analytical reagent grade.

cis-DDP was obtained from Sigma Chemical Co. *cis*-BAFDP was synthesized and is structurally related to *cis*-DDP, containing a flavone molecule instead of amine with two *cis* bound labile chloride ligands [45]. Before experiments the compounds were dissolved in DMSO (Sigma).

2.3. Electrochemical procedure

Each measurement involved the immobilization of the nucleic acid interaction with *cis*-DDP or *cis*-BAFDP detection cycle at a new PGE surface. Repetitive measurements were carried out by renewing the surface and repeating the above assay formats by using PGE. The analytical signals represent the differences in guanine and/or adenine peak height magnitudes obtained with dsDNA or poly[A] modified PGEs.

All the experiments were performed at room temperature $(25.0 \pm 0.5)\,^{\circ}C$.

2.4. Disposable electrode preparation for designing of genosensor

The renewable PGE that was described in the studies [27,28] was used in voltammetric measurements for the electrochemical detection of DNA interactions. A Noki Pencil model 2000 (Japan) was used as a holder for the graphite lead. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically with 12 mm of the lead extruded outside (10 mm of which was immersed in the solution).

2.5. Interaction of surface-confined DNA with cis-DDP

PGE was pretreated by applying +1.40 V for 30 s in blank 0.50 M acetate buffer solution (pH 4.80) without stirring. The dsDNA was immobilized on a pretreated PGE by applying potential at +0.5 V during 5 min in 10 μg/mL DNA in 0.50 M acetate buffer solution containing 20 mM NaCl. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 10 s. The dsDNA-modified PGE was then immersed into 0.50 M acetate buffer solution (pH 4.80) containing different concentrations of *cis*-DDP with 200 rpm stirring for 5 min at open circuit system. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 5 s. The oxidation signals of guanine and adenine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

2.6. Interaction of surface-confined DNA with cis-BAFDP

PGE was pretreated by applying +1.40 V for 30 s in blank 0.50 M acetate buffer solution (pH 4.80) without stirring. The dsDNA was immobilized on a pretreated PGE by applying potential at +0.5 V during 5 min in 10 μg/mL DNA in 0.50 M acetate buffer solution containing 20 mM NaCl. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 10 s. The dsDNA-modified PGE was then immersed into 0.50 M acetate buffer solution (pH 4.80) containing different concentrations of *cis*-BAFDP with 200 rpm stirring for 5 min at open circuit system. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 5 s. The oxidation signals of guanine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

2.7. Interaction of surface-confined poly[A] with cis-BAFDP

PGE was pretreated by applying +1.40 V for 30 s in blank 0.50 M acetate buffer solution (pH 4.80) without stirring. The poly[A] was immobilized on a pretreated PGE by applying potential at +0.5 V during 5 min in 10 μg/mL poly[A] in 0.50 M acetate buffer solution containing 20 mM NaCl. The electrode was then rinsed with 0.50 M acetate buffer so-

lution (pH 4.80) for 10 s. After transfer of poly[A] modified PGE into blank solution, the oxidation signals of adenine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

The poly[A] modified PGE was then immersed into 0.50 M acetate buffer solution (pH 4.80) containing 1 μg/mL of *cis*-BAFDP with 200 rpm stirring for 5 min at open circuit system. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 5 s. The oxidation signals of adenine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

2.8. Interaction of solution-phase poly[A] with cis-BAFDP

PGE was pretreated by applying +1.40 V for 30 s in blank 0.50 M acetate buffer solution (pH 4.80) without stirring. The poly[A] was immobilized on a pretreated PGE by applying potential at +0.5 V during 5 min in 10 μg/mL poly[A] in 0.50 M acetate buffer solution containing 20 mM NaCl. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 10 s. After transfer of poly[A] modified PGE into blank solution, the oxidation signals of adenine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

cis-BAFDP in the concentration level of 1 μg/mL was added into 0.50 M acetate buffer solution containing 10 μg/mL poly[A]. The constituents of the mixture were then immobilized on a new pretreated PGE by applying a potential of +0.50 V for 5 min with 200 rpm stirring. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 5 s. The oxidation signals of adenine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

3. Results and discussion

Fig. 2 shows the differential pulse voltammograms (DPV) for the interaction of *cis*-DDP and *cis*-BAFDP with dsDNA as showing the oxidation signal of guanine and adenine before and after interaction of the compounds with dsDNA-modified PGE as the one with *cis*-DDP (A) and *cis*-BAFDP (B) in concentration level of 6.1 μM and 40.7 μM, respectively. No oxidation signal related to the compound *cis*-DDP was obtained with the bare PGE at the potential range between +0.80 V and +1.4 V. Thus, the changes at oxidation signals of guanine and adenine obtained with the dsDNA-modified PGE before and after the interaction with *cis*-DDP. On the other hand, the oxidation signal related to the compound *cis*-BAFDP was observed at around +1.0 V as the similiar peak potential of guanine; because of this reason, we focused on the changes at the oxidation signals of adenine obtained with the dsDNA-modified PGE before/after the interaction of *cis*-BAFDP with DNA or PolydA at PGE surface or in solution phase.

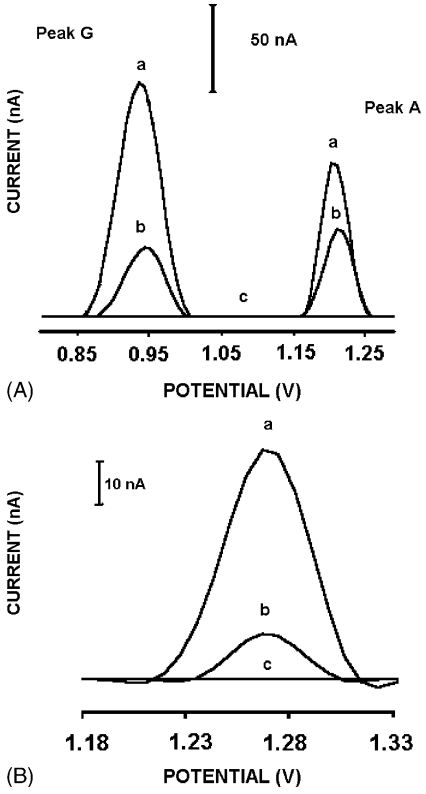

Fig. 2. (A) Differential pulse voltammograms for the interaction of *cis*-DDP with dsDNA as showing the oxidation signal of guanine (peak G) and adenine (peak A) before interaction of *cis*-DDP (a); after interaction of *cis*-DDP (b) with dsDNA at PGE surface in 0.05 M acetate buffer (pH 4.80). *dsDNA accumulation* on a pretreated bare PGE for 5 min at +0.50 V in 10 μg/mL dsDNA containing 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl with 200 rpm stirring. *cis*-DDP *accumulation*, 5 min at open circuit system, 6.1 μM for *cis*-DDP in 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl. *Measurement*, scanning between +0.80 V and +1.40 V at 50 mV s^{-1} pulse amplitude, in blank 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl. The lines labelled with (c) denote the voltammetric response of the bare PGE in a blank as 0.50 M acetate buffer (pH 4.80). (B) Differential pulse voltammograms for the interaction of *cis*-BAFDP with dsDNA as showing the oxidation signal of adenine before interaction *cis*-BAFDP (a); after interaction of *cis*-BAFDP (b) with dsDNA at PGE surface in 0.05 M acetate buffer (pH 4.80). *cis*-BAFDP *accumulation*, 5 min at open circuit system, 40.7 μM for *cis*-BAFDP in 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl. The lines labelled with (c) denote the voltammetric response of the bare PGE in a blank as 0.50 M acetate buffer (pH 4.80). Other conditions are as in (A).

A series of three repetitive DPV signals of guanine and adenine at 10 μg/mL concentration level of dsDNA at PGE surface resulted in reproducible results such as a mean response of 128.5 nA and 70 nA, respectively, with a relative standard deviation of 7.3% and 10.2%.

When the concentration of the compound was increased to different levels, there was observed a gradual decrease/increase in different altitude at guanine and/or adenine oxidation signal by using dsDNA-modified PGE. In Fig. 3, the changes at the oxidation signals of guanine (A) and adenine (B) before and/or after interaction of dsDNA at PGE surface with *cis*-DDP in different concentrations, 6.1 μM, 1.5 μM and 0.5 μM, were presented. A dramatic decrease at

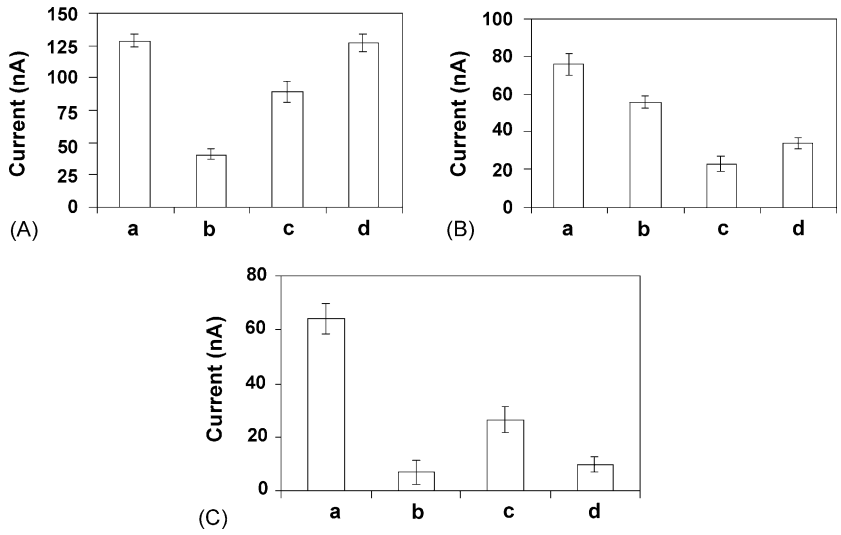

Fig. 3. Histograms presenting the changes at guanine and/or adenine oxidation signals for each compound before and after interaction with dsDNA at PGE surface: (A) Oxidation signals of guanine (a) at bare PGE before and after interaction of *cis*-DDP at concentration level of (b) 6.1 μM; (c) 1.5 μM; and (d) 0.5 μM with dsDNA concentration level of 10 μg/mL in 0.50 M acetate buffer (pH 4.80). Other conditions are as in Fig. 2A. (B) Oxidation signals of adenine (a) at bare PGE before and after interaction *cis*-DDP at concentration level of (b) 6.1 μM; (c) 1.5 μM; and (d) 0.5 μM with dsDNA concentration level of 10 μg/mL in 0.50 M acetate buffer (pH 4.80). Other conditions are as in Fig. 2A. (C) Oxidation signals of adenine (a) at bare PGE before and after interaction *cis*-BAFDP at concentration level of (b) 40.7 μM; (c) 10 μM; and (d) 1 μM with dsDNA concentration level of 10 μg/mL in 0.50 M acetate buffer (pH 4.80). Other conditions are as in Fig. 2A.

guanine signal to about 71% was observed after interaction with 6.1 μM of *cis*-DDP in comparison to guanine signal obtained in the absence of *cis*-DDP. When the concentration of *cis*-DDP was decreased up to 1.5 μM and 0.5 μM, we observed a smaller decrease to about 37% and 9%, respectively. A decrease at adenine signals was also observed to about 34%, 75% and 60% according to the same concentrations of *cis*-DDP, respectively, 6.1 μM, 1.5 μM and 0.5 μM in comparison to adenine signal obtained in the absence of *cis*-DDP.

A series of three repetitive DPV signals of guanine and adenine for interaction between 6.1 μM *cis*-DDP and 10 μg/mL concentration level of dsDNA at PGE surface resulted in reproducible results such as a mean response of 41 nA and 55 nA, respectively, with a relative standard deviation of 4.2% and 6.1%. The detection limit estimated from S/N = 3 corresponds to 122 nM for *cis*-DDP at dsDNA-modified PGE in 5 min accumulation time.

The possibility of a similar binding event between adenine at dsDNA and the analogue *cis*-BAFDP is shown in Fig. 3C. The changes at the oxidation signals of adenine before and/or after interaction of dsDNA at PGE surface with this compound in different concentrations, 40.7 μM, 10.2 μM and 1 μM, were presented. A dramatic decrease at adenine signal was found to about 87.5% in concentration level of 40.7 μM of this analogue in comparison to adenine signal obtained in the absence of *cis*-BAFDP. A decrease was also observed at adenine signals to about 60% and 80% in the concentrations of *cis*-BAFDP, 10.2 μM and 1 μM, respectively.

A series of three repetitive DPV signals of adenine for interaction between 40.7 μM *cis*-BAFDP and 10 μg/mL concentration level of dsDNA at PGE surface resulted in reproducible results such as a mean response of 10 nA, with a relative standard deviation of 5.6%. The detection limit estimated from S/N = 3 corresponds to 600 nM for *cis*-BAFDP at dsDNA-modified PGE in 5 min accumulation time.

The decrease in both the oxidation signals of guanine and/or adenine was attributed to the binding of these compounds to these electroactive bases: this phenomenon could be explained by the shielding of oxidizable groups of electroactive bases such as guanine and/or adenine while *cis*-DDP and *cis*-BAFDP interact with DNA at electrode surface or in solution phase.

In one of the previous studies, it was reported that platin compounds bind covalently to DNA with cross-links between two bases on opposite strands of DNA helix and intra-strand cross-links of two bases on the same DNA strand [15]. Brabec presented the results that show a linear decrease at guanine signal upon increasing concentrations of submicromolar levels of $[Pt\text{-}(dien)(H_2O)]^{2+}$ in 10 min as accumulation time with DNA by using the paraffin wax-impregnated spectroscopic graphite electrode (PWISGE) [17]. This decrease in guanine signal was explained that it appears to reflect the formation of DNA adducts of $[Pt\text{-}(dien)(H_2O)]^{2+}$.

The increase at the guanine and/or adenine signals may also be attributed to the cleavage and/or unwinding of the dsDNA helix. A similar increase in the oxidation signals of guanine were observed for the cleaving agent by using hanging mercury electrode [13] and by using CPE/PGE [26–28]. As these compounds may also cleave dsDNA, the guanine bases could become available to the oxidation. After 6.1 μM *cis*-DDP application, the guanine signals remained constant indi-

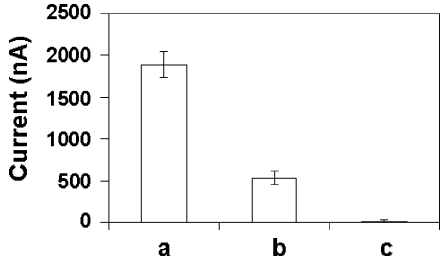

Fig. 4. Histograms presenting the changes at adenine oxidation signals at bare PGE (a) before and after interaction of *cis*-BAFDP with poly[A] at PGE surface (b) and in solution phase (c). *Poly[A] accumulation* on a pretreated bare PGE for 5 min at +0.50 V in 10 μg/mL *Poly[A]* containing 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl with 200 rpm stirring. *cis*-BAFDP *accumulation*, 5 min at open circuit system, 1 μM for this compound in 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl. In solution phase, *ds-DNA and cis*-BAFDP *accumulation* on a pretreated bare PGE during 5 min at +0.50 V in 10 μg/mL dsDNA and 1 μM *cis*-BAFDP containing 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl with 200 rpm stirring. *DPV measurement* from +0.80 V to +1.40 V in the blank 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl. Other conditions are as in Fig. 2A.

cating that all/some guanine bases on dsDNA were exposed to damage by *cis*-DDP as in parallel results obtained after interaction between other compounds and dsDNA [13,26–28].

After poly[A] was interacted at electrode surface or in solution phase with *cis*-BAFDP in concentration level of 1 μM, a dramatic decrease was observed as the signal of adenine to about 70% at PGE surface. The adenine signal almost disappeared to about 99.5% when the interaction occured between poly[A] with *cis*-BAFDP in solution phase. These results (shown in Fig. 4) based on adenine signals obtained using poly[A] are in parallel to the ones obtained using dsDNA in presence of the analogue, *cis*-BAFDP and also in agreement to the results presented by Oliveria Brett et al. [15]. Thus, this similar result could be explained as this decrease in the oxidation signal of adenine attributed to the preferential binding of *cis*-BAFDP to the electroactive base as adenine.

MTT assay [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is the first step in evaluation of cytotoxic properties of new chemical compounds in vitro especially with their potential pharmacological application [46]. It was used to determine energetic cell metabolism by measuring the activity of one of the oxidative enzymes. The dye is reduced in mitochondria by succinic dehydrogenase to an insoluble violet formazan product [41]. A higher cytotoxic effect of *cis*-DDP in normal human lymphocytes in comparison with *cis*-BAFDP was noticed by Kosmider et al. [40]. *cis*-DDP cytotoxicity is connected with induction of mainly DNA cross-links, reactive oxygen species generation and ability of this compound to bind with proteins which causes many side-effects [47–49]. On the other hand, *cis*-BAFDP contains flavonoid ligand, whose anticancer properties were proved [33,50,51]. It has been shown that *cis*-BAFDP was less cytotoxic than *cis*-DDP. The concentrations causing 50% inhibition of lymphocyte growth (IC_{50}) were 40.7 μM for *cis*-BAFDP and 6.1 μM for *cis*-DDP [40]. These results indicate that *cis*-DDP inhibits normal cell metabolic activity several times more efficiently than *cis*-BAFDP. It should be stressed that cytotoxic activity of the platinum complexes is determined by its reactivity towards DNA, e.g. the formation of intra-strand and inter-strand DNA cross-links, binding with mitochondrial DNA and also by other phenomena, e.g. interactions with phospholipids and phosphatidylserine in the cell membrane and proteins, which results in nephrotoxic and ototoxic effects and neuropathy. Moreover, *cis*-DDP induces reactive oxygen species, superoxide anions and hydroxyl radicals causing oxidative renal damage, which may increase nephrotoxicity [40,49]. Jakupec et al. [32] reported that *cis*-DDP enters the cell through passive diffusion only. On the other hand, the hydrophobicity of the 3-aminoflavone ligand could change the kinetics of transport of the *cis*-BAFDP through the membrane of the cells in comparison with *cis*-DDP.

From this point of view it was important to pay attention to the results of MTT assay in normal cells [40] studying electrochemical genosensing of the interaction between tested compounds and DNA. Therefore, in our studies IC_{50} (6.1 μM), $1/4 \times IC_{50}$ (1.5 μM) and $1/12 \times IC_{50}$ (0.5 μM) concentrations of *cis*-DDP as well as IC_{50} (40.7 μM), $1/4 \times IC_{50}$ (10.2 μM) and $1/40 \times IC_{50}$ (1 μM) of *cis*-BAFDP were applied.

As a conclusion, electrochemical studies were prompted by beneficial biological properties of *cis*-BAFDP in comparison with *cis*-DDP, which were proven in vitro both in human normal and cancer cells and in vivo in mice [39–41,43,44]. Such experiments are very important because of the potential anticancer properties of these kinds of complexes and detailed understanding of determination for recognition of DNA sites would be valuable in the rational design of new DNA-targeted molecules for application in chemotheraphy and in the development of new tools for the point-of-care tests and diagnosis based on DNA.

4. Conclusion

The completion of the first draft of the human genome has made it possible to foresee major steps forward in our understanding of the molecular basis of disease, both from attack by external pathogens and internally from variations within the human genome resulting in a plethora of new molecular therapeutic targets for drug design and discovery [7]. The extraordinary pace and scale of developments in the field of genomics has forced a paradigm shift in the manner with which the pharmaceutical industry approaches the discovery and development of new drug compounds.

The analysis of cytotoxic properties of new chemical compounds in vitro is very important especially in case of potential pharmaceuticals. Moreover, the reported electrochemical method here is experimentally convenient and sensitive so that it requires only small amounts of materials. The experimental conditions were optimized by using disposable pencil graphite electrodes and this electrode

improved the reproducibility and the inexpensive cost of this developed assay. The success of PGE over existing carbon electrodes is its commercial availability. In principle, it can be applied to a wide range of DNA-targeted molecules, provided they bear an electrochemically active moiety. Detecting the voltammetric behavior of several drugs that interact with DNA would be valuable in the design of sequence-specific DNA binding molecules for application in chemotherapy and in the development of biotechnological tools for the point-of-care diagnosis based on DNA.

The utility of electrochemical recognition of interaction between this potential novel chemotherapeutic agent, *cis*-BAFDP and DNA in comparison to *cis*-DDP is very simple, cost-effective and provides rapid detection.

An understanding of the structural orientations, kinetics and thermodynamics associated with these complexes is pivotal to design and development of novel "next-generation" chemotherapeutic agents. Elucidation of the forces that drive the thermodynamics and kinetics properties associated with complex formation coupled with structural and chemical features of the DNA binding ligands provides new insight into rational drug design. These studies can play a key role in developing novel chemotherapeutic agents that could be pivotal in targeting specific genes and thereby provide selective control of gene expression [8].

Progress in these laboratories is towards the goal of determining the cytotoxic and electrochemical behavior of newly synthesized compounds with DNA, thus introducing the novel methods to solve the phenomenal drug–DNA interaction mechanisms.

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