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Interaction of anticancer drug methotrexate with DNA analyzed by electrochemical and spectroscopic methods

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ABSTRACT

Electrochemical DNA biosensor was used to study the interaction of methotrexate (MTX) with DNA immobilized on the bare surface of glassy carbon electrode (GCE). The binding mechanism of MTX with DNA was elucidated by using constant current potentiometric technique further supported by UV–Visible and FT-IR studies. The decrease in guanine peak area was used as an analytical signal for the interaction of drug with DNA in acetate buffer solution at pH 4.2 (20% ethanol). The binding constant (K) value calculated for MTX was $3.821 \times 10^5\ M^{-1}$. UV–Visible studies indicated hyperchromic and hypsochromic shifts in the maximum absorption bands of MTX after interaction with DNA. FT-IR investigations of MTX–DNA interaction revealed significant changes in the characteristic IR absorption bands of all the bases and phosphate groups of DNA. Furthermore, the shift of characteristics bands of C=O, N–H, C–H and O–H groups of MTX endow evidence for the interaction of MTX with DNA supporting the intercalative binding between them.

1. Introduction

There are several types of interactions associated with drugs that bind DNA. These include: intercalation, non-covalent groove binding, covalent binding/cross linking, DNA cleaving and nucleoside-analog incorporation. These binding interactions involve changes to both the DNA and drug molecules due to complex formation. Side groups of drug molecules can make additional contacts with edges of base pairs or sugar-phosphate backbone in major or minor groove or at the surface-phosphate backbone, or at the surface of the DNA double-helix either by hydrogen bonding or electrostatic interactions.

Nuclear magnetic resonance (NMR), mass spectroscopy (MS), FT-IR and Raman spectroscopy, molecular modeling techniques, equilibrium dialysis, electric linear dichroism, capillary electrophoresis, surface plasmon resonance, DNA foot printing etc. are different techniques that have been used to study the drug–DNA interactions. Electrochemical methods have witnessed wide applications that can be applied not only for fundamental studies but also in the practical applications due to their high selectivity, rapidness and low cost instrumentation (Rauf et al., 2005). Different electrochemical techniques being used to study drug–DNA interaction are cyclic voltammetry (CV), anodic stripping voltammetry (ASV), chronopotentiometric stripping analysis (CSA) and potentiometric stripping analysis (PSA; Ozkan, 2009).

The interaction of sildenafil citrate (Viagra) and ciprofloxacin with DNA was studied using an electrochemical DNA biosensor by Nawaz et al. (2006) and Rauf et al. (2007). PSA and differential pulse voltammetry (DPV) at DNA-modified glassy carbon electrode was used to elucidate the binding mechanism of these drugs. The decrease in the guanine oxidation peak area or peak current was used as an indicator for the interaction in 0.2 M acetate buffer (pH 5).

Methotrexate (MTX), 2, 4-diamine-N10-methylpteroyl-glutamic acid)), is a folic acid analog with an amino group substituted for the hydroxyl group at the C4 position on the pyridine ring, which converts the molecule to a tight-binding inhibitor of the enzyme dehydrofolate reductase (DHFR). MTX prevents cancer cells to sustain purine and pyrimidine synthesis (Chow and Rubin, 1998). The affinity of MTX for DHFR is about one thousand-fold to that of folate, thereby, MTX inhibits the synthesis of DNA, RNA, thymidylates and proteins. MTX is used in the clinical treatment of various human neoplastic disorders i.e., childhood acute leukemia (Farber et al., 1948; Saxena et al., 2009), head and neck cancer (Levitt et al., 1973), and micro metastases of osteosarcoma (Frei et al., 1975), whereas its polyethylene esters have been used as drug delivery system (Yousefi et al., 2010).

Many mutagens including methotrexate fit into the space between two adjacent base pairs, this is called intercalating. Most intercalators are aromatic and planar molecules included ethidium,

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daunomycin, doxorubicin, thalidomide, etc. In order for an intercalator to fit between base pairs, the bases must separate, distorting the DNA strands by unwinding of the double helix. This inhibits both transcription and DNA replication, causing toxicity and mutations. DNA intercalators are often carcinogens, and other molecules like benzopyrene diol epoxide, acridnes, and aflatoxin and ethidium bromide being well known examples.

Interaction of methotrexate with DNA has been studied using different electrochemical techniques. The anodic voltammetric behavior of methotrexate at GCE in acetate buffer (pH=3.6) solution using cyclic, square-wave voltammetric and chronocoulometric techniques was investigated by Gao et al. (2007), who reported oxidation of methotrexate was an irreversible diffusion-controlled process and the method was applied for the determination of methotrexate in diluted human urine successfully. Interaction of dsDNA with the combination of methotrexate and emodin showed these drugs forming intermolecular hydrogen bonding that enhanced the anticancer activity of methotrexate (Zhou et al., 2009). Moreover, methotrexate can be used to discriminate between dsDNA and ssDNA due to its different interaction with these molecules. Wang et al. (2009) explored the adsorptive voltammetric behaviors of methotrexate on DNA-modified electrode using cyclic voltammetry (CV) and square wave voltammetry (SWV) and observed that currents measured by SWV presented a good linear function of the concentrations of methotrexate with LOD of 5.0×10^{-9} mol L^{-1}. This method was successfully applied for the determination of methotrexate in pharmaceutical dosage and diluted human urine. Resonance light scattering (RLS), UV–Vis, fluorescence and NMR spectra have been used to study the interaction of methotrexate (MTX) with nucleic acids in aqueous solution in the presence of cetyl trimethyl ammonium bromide (CTMAB) in Briton–Robinson (BR) buffer at pH 9.30 by Cai et al. (2009).

This is the first report for methotrexate interaction with dsDNA using potentiometric stripping analysis (constant current) supported by UV–Visible and FT-IR spectra.

2. Materials and methods

2.1. Apparatus

The electrochemical DNA biosensor consisted of a glassy carbon electrode (Metrohm 6.1204.110, diameter=2 mm), an Ag/AgCl reference electrode (Metrohm 6.0733.100) and a glassy carbon rod (as counter electrode). The analytical signals of the oxidation of guanine were obtained by using CCPS technique with an Auto lab PGSTAT 12 (Eco Chemie, The Netherlands) and the GPES 4.9 software. The moving average baseline correction (peak width=0.001) was applied during data treatment which was smoothed by the Switzky and Golay method (level 4). The UV–Visible spectra were recorded by using a T80 UV–Vis spectrometer (PG Instruments Limited). FT-IR spectroscopy was performed using BX-FT-IR system (Perkin Elmer).

2.2. Reagents and preparation of stock solutions

Salmon sperm DNA (D1626), acetic acid (A6283) and sodium acetate (S653) were obtained from Sigma. Methotrexate tablets (2.5 mg) were purchased from Werrick Pharmaceuticals, Islamabad-Pakistan. Stock solution of different DNA concentrations were prepared by dissolving 2, 4, 6, 8, 10 and 12 mg of Salmon sperm DNA (D1626) in 1 ml of double distilled deionized water. The solution was stirred for 2 h till complete solubility of DNA.

Stock solution of methotrexate (2.5×10^{-5} M) was prepared in 0.2 M NaOH by stirring for 30 min. freshly prepared stock solutions were used during all experiments. Sodium acetate buffer solution 0.16 M HAc–NaAc (20% ethanol, pH 4.2) was used as supporting electrolyte

2.3. Electrode Pretreatment and immobilization of DNA

Before DNA immobilization, glassy carbon electrode (GCE) was polished with Alumina cleaning kit (Metrohm 62.80.2000) to obtain a mirror-like surface. The alumina was removed from the surface of the electrode by sonication, thoroughly washed by deionized water, and dried. For immobilization of DNA on the tip of GCE, it was inverted upside and Salmon sperm DNA solution was dropped on the electrode surface tip with the help of a micropipette. It was allowed to dry for 40 min at room temperature. Immobilization time was kept constant in all experiments. Potentiometric stripping conditions used were equilibration time, 60 s; constant current, +8 μA; and potential range, from 0 to +1.2 V. The amount of DNA immobilized on electrode tip was estimated by plotting the guanine peak area versus DNA concentration.

2.4. Interaction of MTX with DNA

DNA electrode was immersed into methotrexate solution in 0.16 M acetate buffer ($c_{HAc-NaAc}$) at pH 4.2 at open circuit for selected times. The electrode was rinsed after the accumulation of MTX and placed in MTX-free acetate buffer solution and constant current potentiometric voltammogram was recorded.

3. Results and discussion

3.1. Optimization of immobilization of DNA

Optimization of immobilization of dsDNA on the glassy carbon electrode (GCE) was carried out by immobilizing 2 μL of DNA from solutions of different concentrations (2, 4, 6, 8, 10 and 12 mg/ml). The dt/dE signal was plotted against voltage (V). The guanine base in the DNA was oxidized at +0.89 V that gradually shifted to +0.86 V with increase in initial concentration of immobilized DNA. The guanine oxidation peak area was determined and attributed to the concentration of DNA immobilized on the electrode surface. The guanine oxidation peaks showed a gradual increase in peak area with increasing DNA concentrations onto the glassy carbon electrode from (a–e in Fig. 1) and then leveled off (Fig. 1f) ensuring maximum surface coverage of GCE.

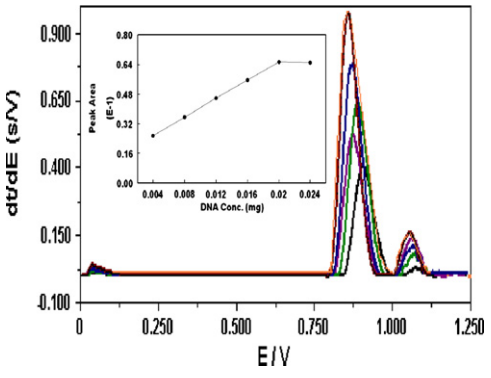

Fig. 1. Optimization of DNA immobilization on the glassy carbon electrode by using constant current potentiometric stripping analysis. Inset: plot of the different DNA concentrations against the guanine peak area obtained by immobilizing the corresponding DNA concentrations on glassy carbon electrode.