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Off-line coupling of automated pipetting system with square wave voltammetry

as a tool for study of drug-DNA interaction

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ABSTRACT *Manual handling with DNA and drugs can be a source of errors, which can be eliminated only by automation of whole process of sample preparation. The aim of this work was to study interaction between DNA and ellipticine by using of off-line coupled automated pipetting system with adsorptive transfer stripping technique (AdTS) square wave voltammetry (SWV). Interaction between dsDNA and ellipticine was evident from the decrease of CA signal. Six ng of ellipticine per ml resulted in a statistically significant decrease in CA signal (the decrease was higher than the standard deviation of determinations, Student t-test). The decrease in CA signal corresponded to increasing ellipticine concentrations.*

INTRODUCTION

Malignant tumours are the leading cause of death in many countries. Little improvement in therapeutic options has been made in the last decade, requiring a need for the development of new therapies. Therefore, development of novel therapeutic strategies is one of the most important aims for clinical and analytical chemists, biologists and other specialists (1). Chemotherapeutics belong to the main therapeutic modalities. The mechanisms of action of these agents are different, but very often interfere with processes of cell proliferation. Cell proliferation can be interfered by DNA damage including intercalation of small organic and/or inorganic molecules into nuclear DNA. DNA damage of cancer cells is, thus, of great interest for the potential to treat a tumour. Ellipticine (5,11-demethyl-6H-pyridol(4,3-b)carbazole) and its derivates belong to prototype compounds acting predominantly by DNA-damaging mechanisms (2). It has been shown that this drug involves in interfering with several tumour suppressors proteins such as protein p53 (3-7) and protein p21 promoter (7). Ellipticine was electrochemically studied in early eighties last century (8-13). Now, because of its therapeutic action, this anti-tumour drug attracts again greater attention to be studied (14-18). Several chemical and biological methods have been used to investigate a mechanism of ellipticine-mediated DNA damage such as intercalation into DNA and/or formation of covalent DNA adducts and have been reviewed in (2, 19, 20). In the case of ellipticine intercalation into dsDNA, the size and shape of the ellipticine chromophore closely resemble those of a purine-pyrimidine complementary base pair, providing favourable conditions for its intercalation in double-stranded DNA. Furthermore, the polycyclic aromatic character of the molecule may, moreover, result in tight interactions with appropriately conformed hydrophobic regions in DNA. Interactions between the methyl groups of the drug and the thymine bases at the intercalation site appear important in determining the orientational preferences of the drug (21, 22). Electrochemical techniques have, however, not been used to investigate ellipticine intercalation into DNA, despite the fact that electrochemistry can be successfully used to study the interaction of DNA with biologically active molecules (23). The aim of this work was to study interaction between DNA and ellipticine by using of off-line coupled automated pipetting system with adsorptive transfer stripping technique (AdTS) square wave voltammetry (SWV).

MATERIAL AND METHODS

Chemicals and material

Ellipticine and other used chemicals were purchased from Sigma-Aldrich (Sigma Aldrich corp., USA). All chemicals of ACS purity used and parafilm were purchased from Sigma Aldrich Chemical Corp. (Sigma-Aldrich, USA). Lyophilised

polymerized DNA (Reanal, Hungary) was isolated from chicken erythrocytes (MW = 400 000 g/mol). All other used chemical in ACS purity were purchased from Sigma Aldrich. Final concentration DNA was determined spectrophotometrically (wave length 260 nm, instrument Spectronic Unicam, type Helios Beta NC9423UVB1002E, England).

Stationary electrochemical measurements

Electrochemical measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland) and 797 VA computrace (Metrohm, Switzerland) using a standard cell with three electrodes. A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was employed as the working electrode. An Ag/AgCl/3M KCl electrode served as the reference electrode. Glassy carbon electrode was used as the auxiliary electrode. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed. Square wave voltammetric (SWV) measurements were carried out in the presence of 0.2 M Na-acetate buffer pH 5.0. SWV parameters: potential step 5 mV, frequency (260 Hz on HMDE) and (50 Hz on carbon electrode) (24). The analysed samples were deoxygenated prior to measurements by purging with argon (99.999 percent), saturated with water for 120 s. All experiments were carried out at room temperature. The temperature of supporting electrolyte was maintained by the flow electrochemical cell coupled with thermostat JULABO F12/ED (Labortechnik GmbH, Germany).

Automated pipetting systems

Fully automated isolation was carried out on automated pipetting system epMotion 5075 (Eppendorf, Germany). The position of B4 is a magnetic separator (Promega). The positions of C1 and C4 can be thermostated (Epthermoadapter PCR96). The pipetting provides a robotic arm with adapters (TS50, TS300, TS1000) and Gripper (TG-T). The samples are placed in the position B3 in adapter Ep0.5/1.5/2ml. Module Reservoir is located in the position B1, where washing solutions and waste are available. The device is controlled by the ep*Motion* control panel. The tips are located in the A4 (ePtips 50), A3 (ePtips 300) and A2 (ePtips 1000) positions. PCR 96 plates are used. The resulting volumes of collected samples ranged from 10 to 30 µl depending on the procedure. The instrument is shown in Figure 1A.

Descriptive statistics

Data were processed using MICROSOFT EXCEL® (USA). Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®). Differences with $p < 0.05$ were considered significant and were determined by using of Student *t*-test, which was applied for means comparison.

RESULTS AND DISCUSSION

DNA still belongs to the most studied molecule, because there are still a lot of un-answered questions. Interaction between short DNA sequences forming loops is still not clearly understood (25, 26), however, their biological importance is high. In the area of DNA studies, there was recently discovered a regulatory nucleic acid with the ability to control the transcription (27, 28). Besides, drugs whose mechanism of action is based on the chemically modified nucleotide are commonly used for the treatment of many diseases (29). The insertion of these nucleotides into DNA and/or modification of nucleotides in intact DNA by DNA-modifying drugs result in stopping cell growth or viral replication. All of these biologically important processes are still not clearly explained. The first step in understanding of them and many other processes is to develop simple methods capable of selective and sensitive detection of changes in DNA. Electrochemical techniques, which are inexpensive, fast and accurate tool for detection of nucleic acids and their interactions with other molecules, seem to be good candidates for such purposes.

Automated pipetting system off-line coupled with AdTS SWV

Manual handling with DNA and ellipticine is a source of errors, which can be eliminated only by automation of whole process of sample preparation. Therefore, further experiment was focused on the study of ellipticine intercalation into dsDNA by using automated pipetting system off-line coupled with AdTS SWV. Using a pre-programmed procedure the same concentration and volume of genomic dsDNA (standard, 30 µl, 10 µg/ml) was pipetted into wells in microtiter plate. Subsequently various concentrations of ellipticine (30 µl) were added into wells with DNA and the mixture was incubated at 37 °C for 120 min (Figure 1A). During incubation, samples for monitoring ellipticine intercalation into dsDNA were taken. Detection was performed by using AdTS SWV. Briefly, genomic dsDNA (5 µl) is adsorbed onto surface of HMDE for 240 s. Then, the electrode was washed and transferred to pure supporting electrolyte. Typical square wave voltammograms of DNA and ellipticine-DNA (5 min. long interaction) are shown in Figure 1B. Interaction between dsDNA and ellipticine is evident from the decrease of CA signal.

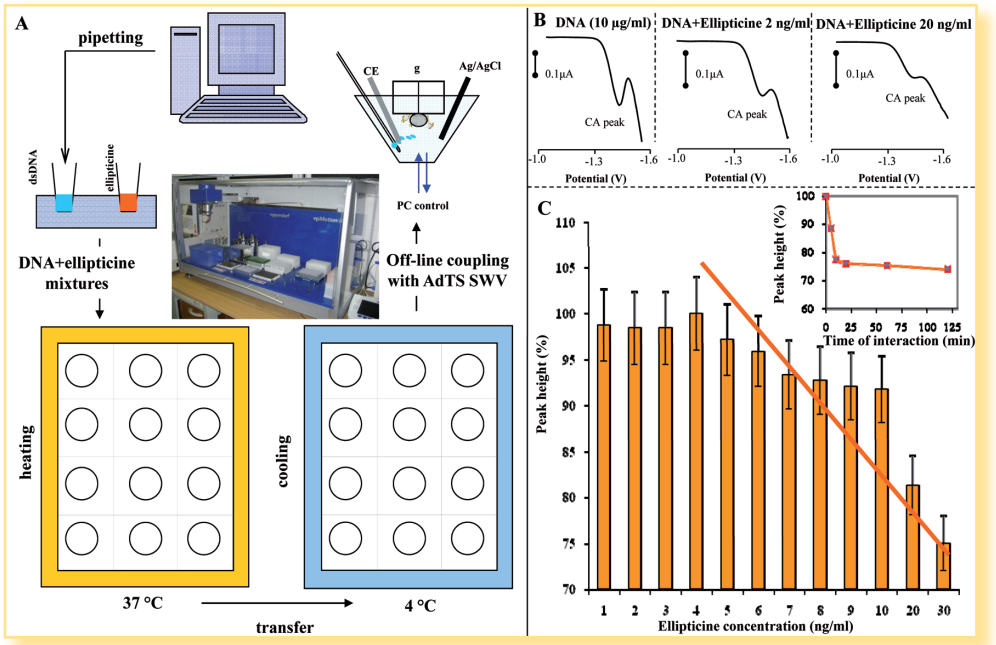

Figure 1. (A) Simplified scheme of automated preparation of dsDNA-ellipticine mixtures. dsDNA and ellipticine were pipetted using automated system into wells of microtiter plate (37 °C). During incubation, samples for monitoring ellipticine intercalation into dsDNA were taken and pipitted into wells of cooled microtiter plate (4 °C). Inset: photo of the Automated pipetting System (Ependorf) (B) Typical square wave voltammograms of DNA and ellipticine-DNA (5 min. long interaction). (C) Changes in cytosine/adenine signal of dsDNA with increasing concentration of ellipticine (incubation: 20 min.); in inset: the influence of increasing time of incubation (5, 10, 20, 60 and 120 min) on cytosine/adenine signal (dsDNA 30 µl, 10 µg/ml; ellipticine 30 µl, 20 ng/ml).

Moreover, CA signal slightly shifted to more negative potential. A new signal corresponded to intercalated ellipticine at -0.7 V was detected. In addition, we investigated changes in CA with increasing time of incubation. Ellipticine (30 µl, 20 ng/ml) was incubated with dsDNA (30 µl, 10 µg/ml) and, in the certain time intervals (5, 10, 20, 60 and 120 min), samples were taken and analysed. It clearly follows from the results obtained that CA signal decreased with increasing time of incubation rapidly to 20 min., then the decrease was much more slower (Figure 1C). This phenomenon can be associated with saturation of dsDNA chain with ellipticine molecules, which inhibit binding of the other ones. The effect of increasing concentration of ellitpicine on CA signal of dsDNA is shown in Figure 1C. Both substances were incubated for 30 min. and, the mixture was sampled in the certain time intervals and analysed. The first considerable decrease in CA signal was determined after five minutes long incubation. The signal steadily decreased up to 7th minute. The decrease was slower within time interval from 8 to 10 minutes compared to previous values. This phenomenon can be associated with conformation changes in dsDNA due to previously bounded ellipticine, which cause lowering of ellipticine binding rate into dsDNA. The steadily decrease in CA signal was determined both in 20th and 30th minute of incubation (Figure 1C). Moreover, we calculated the amount of ellipticine bounded into dsDNA and found that six ng of ellipticine per ml resulted in a statistically significant decrease in CA signal (the decrease was higher than the standard deviation of determinations, Student *t*-test). The decrease in CA signal corresponded to increasing ellipticine concentrations.

CONCLUSION

An interaction of drugs with biologically active molecules including DNA is still of great interest. Due to enhancing demands on all aspects of such studies, cooperating between various branches and looking for new techniques for such purposes is needed. In this study, off-line coupling of automated sample preparation system with electrochemical method (adsorptive transfer stripping technique square wave voltammetry) is shown to be suitable for intercalation experiments. The main improvement of our automatic method over the existing ones lies in the more accurate and precise sample preparation followed by sensitive electrochemical detection. This method, thus, represents versatile tool for anticancer drug-DNA studies.

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