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Redox mechanism of anticancer drug idarubicin and in-situ evaluation of interaction with DNA using an electrochemical biosensor

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ABSTRACT

Idarubicin (IDA), 4-demethoxydaunorubicin, is an anthracycline derivative and widely used treatment of leukemia. The electrochemical behavior of IDA was examined at a glassy carbon electrode (GCE) in different aqueous supporting electrolyte using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The oxidation process of IDA was found to be pH dependent and irreversible proceeding with a transfer of 1 proton and 1 electron under the diffusion controlled mechanism. The electroactive center is the hydroxyl group on the aromatic ring which produces a final quinonic product. The diffusion coefficient of IDA was calculated to be $D_{IDA} = 7.47 \times 10^{-6}$ cm^2 s^{-1} in pH = 4.3 0.1 M acetate buffer.

The interaction of IDA and double stranded deoxyribonucleic acid (ds-DNA) was investigated using electrochemical ds-DNA biosensor and incubation solution by means of DPV. The DNA damage was detected following the changes in the oxidation peaks of guanosine and adenosine residues. The results obtained showed that IDA interacts with DNA which causes the change in the DNA morphological structure. In addition to these polynucleotides, PolyG and PolyA, biosensors were also used to confirm the interaction between ds-DNA and IDA. However, no oxidation peaks of the purine base oxidation products, 8-oxoGua and 2,8-oxoAde, were observed.

1. Introduction

Idarubicin (IDA), 4-demethoxydaunorubicin, is an anthracycline derivative and widely used treatment of leukemia. It is a synthetic analogue of daunorubicin, Scheme 1. The absence of a methoxy group at position 4 of the anthracycline structure gives the high lipophilic character, therefore it is used orally. It binds in a non-covalent interaction to deoxyribonucleic acid (DNA), inhibits nucleic acid synthesis and interacts with the enzyme topoisomerase II [1,2].

Very few methods appear in the literature for the determination of IDA and its metabolites using high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in bulk solutions, plasma, saliva, and urine with amperometric [3], UV [4], fluorescence [5–7], and mass spectrometry (MS) [8,9] detectors. In addition to these studies, only one voltammetric technique was used to investigate the electrochemical characterization of IDA using a multiwalled carbon nanotube modified glassy carbon electrode and pyrolytic graphite electrodes [10]. The modified electrodes were constructed for the determination of IDA in pharmaceutical dosage form using adsorptive stripping DPV.

DNA plays a major role in the life process because it carries heritage information and is responsible for the replication and transcription of genetic information in living cells. DNA is a cellular target for smaller molecules like drugs, metals, and carcinogens. Studies on binding mechanism are of great help to understand the mutation of genes and the origin of some diseases. Moreover the investigation of drug–DNA interaction is important for designing and monitoring new DNA-target drugs. There are two well characterized binding modes for small molecules with DNA: covalent and non-covalent. Non-covalent interaction includes groove binding and intercalation which are the most common modes to bind directly and selectively to DNA [11,12].

The interaction of DNA with drugs is among the important aspects of biological studies in drug discovery and pharmaceutical development. Various methods have been applied for the characterization of the interaction of drug with DNA such as UV–vis spectroscopy [13], IR and Raman spectroscopy [14], molecular modeling techniques [15], circular dichroism [16], fluorescence spectroscopy [17], capillary electrophoresis [18], and HPLC [19]. However, these methods need time, cost and more chemicals and also some of them have low sensitivity.

At the beginning of the sixties after discovery of electroactivty in nucleic acids, there has been a growing interest in the electrochemical investigation of interaction between anticancer drugs and DNA. The electrochemical techniques allow to evaluate and predict interactions and damage caused to DNA. These methods are suitable for investigation of drug–DNA interaction due to their advantages such as high sensitivity, fast response time, and low cost. In these techniques, DNA-electrochemical biosensors are one of the most important groups. Biosensors are small devices, which utilize biological reactions for detecting target analytes. Electrochemical DNA biosensors comprise a nucleic acid recognition layer, which is immobilized over an electrochemical

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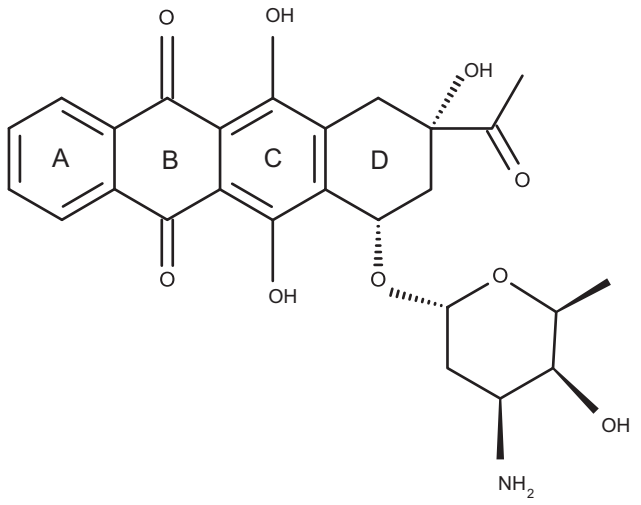

Scheme 1. Structural formula of idarubicin.

transducer. Different immobilization procedures are used for electrode surface modification such as formation of mono- or multi-layer DNA film, electrostatic adsorption, and evaporation. The investigations of interactions are based on the differences in the electrochemical behavior of DNA such as the decreases/increases of the peak currents and the shifts of the potentials related to guanine and adenine. These DNA probes using differential pulse voltammetry have great sensitivity for detecting small changes of DNA structure and have been successfully used to identify the interaction of anticancer drug with DNA [20].

In order to better understand the interaction, the other method used is the incubation procedure. For investigation of interaction in solution, drug and DNA are placed in the same solution and the changes in the electrochemical signals of drug or DNA are compared with the signals obtained with DNA or drug alone in the solution.

In previous studies, interaction of idarubicin and DNA was identified by electrochemical and spectroscopic techniques. These studies are based on the changes of spectra or only cyclic voltammograms before and after interaction. [21,22]. The electrochemical part of these studies is based on the following changes in the oxidation peak of IDA in the absence and presence of DNA.

In this study, the electrochemical oxidation of IDA and the mechanism of interaction of IDA with ds-DNA and polyhomonucleotides, poly[G] and poly[A], were carried out using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at a glassy carbon electrode (GCE). The results indicated that the intercalation played a predominant role in the interaction of IDA with DNA.

2. Experimental

2.1. Materials and reagents

Idarubicin (IDA) was kindly supplied by Mustafa Nevzat (Istanbul, Turkey). Double stranded (ds-DNA), polyadenylic acid (Poly[A]) and polyguanilic acid (Poly[G]) were obtained from Sigma and used without further purification. A stock solution of 1 mM IDA was prepared in deionized water and stored at 5 °C. Solutions of different concentrations of IDA were prepared by dilution of the appropriate quantity in supporting electrolyte. Stock solutions of 138 μg mL^{-1} ds-DNA, 83.2 μg mL^{-1} Poly[G], and 136.4 μg mL^{-1} Poly[A] were prepared in deionized water and diluted to the desired concentrations in 0.1 M pH = 4.5 acetate buffer. After dissolving the DNA fibers in deionized water, the purity of this solution was checked from the absorbance ratio A_{260}/A_{280}. The ratio of absorbance for the ds-DNA solution at 260–280 nm provides an estimate of the purity of DNA with respect to contaminant that absorbs in UV. DNA samples purified from biological sources such as tissue or cells have proteins as contaminants. Proteins absorb more strongly around 280 nm due to the absorbance of tryptophan with a lesser contribution from tyrosine, which decrease the ratio if they are present in a ds-DNA. A value of A_{260}/A_{280} ratio between 1.8 and 1.9 indicates that the protein concentration is negligible and no further deproteinization is required. The ratio of absorbance from ds-DNA solution was found to be 1.8, which indicated that the ds-DNA was free from any contamination.

Acetate (0.1 M, pH = 4.5 and 0.2 M, pH 3–5), phosphate (0.2 M, pH 6–8), borate (0.025 M, pH 8–9) buffers, HCl (0.2 M), and NaOH (0.2 M) were used as supporting electrolytes. All supporting electrolyte solutions were prepared using analytical grade reagents and deionized water.

2.2. Apparatus

Voltammetric experiments were carried out using a Gamry potentiostat (Model Reference 600, USA) with a three-electrode cell. A three electrode system was used, including a glassy carbon working electrode (GCE, d = 1.6 mm, BAS) (unmodified or modified), the Pt wire counter electrode and the Ag/AgCl (3 M KCl, BAS) reference electrode. The pH measurements were done by using a combined pH electrode with an Orion model 720 A pH meter. A Bondelin Sonorex RK 100H-type sonicator was used throughout this study. All experiments were done at room temperature.

The experimental conditions for DPV were: pulse amplitude of 50 mV, pulse width of 70 ms and scan rate of 5 mV s^{-1}.

Before each measurement the GCE was polished mechanically using the Al_2O_3 slurry. After polishing, it was rinsed thoroughly with deionized water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

2.3. Incubation procedure and DNA-biosensor preparation

IDA and ds-DNA interaction was identified in two ways; ds-DNA biosensor and incubation procedure. Before modification, a working electrode was mechanically polished with slurry of alumina powder. The thin layer ds-DNA-modified electrode was prepared depositing three drops of 5 μL each containing 50 μg mL^{-1} ds-DNA on the GCE surface. After each dropping step, the biosensor was allowed to dry. The prepared ds-DNA-electrochemical biosensor was incubated in 100 μM IDA solution during different times. Then, an electrode was rinsed carefully with deionized water to remove unbound IDA molecules from the electrode surface and transferred to a fresh supporting electrolyte. After DP voltammograms were recorded, the ds-DNA film was removed from the electrode surface. A new biosensor was prepared for each experiment. Poly[G] and Poly[A]-electrochemical biosensors were prepared from a 25 μg mL^{-1} solution, using the same procedure. pH = 4.5 0.1 M acetate buffer was used as a supporting electrolyte for all procedures.

In the incubation procedure, 100 μg mL^{-1} ds-DNA was mixed with 2 μM IDA in pH = 4.5 0.1 M acetate buffer, and 100 μg mL^{-1} Poly[G] or Poly[A] was mixed with 5 μM IDA in pH = 4.5 0.1 M acetate buffer, and then incubated at room temperature during different time periods. Control solutions of IDA, ds-DNA, Poly[G] and Poly[A] were also prepared in pH = 4.5 0.1 M acetate buffer and stored during the same time periods and in similar conditions as the IDA–ds-DNA, IDA–Poly[G] and IDA–Poly[A] incubated solutions. Systematic studies to clarify the interaction mechanism of IDA with ds-DNA, Poly[A] and Poly[G] were carried out at the modified GCE using DPV that enables the rapid detection of minor changes in the ds-DNA morphological structure and of DNA oxidative damage, because of its high sensitivity and selectivity.