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

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Abstract

Cyclic voltammetry coupled with different spectroscopic (UV/Vis, fluorescence and Raman) techniques were used to study the interaction of mitoxantrone (MTX), an antitumor drug, with calf thymus DNA in acetate buffer solutions (pH 4.5). The interaction of MTX with DNA could result a considerable decrease in the MTX peak currents and a hypochromic and bathochromic shift in the maximum adsorption bands of MTX as well as the emission quenching in the MTX fluorescence spectra. The variations in the electrochemical and spectral characteristics of MTX indicated MTX bind to DNA by an intercalative mode. This conclusion was reinforced by Raman data. The merely particular vibrations were affected in Raman, suggesting that only a portion of the chromophore of MTX was involved in the intercalation into DNA duplex. These studies are valuable for a better understanding the detailed mode of MTX–DNA interaction, which should be important in deeper insight into the therapeutic efficacy of MTX and design of new DNA targeted drug.

Keywords: Mitoxantrone; DNA; Interaction; Electrochemistry; Spectroscopic methods

1. Introduction

In the last decades, much attention was paid to the binding of small molecules with the DNA, as a result of decided advantages of these molecules as potential drugs. Many natural or synthetic drugs serve as analogues in the research of protein-nucleic acid recognition and provide site-specific reagents for molecular biology. Therefore, the investigation of drug–DNA interaction is important for understanding the molecular mechanisms of the drug action and designing specific DNA-targeted drug [1]. Since the concept of intercalation into DNA was first formulated by Lerman [2] in 1961, it has become widely recognized that many compounds of pharmacological interest, including anticancer drugs and antibiotics correlate their biological and therapeutic activities with the ability of intercalative interaction with DNA [3]. This noncovalent binding has an important function in life phenomena at the molecular level, deciding the interaction specificity of drug with DNA.

The anthracyclines was one of the widely studied types of such drugs [4] owing to their notably clinical efficacy against a wide range of malignancies [5]. As a member of the anthracycline antibiotic, mitoxantrone (MTX) was regarded as the most promising of anticancer drugs. It has major clinical value in the treatment of several leukemia as well as ovarian and breast cancer [6]. Widespread interest in the agent MTX has arisen because of its apparent lower risk of cardiotoxic effects compared with the naturally occurring anthracycline doxorubicin and daunorubicin [7]. The structure of MTX is given in Fig. 1. It has a planar anthraquinone ring intercalating between DNA base pairs and the nitrogen-containing side chain electrostatic binding the negatively charged phosphate backbone of DNA [8]. The molecular mechanism of action of MTX is complex, for example, free radicals generation [9] and the induction of

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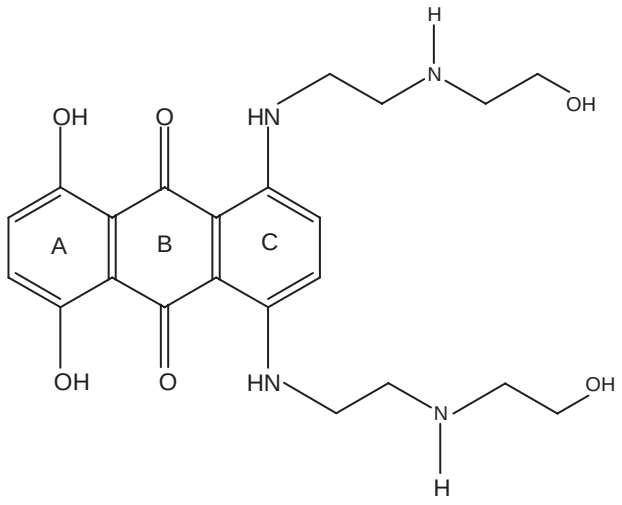

Fig. 1. Structure of mitoxantrone.

long-term DNA damage [10,11] as well as intercalative binding [12]. Some investigators have reported G–C base pair specificity [13], while others argued no such high degree of sequence specificity [14]. However, the ability of MTX intercalative interaction with DNA has been proved to correlate with antitumor properties [15,16].

The interaction of MTX–DNA has been examined by various biochemical and physicochemical methods involving DNA foot printing assay [17], molecule dynamics simulation [18], electrical linear dichroism [19], scanning electron microscopy [20], and so on. In recent years, electrochemical methods have gained growing interests in the investigation of DNA–drug interactions [21–23], due to its simplicity, rapidness and economy. In previous electrochemical investigation of such interactions, differential pulse and square wave voltammetry were applied to study MTX in a DNA biosensor [24]. Additionally, the electrochemical behaviors of MTX–DNA were also reported at a waxed graphite electrode [25], a Ni/GC ion implantation modified electrode [26] and a carbon paste electrode [27]. On the other hand, important information could be gained by spectroscopy in bioanalytical science. More importantly, the strong absorbance and fluorescence as well as Raman characteristics of MTX could provide a sensitive handle to study the binding of MTX to DNA.

There has not yet any report about the detection of the MTX–DNA interaction based on the electrochemical behaviors at a glass carbon electrode (GCE), and especially on the change of various spectroscopic characteristics. Accordingly, in this work, detailed investigations of the electrochemical behavior of MTX upon addition of DNA were carried out. Moreover, the changes in the electronic-absorption spectra, fluorescence emission spectra and Raman vibration spectra when MTX binding to DNA were used to study the mode of such interaction. The agreement of the various methods is quite good. Thus it can be seen, there is a mutual complement between electrochemical method and spectroscopy techniques, which can provide fruitful information about the mechanism of interaction and the conformation of adduct from different aspects.

2. Experimental

2.1. Materials

MTX and calf thymus DNA (Sigma, USA) were used without further purification. The MTX stock solution of 1×10^{-3} M was kept away from light to avoid photochemical decomposition and was diluted just before use. The concentration of DNA ($\approx 3 \times 10^{-3}$ M, base pairs) was spectroscopically determined using molar absorption coefficient of 13 200 cm^{-1} M^{-1} at 260 nm. The universal 0.3 M ionic strength buffer was prepared by mixing 0.8 M NaOH, 1.34 M KCl, and an acid solution (0.16 M acetic acid, 0.16 M phosphoric acid, and 0.16 M boric acid). If not specially stated, the supporting electrolyte was acetate buffer solution (NaAc–HAc, 0.2 M, pH 4.5). All reagents were analytical grade and aqueous solutions were prepared using doubly distilled deionized water.

2.2. Apparatus

Cyclic voltammetry studies were carried out by CHI 800 (Shanghai, China). The three-electrode system consisted of a glassy carbon working electrode, an Ag/AgCl-saturated KCl reference electrode and a platinum wire counter electrode. All potentials were referred to the reference electrode.

UV/Vis absorbance spectra were obtained by Cary 500 UV/Vis–NIR spectrophotometer (Varian, USA) equipped with a quartz micro-colorimetric vessel of 1-cm path length.

Fluorescence experiments were carried out on Spectrofluorometers of QuantaMaster Systems. A quartz cuvette of 1 cm was used. The fluorescence titrations were performed by keeping the concentration of the drug constant while varying the concentration of DNA. Continuous stirring was made throughout the course of the titration.

Raman spectra were recorded with Jobin Yvon, model T64000 Raman spectrometer, supplied with a multichannel detector. The argon laser was used for excitation (514.5 nm).

3. Results and discussion

3.1. Electrochemical confirmation of the interaction of MTX with DNA

For the electrochemical oxidations of MTX in the universal buffer with a wide range of pH (1.0–13.0), it was observed that the peak potentials shifted to negative direction and the peak currents diminished with increasing pH (figure not shown). In order to obtain better detecting

sensitivity, the NaAc–HAc buffer (pH 4.5) was chosen as the supporting electrolyte.

The cyclic voltammogram of MTX in NaAc–HAc solutions (pH 4.5) clearly showed that the electrochemical oxidation of MTX at a GCE was an irreversible process (in the insert of Fig. 2). The first oxidation peak at +0.53V was corresponded to the two-electron oxidation process of 5,8-hydroxyl substituents on MTX. The second peak at +0.78 V was attributed to the oxidation of the aminoalkyl substituents after tautomeric structural rearrangements [28]. Thus, the electrochemical oxidation mechanism of MTX was a multi-step process [29].

The linear sweep voltammetric behavior of 2.5×10^{-5} M MTX in the absence and presence of DNA was illustrated in Fig. 2. In the presence of DNA, both peak currents of MTX decreased considerably due to the decreasing of equilibrium concentration of MTX, which is in good agreement with the report of Erdem [27] regarding the MTX–DNA interaction at carbon paste electrodes (CPE). Besides, Bard and co-workers [30] reported that positive shifts in the peak potential of intercalators were observed in the binding form via hydrophobic interactions (intercalation) while electrostatic interactions led to negative shifts. Based on this report, the positive shifts in the peak potential of MTX upon binding to DNA should be as a result of specific intercalation to DNA. In addition, the effect of scan rate (v) on peak current (i_p) of MTX was tested before and after its interaction with DNA (Fig. 3). Both peak currents of MTX and MTX–DNA adduct were linearly dependent on the square root of the scan rate, suggesting that the oxidation process was controlled by diffusion of the electroactive species to the electrode surface [31]. Furthermore, the smaller linear slop of MTX–DNA complex demonstrated that MTX could intercalate with DNA in solution, forming MTX–DNA adduct with large molecular weight, resulting in a considerable decrease in the apparent diffusion coefficient [25].

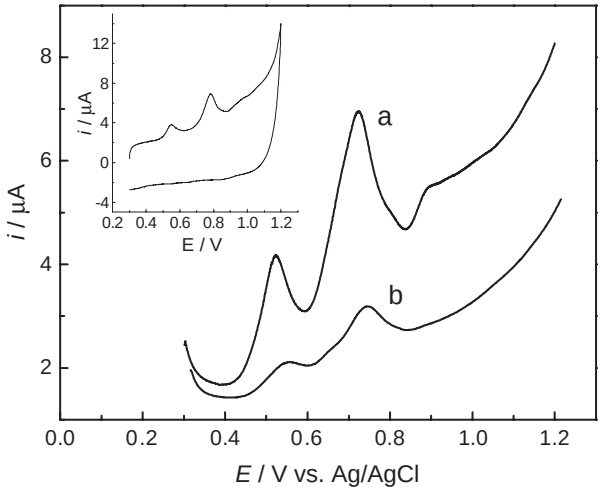

Fig. 2. Linear sweep voltammograms of NaAc–HAc buffer solution (0.2 M, pH 4.5) containing 2.5×10^{-5} M MTX in the absence (a) and presence (b) of 1.0×10^{-4} M DNA. Inset: cyclic voltammograms of MTX in the same condition. Scan rate: 100 mV/s.

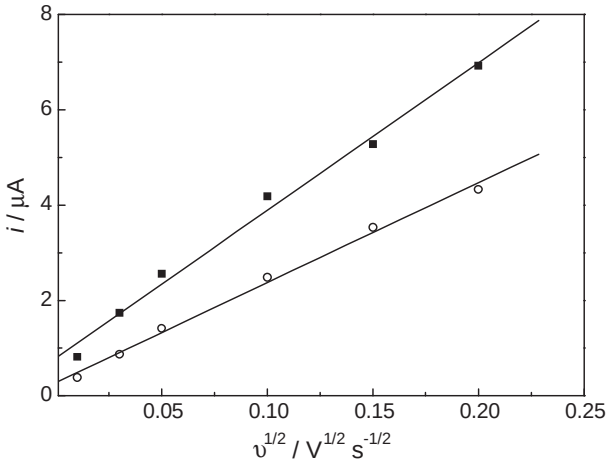

Fig. 3. Relationship between the peak currents of the first oxidation wave of MTX in the absence (■) and presence of (○) DNA and the scan rates.

3.2. Spectroscopic confirmation of the interaction of MTX with DNA

3.2.1. UV/Vis spectra

Fig. 4 showed the UV/Vis absorption spectra of MTX in the absence and presence of different concentrations of DNA. The maximum absorbance of MTX was located around at 608 and 660 nm. It is well established that the absorption bands of the anthraquinone chromophore, usually in the visible region, are attributed to substitution of the anthraquinone ring by electron-donating substituents such as amino and hydroxyl groups [32]. In MTX molecular, these types of hydroxyl and amino groups are both present, so, the above-mentioned maximum absorbance bands were attributed to the charge transition from the hydroxyl and amino substituents on the anthraquinone ring to the ring itself, respectively. It was observed that a continuous decrease in the absorbance of MTX was followed by the gradually increasing concentration of

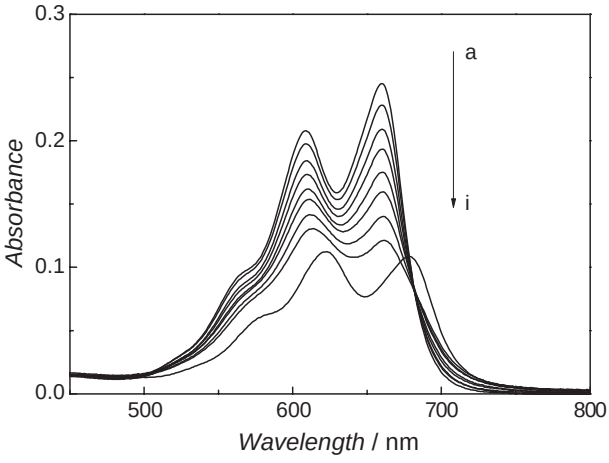

Fig. 4. UV/Vis absorption spectra of NaAc–HAc buffer solution (0.2 M, pH 4.5) containing 1.0×10^{-5} M MTX in the presence of DNA (μM): (a) 0.00; (b) 10.00; (c) 20.00; (d) 30.00, (e) 40.00; (f) 50.00; (g) 60.00; (h) 70.00; (i) 80.00.

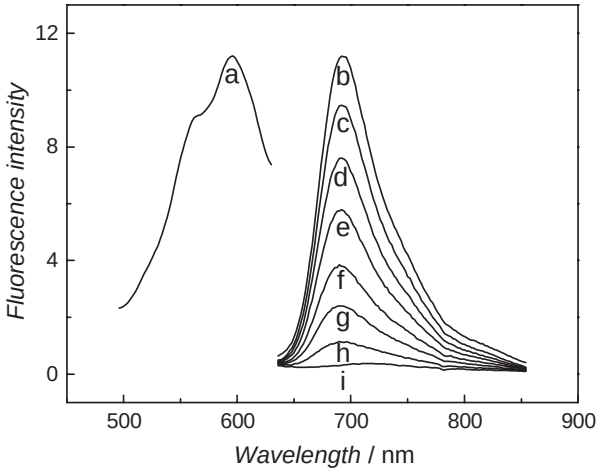

Fig. 5. (a) Fluorescence emission and excitation spectra of 1×10^{-5} M MTX in NaAc–HAc solution (0.2 M, pH 4.5). The excitation spectra (a) of MTX were recorded with λ_{em}=688 nm; the emission spectra of MTX were recorded with λ_{ex}=596 nm in the absence (b) and presence of DNA (μM): (c) 5.00; (d) 10.00; (e) 15.00; (f) 20.00; (g) 25.00; (h) 30.00; (i) 35.00.

DNA in solution. This hypochromic effect is thought to be due to the interaction between the electronic states of the intercalating chromophore and those of the DNA bases [33]. It is expected that the strength of this electronic interaction would decrease as the cube of the distance of separation between the chromophore and the DNA bases [34]. So, the obviously large hypochromism observed in our experiments suggested the close proximity of the MTX chromophore to the DNA bases. In addition, with a high concentration of DNA, the red shift in both of two maximum absorption peaks was observed by 20 nm. This phenomenon indicated that upon binding to DNA the ring substituents on the chromophore could slide into the base pairs, resulting in that they were in an environment in which it was unable to H bond with the solvent water molecules. The MTX solution exhibited peculiar hypochromic effect and bathochromic shift in UV/Vis spectra upon binding to DNA, a typical characteristic of an intercalating mode [35].

Based on the variations in the absorbance spectra of MTX upon binding to DNA, the binding constant, K, was calculated according to the equation [36]:

$$\frac{A_0}{A-A_0}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K[DNA]}, \quad (1)$$

where A_0 and A are the absorbances of drug in the absence and presence of DNA, ε_G and ε_{H-G} are the absorption coefficients of drug and its complex with DNA, respectively. The plot of $A_0/(A-A_0)$ versus 1/[DNA] was constructed (figure not shown) using the data from the absorbance titrations and a linear fitting of the data yielded the binding constant, $K=1.15\times10^5$ M^{-1}. This indicated that MTX has a high affinity with DNA. The values of K obtained here are consistent with that reported for the interaction of anthracycline molecules with DNA ($K\approx10^4$ to 10^5 M^{-1}) [37,38].

3.2.2. Fluorescence spectra

The interaction of MTX with DNA was also examined using fluorescence titrations. The fluorescent excitation and emission spectra of MTX as well as the effect of DNA concentrations on the fluorescence emission spectra of MTX were illustrated in Fig. 5. MTX exhibited an excitation maximum at 596 nm (Fig. 5, curve a) and an emission maximum at 688 nm (Fig. 5, curve b). The fluorescence emission was gradually decreased with increasing amount of DNA, showing that the MTX fluorescence was efficiently quenched upon binding to DNA. When the concentration of DNA was up to 3.5×10^{-5} M, the fluorescence intensity of MTX was almost completely quenched, indicating the binding reached saturation. This could also be explained by lack of H bonding of the ring substituents with solvent water molecules upon binding to DNA, similar to that clarified in absorption spectra. Thus, the intercalative mechanism of MTX with DNA was further confirmed by quenches in the emission spectra of MTX upon DNA addition.

The Stern–Volmer quenching plot from the fluorescence titration data was shown in Fig. 6. The fluorescence quenching constant (K_{SV}) was evaluated using the Stern–Volmer equation [39]:

$$I_0/I=1+K_{SV}[DNA], \quad (2)$$

where I_0 and I are the fluorescence intensities in the absence and presence of DNA, respectively, K_{SV} is the Stern–Volmer quenching constant, which is a measure of the efficiency of quenching by DNA. The titration data were used to construct a plot of I_0/I versus [DNA]. K_{SV} obtained from the slop of the linear line was 7.59×10^5 M^{-1}. The Stern–Volmer plot is linear, indicating that only one type of quenching process occurs, either static or dynamic quenching [40]. The research of its anthraquinone analog showed that the fluorescence quenching was static [41].

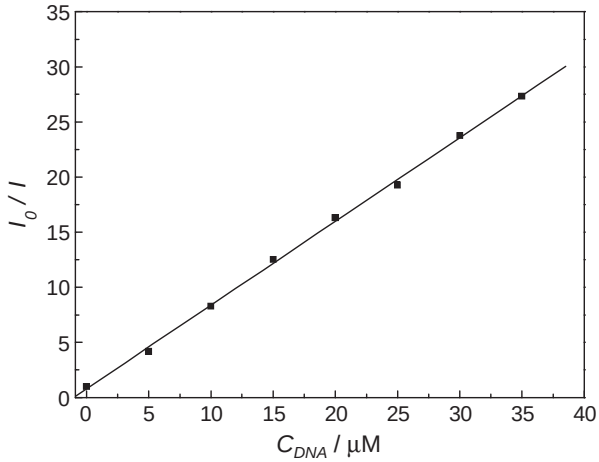

Fig. 6. A Stern–Volmer quenching plot of MTX with DNA concentrations.

3.2.3. Raman spectra

Raman spectroscopy has been used as a powerful tool to obtain information for drug chromophores and their interactions with the targets [42]. The Raman spectra of MTX and its complex in buffer solution were shown in Fig. 7. Since the Raman spectra involve only the vibrations of the anthraquinone chromophore [43], a considerable simplification in elucidation for these observed bands. The band at 1653 cm^{-1} in the spectra of free MTX was mainly assigned to the ν(C=O) mode. The presence of this band reflected the influence of the intramolecular bond C=O···H···N–C between rings B and C of the chromophores on the vibrational frequency [44]. The bands in the 1610–1400 cm^{-1} region were attributed to the ν(C–C) vibrations of the A-type symmetry in the phenolic rings A and C of the chromophore. Thereafter, we called these vibrations "ring stretches" [45]. The band at 1567 cm^{-1} was assigned to the ring stretch vibration of phenolic ring A. The assignments of the band at 1501 and 1450 cm^{-1} to the ν(C=C) motions were reasonable. The band at 1360 cm^{-1} was corresponded to the ν(C–O) motions coupling the vibration with the chelate system of the chromophore. The band at ca. 1306 cm^{-1} was the most intense one in the Raman spectra of MTX. It could be assigned to the ring stretch mode coupled with the ν(C–O) mode because it is sensitive to the existence of the C–O group in ring A of the MTX chromophore [45]. The region 1400–1200 cm^{-1} seemed to be the result of overlapping of several bands. The bands at ca. 1185 and 1115 cm^{-1} had frequency characteristics of δ(CC–H) vibration coupled with both C–O and C–N motions [46]. The skeletal deformation was occurred at 990 and 826 cm^{-1}. It maybe that the δ(CC–O) motions might contribute largely to the band at ca. 467 cm^{-1}, the other possible assignments of this band, e.g., the δ(CC=O) vibration or ring deformation was less probable. The band at 436 cm^{-1} could be assigned to the δ(CC–N) vibration because the δ(CC–N) vibration was expected to be at a lower frequency compared to the δ(CC–O) [45]. A comparison between the difference spectra of MTX and MTX–DNA complexes showed that no new bands appeared upon binding. However, the bands at 1653 cm^{-1}, 1306 cm^{-1}, 1185 cm^{-1}, and 1115 cm^{-1} that attributed to ν(C=O) mode, ν(C–O) mode, and δ(CC–H) vibrations, exhibited a lower intensity upon binding. It was different from adriamycin, where distinct vibrational shifts were observed upon interaction [47]. This hypochromism in Raman spectra would be the same reason as the red shifting of the visible band upon binding to DNA, as well as decreased excitation coefficient of the fluorescence band. On the other hand, the bands at 436 cm^{-1} resulting from the C–N vibrations were unchangeable upon formation of the complex. This revealed that the vibrations localized on ring C of the chromophore were not changed upon interaction with DNA. The experimental data presented here clearly demonstrated that the interaction with the base pairs involved the formation of intermolecular hydrogen bond with rings A and B of MTX chromophore. Importantly, the phenomenon that merely particular vibrations were affected upon binding to DNA demonstrated only a portion of the chromophore of MTX including rings A and B was involved in the intercalative base pairs. Thus, the binding mode of MTX interaction with DNA was more clearly elucidated by Raman data.

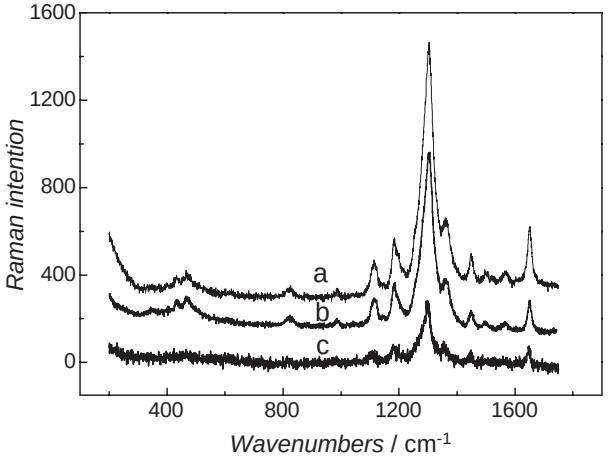

Fig. 7. Raman spectra of NaAc–HAc buffer solution (0.2 M, pH 4.5) containing 5.0×10^{-4} M MTX in the absence (a) and presence (b) of 1.0×10^{-4} M DNA, and their difference spectrum (c).

4. Conclusion

In this work, the interaction of MTX with DNA was studied by cyclic voltammetry, especially by various spectroscopic methods. The binding of MTX to DNA resulted in a series of changes in the electrochemical behavior and spectra characteristics. Upon binding to DNA, the adsorption spectra of MTX showed peculiar hypochromic effect and bathochromic shift and the fluorescence emission of MTX was efficiently quenched as well as only particular vibrations were affected. From these experimental results, it could be affirmed that the interaction of MTX with DNA through intercalative mode, and furthermore, only a portion of the chromophore of MTX including rings A and B was involved in the intercalation into DNA base pairs. Moreover, the large binding constant indicated MTX has a high affinity for the DNA base pairs and the intercalation of MTX into the DNA duplex appears to be critical for this high affinity. The electrostatic attraction between the cationic charge on MTX and the DNA phosphates is also expected to improve the DNA binding affinity. These investigations showed that electrochemistry coupled with spectroscopy techniques could provide a convenient way to characterize both the binding mode and the interaction mechanism of MTX binding to DNA, which is important for the design of new anticancer drugs.

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