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Notes

Electrochemical Investigation of Interactions between Potential DNA Targeted Compounds, 2,4-Di- and 2,3,4-Trisubstituted Benzimidazo[1,2-a]pyrimidines and Nucleic Acid

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The electrochemical aspects of interactions between DNA and two organic compounds are discussed herein. Potential DNA targeted compounds, 2-methyl-4-phenyl-benzo[4,5]imidazo[1,2-a]pyrimidine (**C1**) and 2,3,4-trimethyl-benzo[4,5]-imidazo[1,2-a]pyrimidine (**C2**), were synthesized and their cytotoxic and/or growth inhibitory effects were studied previously. Disposable sensor technology was used to explore the interaction between the compounds and nucleic acid, such as fish sperm DNA at the electrode surface and in the solution phase. The changes upon encountering oxidation signals of electroactive DNA base-guanine and these compounds were monitored electrochemically.

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Introduction

A recent exciting aim of scientific research is to study the nature and dynamics of binding small molecules to various biomacromolecules. Designing site-spesific and conformation-specific models leads to a more rational drug design.[1-8] Nucleic acids offer a powerful tool in the recognition and monitoring of many important compounds.[1-4] The interaction of deoxyribonucleic acid (DNA) with other molecules represents an important fundamental issue in life sciences.

The ability to interact with DNA forms the basis for numerous antitumor compounds. The interactions of some anticancer drugs with DNA by a variety of techniques have been reported.[9-11] Investigations of DNA interactions are helpful for understanding the mechanisms of the actions of some antitumor and antiviral drugs, as well as some π-carcinogenic molecules. Such studies may contribute to the design of new DNA targeted drugs and their *in vitro* screening.

The binding of small molecules to DNA can occur primarily in three modes: intercalation into the base pairs, in the grooves ("major" or "minor") and outside the helix by electrostatic interactions.[3]

Electrochemistry offers some advantages over devices based on optical schemes. Electrochemical techniques provide rapid, simple and low-cost detection of specific nucleic acid sequences.[2,3,12,13] Electrochemical genosensors are important in pharmaceutical, clinical, environmental and forensic applications. In recent years, there is a growing interest in the design of electrochemical DNA biosensors that can be used to examine the interactions between surface-modified DNA and target drugs for their rapid screening.[3-8,14-24] However, there is only a few data concerning electrochemical evaluations of the interactions between potential chemotherapeutic agents, anticancer compounds and other drug candidates and DNA. Data obtained include the IC_{50} (concentration required to inhibit the cellular growth by 50%) values of these agents or drug candidates. Such experiments are of vital importance in understanding the recognition of DNA sites that would be helpful in the rational design of new DNA targeted molecules which may be used in chemotheraphy, and also may help in the development of new tools for testing.[7]

In a previous study performed by our group,[7] the interactions of *cis*-diaminedichloroplatinum(II) (*cis*-DDP) and a potential novel chemotherapeutic agent, *cis*-bis(3-aminoflavone)dichloro-platinum(II) (*cis*-BAFDP), with calf thymus double-stranded DNA (dsDNA) were studied electrochemically by using differential pulse voltammetry (DPV) connected with a pencil graphite electrode (pGE). These studies were prompted by beneficial biological properties of *cis*-BAFDP compared with *cis*-DDP, which were proved in *in vitro* both in human normal and cancer cells and *in vivo* by using their IC_{50} values. After the interaction of *cis*-DDP with dsDNA, a decrease in the DPV signal of electroactive DNA bases, guanine and adenine, was found. In comparison to the study performed by *cis*-DDP, a dramatic decrease in the adenine signal was also obtained after interactions of *cis*-BAFDP and dsDNA.

An investigation was made concerning to the electrochemical detection of interaction between potential DNA targeted compounds; some benzimidazo[1,2-a]pyrimidine derivatives and DNA were succesfully obtained by using disposable pGE in our study. Benzimidazo[1,2-a]pyrimidines have been reported in the literature concerning their antibiotic, antihypertensive, antidiabetic, diuretic, antidepressant, antiulcer and immunotropic properties.[25-27] These compounds were also evaluated for their interactions with DNA,[28] and their benzodiazepine receptor binding affinity.[29]

Among bicyclic aza compounds, it is well known that imidazopyrimidines have exhibited various activities ranging

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from antiinflammatory, antiulcer to antineoplastic and antiviral. One of the latest papers has addressed the ability of these compounds to bind a benzodiazepine receptor.[30] Our interest in derivatives of imidazo[1,2-a]pyrimidine having potential anticancer activity prompted us to focus on above mentioned bridgehead nitrogen compounds.

As a first approach, we synthesized 2-methyl-4-phenyl-benzo[4,5]imidazo[1,2-a]pyrimidine (**C1**) and 2,3,4-trimethyl-benzo[4,5]imidazo[1,2-a]pyrimidine (**C2**) (shown in Table S1) and reported their cytotoxic properties related to cancer (5RP7) and non-cancer (F2408) cell lines in our previous study.[31] Disposable sensor technology was used in order to detect the interaction between the compounds and nucleic acid, such as fsDNA both at an electrode surface and in the solution phase before/after the interaction process. Consequently, the changes in the oxidation signal of guanine and those of these compounds were monitored electrochemically. Furthermore, a literature survey revealed that there were no previous attempts made to evaluate the electrochemical sensing of interaction between the potential DNA targeted compounds (**C1** and **C2**) and DNA. The features of this assay for electrochemical monitoring of interaction between the compounds and DNA were considered in contrast to earlier reports for other DNA targeted agents or drug candidates mentioned in the literature.

Experimental

Materials

Synthetic and biochemical background of compounds. The chemicals and solvents were purchased from Merck and Aldrich Chemical. Herein considered compounds (**C1** and **C2**) were re-synthesized, and their structures were confirmed by NMR and mass spectrometry; also, their cytotoxicities against F2408 (non-cancer) and 5RP7 (cancer) cells by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay were previously evaluated.[31]

The results of IC_{50} evaluations for **C1** and **C2** suggested that the existence and abundance of a methyl substituent on the structure increases the cytotoxic activity. Consequently, substances abundantly bearing a methyl substituent were found to be promising for providing potential antineoplastic activity.[30,31]

Methods and chemicals for the electrochemical detection of interaction between compounds and DNA. The oxidation signals of guanine and compounds were investigated by using DPV with an AUTOLAB-PGSTAT 302 electrochemical analysis system, General Purpose Electrochemical Software (GPES 4.9 software) package (Eco Chemie, The Netherlands). Raw data from Autolab were also treated using a Savitzky and Golay filter (level 2)[32] and a moving average baseline correction (peak width 0.01) of the GPES software. The three electrode system consisted of a pencil graphite working electrode, an Ag/AgCl reference electrode (Model RE-1, BAS, W. Lafayette, USA) and a platinum wire as an auxiliary electrode.

Fish sperm dsDNA, fsDNA (as lyophilized powder), was obtained from Sigma (Germany). All DNA stock solutions (100 mg/L) were prepared with a 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.

The compounds considered in this present study, **C1** and **C2**, were prepared at concentration levels of 40 and 18 μg mL^{-1}, respectively, by using dimethyl sulfoxide (DMSO).

Electrode preparation

pGE was used in voltammetric measurements for the electrochemical detection of DNA interactions.[6,7] A Tombo pencil (Japan) was used as a holder for a graphite lead (0.5, HB, Noki, Japan). Electrical contact with this lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically with 14 mm of the lead extruded outside (10 mm of which was immersed in the solution).

Procedure

Each measurement was performed by using a new pGE surface. All of the experiments were performed at room temperature 25.0 ± 0.5°C. The interactions of compounds with DNA were studied (a) at the electrode surface, and (b) in the solution phase.

Interaction at the electrode surface. Immobilization of fsDNA onto a pGE surface: pGE was pretreated by applying +1.40 V for 30 s in a 0.05 M acetate buffer solution containing 20 mM NaCl (ABS; pH 4.80); then 16 μg mL^{-1} fish sperm DNA (fsDNA) was immobilized on pretreated pGE by passive adsorption *via* a dip-coating procedure[5,6,8,33] for 7.5 min in 0.50 M ABS. After the immobilization of DNA, the electrode was rinsed with ABS for 10 s. The oxidation signals of guanine and compounds were monitored by using DPV in blank ABS.

Interaction of compounds with DNA at the electrode surface: fsDNA modified pGE was immersed into a compound solution in a 0.05 M tris buffer solution containing 20 mM NaCl (TBS; pH 7.0) and stirred for 5 min without applying any potential. After the interaction, the electrode was rinsed with TBS for 10 s. The oxidation signals were taken by using DPV in blank ABS.

Voltammetric transduction: The oxidation signals of guanine and compounds, **C1** and **C2** were measured by using DPV[6-8,32] in blank ABS by scanning from +0.20 to +1.40 V at 50 mV pulse amplitude at a 30 mV/s scan rate.

Interaction in the solution phase. Interactions of compounds, **C1** and **C2** with fsDNA were studied in the solution phase by using pretreated pGEs. Firstly, pGE was pretreated by applying +1.40 V for 30 s in ABS. Pretreated pGE was immersed into a solution containing both 16 μg mL^{-1} fsDNA and the required concentration of compounds (**C1** or **C2**) in ABS; the sample was then stirred for 5 min without applying any potential. The oxidation signals were taken by using DPV in blank ABS. An electrochemical measurument was performed, followed by the same procedure for voltammetric transduction mentioned above.

Repetitive measurements were carried out by using a new electrode surface, and repeating the above mentioned assay formats by using an electrochemical transducer.

Results and Discussion

The electrochemical detection of the interaction of DNA targeted compounds (**C1** and **C2**), which had been selected for promising results according to our previous result,[31] with DNA was studied based on the oxidation signals of guanine and these compounds by applying the DPV technique.

In our studies, to present a representative work based on the IC_{50} concentration level of a drug candidate for the **C1**, it was applied at the 30 μg mL^{-1} concentration level of **C1**, as shown in Table S1 (IC_{50} = 30 μg mL^{-1} for F2408 and IC_{50} = 15.4 μg mL^{-1} for 5RP7 cell line for **C1** and no data for **C2**).[31]

Interaction of compounds with DNA at an electrode surface

Figures S1-A and S1-B show the oxidation signals of **C1**

measured at +0.867 V and **C2** measured at +0.801 V, respectively, both bare and DNA modified disposable graphite electrodes. A high level of decreased ratios was obtained in the oxidation signals of guanine and **C1** (respectively, around 74 and 83%) after an interaction with fsDNA at the pGE surface. Similar results were obtained in the presence of an interaction between **C2** and fsDNA at the pGE surface (shown in Fig. S1-B; the level of the decrease ratio shown in Table 1).

Column graphs showing the changes in the oxidation signals of guanine and compounds 1 and 2 (abbreviated as **C1** and **C2**) are presented insets of Figs. S1-A and S1-B. A series of three repetitive DPV measurements, concerning the interaction at 40 and 18 μg mL^{-1} as IC_{50} values of **C1** and **C2**, respectively, at a 16 μg mL^{-1} concentration level of fsDNA at the pGE surface resulted in reproducible results, such as the mean response of the guanine signal with the relative standard deviation (RSD %, n = 3); 833 nA (10.1%) and 392 nA (11.7%) were obtained, respectively.

As a result of the interaction at the electrode surface between these DNA targeted compounds with fsDNA, the high level of decrease in the ratio, 74 and 86%, was obtained in the oxidation signal of guanine in the presence of **C1** and **C2**, respectively. The decrease observed in guanine signals in the presence of their interaction with nucleic acids was found to be similar to results presented in the literature[7,16] by using some anticancer drugs or DNA targeted compounds. Thus this result can be explained similarly to the decrease in the response of a DNA biosensor, which could be attributed to preferentially binding of these compounds to the most electroactive base of DNA, *i.e*., guanine in the case of an interaction with DNA at the electrode surface.

There was also a decrease observed in a different ratio in the oxidation signals of each compound (**C1** and **C2**). This result also could be explained as being due to possible damage to oxidizable groups of each compound after their interaction with DNA immobilized onto the surface of electrodes by the intercalation of the potential DNA targeted compound into the base pairs of the double helix form of DNA in parallel to results obtained in earlier studies performed by using some anticancer drugs and antibiotics, such as: Epirubicine (EPR),[20] Mitomycine C (MC),[15,24,33] Mitoxantrone (MTX),[19,21] Doxorubicin (DXR),[22] or novel DNA targeted compounds; *e.g*., a novel tetracyclic compound, benzothienoindole (BTIN).[17]

Interaction of the compounds with DNA in the solution phase

Figures S1-C and S1-D represent the DPVs and column graphs showing, correspondingly, changes in the oxidation signals of guanine, and those of **C1** and **C2**, before/after the interaction between the potential DNA targeted compounds and fsDNA in the solution phase. The decrease ratios % observed in the oxidation signals of the compounds (**C1** and **C2**), and also in the guanine signal after interactions between these compounds and DNA are also summarized in Table 1. After the interactions between the compounds and fsDNA in the solution phase, there was a gradual decrease obtained at the guanine signal; on the other hand, there was a large decrease in the ratio calculated for the oxidation signals of **C1** and **C2** (summarized in Table 1). This large decrease in the ratio of around 90 – 95% in the signals of these compounds after interactions with fsDNA can be attributed to the a possible strong intercalation to DNA, since the interaction easily occured in the solution phase. This result also could be explained as being due to some possible damage to the oxidizable groups of each compound, as in the same case as the interaction with DNA at the electrode surface by intercalation of the potential DNA targeted compound into the base pairs of the DNA double helix according to results obtained that compliment earlier studies performed by using some anticancer drugs and antibiotics.[15,17,19–22,24] It has been concluded that these compounds (**C1** and **C2**) may be totally exposed to an intercalation process into DNA. The electrochemical sensing of the interaction between these benzimidazo[1,2-a]pyrimidine derivatives and nucleic acid successfully performed here at a lower concentration level of DNA with a shorter interaction time and sensitively by the advantage of disposable graphite sensor technology, in contrast to earlier studies by using different electrochemical transducers, such as a glassy carbon electrode (GCE), a hanging mercury drop electrode (HMDE), explored for DNA interaction with some well-known compounds or drugs, *e.g*., MTX, DXR.[19,22] An investigation for concerning the interaction of an anthraquinone drug, MTX, with double-stranded or single-stranded DNA at high concentration levels was studied electrochemically in an aqueous medium, and at the surface of GCE using different voltammetric methods.[19] The intercalation and behavior of MTX was determined by significant changes in the oxidation signal of MTX and guanine, and the adenine signal after an interaction between the drug and DNA. In another study based on conformational changes of DNA due to the binding of some DNA intercalators, such as the anticancer agent DXR, a decrease in the DXR peak was detected to be less than 40% by using adsorptive transfer stripping voltammetry[22] in the presence of a higher concentration level of calf thymus DNA in contrast to our study. Consequently, the electrochemical method presented here has been found to be experimentally much more convenient and sensitive, and also it requires a smaller amount of these materials (compounds, drugs and DNA) compared to those reported earlier, and discussed here as well.

Table 1 Change % in the oxidation signals of novel potential anticancer compounds (**C1** and **C2**) and an electroactive DNA base, guanine (G), after interactions between the compounds and fsDNA

Interaction of the compounds with DNA	C1	G	C2	G
With fsDNA at the electrode surface	83	74	58	86
With fsDNA in solution phase	90	22	95	38

According to our previous data, **C1**, bearing a methyl substituent at position 2 and a phenyl substituent at position 4 of the imidazopyrimidine system, was found to be cytotoxic, especially for 5RP7 cells; 16 μg mL^{-1} of this compound showed 57% cytotoxicity. The effect was enhanced by increasing the concentrations (40 μg mL^{-1}, 96% cytotoxicity for 5RP7 cells). Since the methyl groups enhance the cytotoxicity in a particular cell line (5RP7), **C2** was found to be more cytotoxic than **C1** for 5RP7 cells, except for the higher concentration levels of 8, 16 and 40 μg mL^{-1} (no data).[31] It can be concluded that a cellular growth inhibition assay of the cancer cell line (5RP7; H-*ras* active rat cells)[31] revealed that the considered compounds exert a cytotoxic effect that is in aggreement with their ability to interact with the DNA. In order to further evaluate this effect, we may also conclude that their electronic and conformational properties, crystallographic planarity,[28,31] would play an important role to set up their structure-activity relationship.

In continuation of our efforts to determine the anticancer activity of benzimidazo[1,2-a]pyrimidines, molecular modelling and biochemical studies regarding to their NCI screening (National Cancer Institute, Bethesda, MD) results are in proggress.

Conclusions

There have been few data concerning the evaluation of electrochemical sensing of the interaction between novel potential DNA targetted compounds, anticancer compounds or any drug candidates and DNA obtained in their IC_{50} values.[7] The utility of the electrochemical recognition of the interaction of potential DNA targeted compounds, **C1** and **C2**, with DNA has been shown in this study by using a faster, more sensitive and less laborious electrochemical technique with the advantages of this disposable graphite sensor (pGE) technology. The success of pGE over the existing carbon electrodes is its commercial availability; also this electrode improved the reproducibility compared to other electrodes, such as a mercury-thin film electrode, a carbon paste electrode (CPE) and a screen-printed electrodes.[6,24,33,34]

The determination of interactions between DNA-targeted molecules and nucleic acids would be valuable in the design of a molecule-specific electrochemical biosensor for applications in diagnosis tests, and also in the further development of drugs for chemotherapy. The results have shown that these studies will have a vital importance for developing newly produced chemotherapeutic compounds; also, the electrochemical monitoring of drug-DNA interactions will provide the discovery of drug-DNA interaction mechanisms and provide rapid, sensitive and cost-effective detection.

Briefly, the development and further improvement of these type electrochemical techniques would constitute suitable methods for detailed analysis of the chemical structures reacting with side chains of amino acid residues in individual proteins, such as p53.

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Supporting Information

Supporting information, Table S1 and Fig. S1 are available on the web site of the journal (http://www.jsac.or.jp/analsci/).

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