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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].

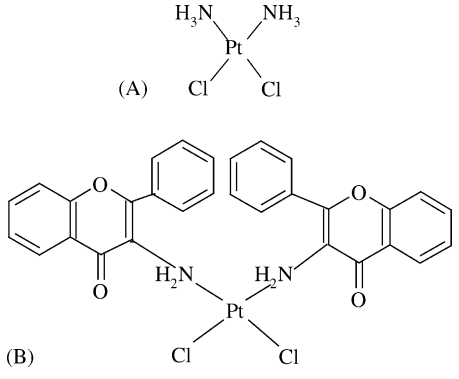

Fig. 1. Chemical structures of the tested compounds: (A) *cis*-DDP; (B) *cis*-BAFDP.

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-