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Voltammetric study of the interaction of lomefloxacin (LMF)–Mg(II) complex with DNA and its analytical application

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Abstract

The voltammetric behavior of the LMF-Mg(II) complex with DNA at a mercury electrode is reported for the first time. In NH_3–NH_4Cl buffer (pH = 9.10), the adsorption phenomena of the LMF–Mg(II) complex were observed by linear sweep voltammetry. The mechanism of the electrode reaction was found to be a reduction of LMF in the complex, and the composition of the LMF–Mg(II) complex is 2:1. In the presence of calf thymus DNA (ctDNA), the peak current of LMF–Mg(II) complex decreased considerably, and a new well-defined adsorptive reduction peak appeared at −1.63 V (vs. SCE). The electrochemical kinetic parameters and the binding number of LMF–Mg(II) with ctDNA were also obtained. Moreover, the new peak currents of LMF–Mg(II)–DNA system increased linearly correlated to the concentration of DNA in the 4.00×10^{-7}–2.60×10^{-6} g ml^{-1} range when the concentrations of LMF–Mg(II) complex was fixed at 5.00×10^{-6} mol l^{-1}, with the detection limits of 2.33×10^{-7} g ml^{-1}. An electrostatic interaction was suggested by electrochemical method.

Keywords: Voltammetry; Lomefloxacin; Lomefloxacin–Mg(II); DNA; Binding mode

1. Introduction

Nucleic acids have an important function in life processes, so study of them has become an important research field of life science. Recently, more attention has been attracted into the study of recognizing special sequence of DNA and measuring DNA damage. But, quantitative determination of nucleic acids was the base of investigation on nucleic acids. Electrochemical signals of nucleic acids are so week that the direct determination is difficult. Many antitumor or antibacterial drugs such as daunomycin [1], nogalamycin [2] are electrochemical active molecules. The study on the interaction of nucleic acids with them, has attracted considerable interest, not only for its mechanism of action, but also for its analytical application.

Adsorptive stripping votammetry has been demonstrated as a high sensitive and selective analytical method. It can achieve low detection limits and decreases the possible interferences in analysis by employing adsorptive preconcentration of the analyte with adsorption capabilities on the electrode. The high sensitive method has been used,

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in particular, for determination of protein and DNA.

Quinolones are a group of compounds widely used as broad-spectrum antibacterial agents. A large amount of biological data indicates that the functional target of there drugs is DNA gyrase, an essential type II DNA topisomerase that caltalyzes the supercoiled form [3]. In 1985, Shen et al. found that norfloxacin, one of the most potent DNA gyrase inhibitors of the quinolone family, did not bind directly to DNA gyrase but bind to DNA itself [4]. However, the report about their interaction by means of voltammetry has never been seen.

In the present paper, electrochemical studies of the interaction of LMF–Mg(II) with ctDNA at a mercury electrode are reported. When LMF–Mg(II) interacted with DNA, the peak current I_p of LMF–Mg(II) decreased, and at the same time a new reduction peak was obtained at −1.63 V. The concentrations of DNA were determined according to the new peak of LMF–Mg(II)–DNA system. The binding-number of LMF–Mg(II)–DNA interaction was also obtained by a titration of nucleotide phosphate with LMF–Mg(II). We used fluorescence and UV techniques to study the interaction of LMF–Mg(II) with DNA. We demonstrated that LMF could bind to ctDNA, fsDNA and yRNA in the presence of Mg(II) ions and focused on the binding mode of LMF–Mg(II) to DNA.

2. Experimental

2.1. Reagents

Calf thymus DNA (ctDNA), fish sperm DNA (fs DNA), yeast RNA (yRNA) were purchased from Sigma (USA). The stock solution of ctDNA, fsDNA and yRNA was prepared by directly dissolving them in quadric distilled water from an all-quartz still. Unless otherwise stated, the DNA in this paper represents ctDNA. Lomefloxacin (LMF) was obtained from Shandong Pharmaceutical Industrial Institute of China. $MgSO_4$, NH_4Cl and NH_3 H_2O are reagent grade. All solutions were prepared from quadric distilled water.

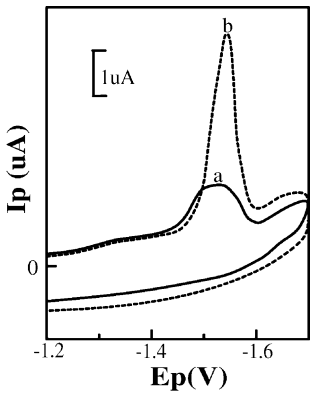


Fig. 1. Cyclic voltammograms of LMF in the presence of Mg(II); (a) 5.00×10^{-6} mol l^{-1} LMF; (b) 5.00×10^{-6} mol l^{-1} LMF mixing 1.25×10^{-5} mol l^{-1}Mg(II); 0.08 mol l^{-1} NH_3–NH_4Cl, pH 9.10; accumulation time: 60 s; scan rate100 mV s^{-1}; potential step: −1.20 to −1.70 V.

2.2. Apparatus

Cyclic voltammetric (CV), linear sweep voltammetric (LSV) experiments were carried out by a model MF-1A voltammeter (Jiangsu Electroanalysis Instrument Factory, China). The working electrode was used in a hanging mercury drop electrode (HMDE) mode. The average electrode surface area was 0.0234 cm^2, as calculated from the weight of a large number of mercury drops. An Hg/Hg_2Cl_2 electrode was used as the reference electrode, and a platinum wire as the counter electrode. The Shimadzu Model UV-240 and Hitachi-850 fluorescence instruments were used for spectrophotometric determinations.

3. Results

3.1. Electrochemical behavior of LMF–Mg(II) system

Typical CV behavior of LMF in the absence and presence of Mg(II) are shown in Fig. 1. LMF has an irreversible reduction peak at −1.53 V (Fig. 1a), but a new well-defined reduction peak (Fig. 1b) at −1.55 V is obtained when mixing Mg(II). From Fig. 2, we found that the reaction of LMF–Mg(II) is of adsorptive characters. As we know, Mg(II) ions are difficult to reduce, so

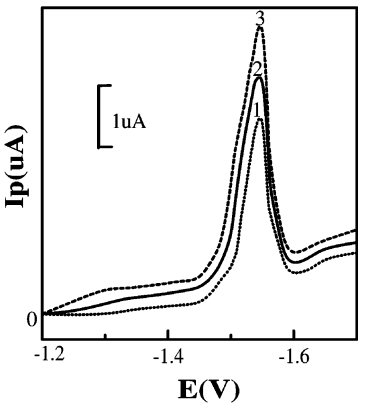


Fig. 2. Linear sweep voltammograms of 5.00×10^{-6} mol l^{-1} LMF at different accumulation time t_A: (1) 30 s; (2) 60 s; (3) 90 s, scan rate: 100 mV s^{-1}, accumulation potential: $E_A=-1.20$ V.

we concluded that LMF was reduced in the LMF–Mg(II) system. Cao et al. [5] have reported the electrochemical behavior of free LMF; it has an irreversible reduction peak and belongs to a two-electron reduction carbonyl moiety.

According to the formula given by Li [6]:$1/I_p=1/I_{p\max}+1/\{\beta_s\ I_{p\max}\ [\mathrm{LMF}]^m\}$, I_p stands for the peak current of LMF–Mg(II), $I_{p\max}$ is the peak current in case of complete metal complexing, [LMF] is the concentration of the LMF, β_s for the condition formation constant, m for the combined number. If Mg(II) and LMF form a complex mLMF-Mg(II), then $\lg(I_p/I_{p\max}-I_p)$—lg [LMF] becomes linear with the slope of m. The results of $m=2$ and $\beta_s=12$ were obtained, which means that only one compound is formed.

3.2. Electrochemical behavior of LMF–Mg(II)-DNA system

The CV Fig. 3 shows a irreversible reduction peak of LMF–Mg(II) at -1.55 V (Fig. 3b). When DNA was added, the reduction peak of LMF–Mg(II) decreased, but a new reduction peak appears at -1.63 V (Fig. 3c) which represents the formation of the complex LMF–Mg(II)–DNA. In this paper, we select 0.08 mol l^{-1} NH_3–NH_4Cl buffer (pH 9.10), $t_A=60$ s as the optimal conditions, because there is a well-defined reduction peak in solution. In 0.08 mol l^{-1} NH_3–NH_4Cl buffer, the reduction potential of LMF–Mg(II)-DNA shifts linearly in negative direction with the increase of pH from 7.70 to 9.80, $E_p(V)=-1.0152-0.096$ pH, which means the reduction of LMF–Mg(II)–DNA involves proton reaction.

Our experimental results show that the electrode reaction of LMF–Mg(II)–DNA is irreversible (see Fig. 3) reduction reaction with adsorptive characters. According to Laviron's equation [7–9]:

$$E_p=E^{0\prime}+\frac{RT}{\alpha nF}\ln\frac{RTk_s}{\alpha nF}\frac{RT}{\alpha nF}\ln\upsilon \qquad (1)$$

Where α is the transfer coefficient, k_s the standard rate constant of the surface reaction, υ the scan rate and $E^{0'}$ the formal potential. According to Eq. (1), the plot of E_p should be linear. From its slope, the αn value can be determined, and from the intercept, the k_s can be calculated, if the value of $E^{0'}$ is known. The value of $E^{0'}$ in Eq. (1) can be determined from intercept of E_p vs. υ plot on the ordinate by extrapolating the line to $\upsilon=0$. Figs. 4 and 5 are plots E_p vs. υ and E_p vs. ln υ, respectively, from slope (-0.02394), intercept (-1.68408) and $E^{0'}(-1.57)$, an$=1.03$, $k_s=0.42$ s^{-1} were obtained. Assuming $\alpha=0.50$, then $n=2.06\approx2$, so $\alpha=0.52$ is reasonable. From the CV of LMF–Mg(II)–DNA, the width of the peak at mid-height ($W_{1/2}$) is 55 mV (15 °C). For an

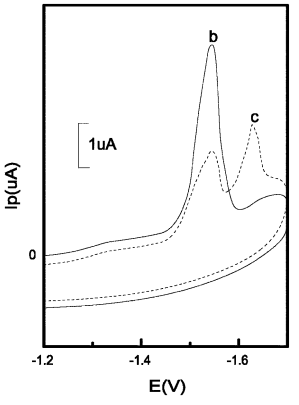


Fig. 3. Cyclic voltammogram of 5.00×10^{-6} mol l^{-1} LMF–Mg(II), (b) in the absence, and (c) presence of 1.00×10^{-6} g ml^{-1} DNA.

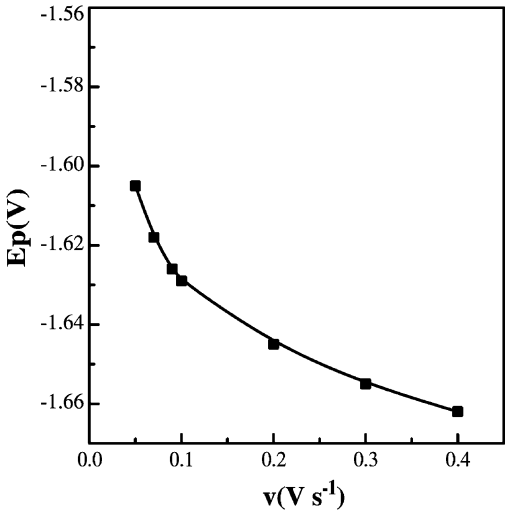


Fig. 4. Dependence of the peak potential E_p on the potential scan rate v for 5.00×10^{-6} mol l^{-1} LMF–Mg(II) mixing 6.00×10^{-7} g ml^{-1} DNA.

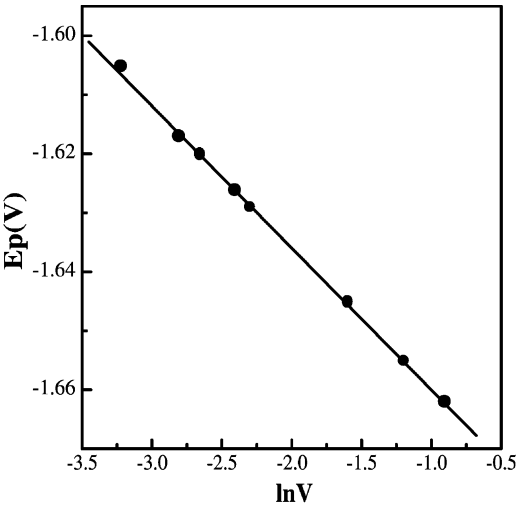


Fig. 5. Semilogarithmic dependence of the peak potential on the potential scan rate ln v for 5.00×10^{-6} mol l^{-1} LMF–Mg(II) mixing 6.00×10^{-7} g ml^{-1} DNA.

irreversible adsorption peak, $W_{1/2}$ is: $W_{1/2}=2.44\ RT/\alpha nF$ mV [10]. Thus, the estimated number of electron (n) is still 2, and α is 0.55, which is consistent with that of above experiment. Therefore, it is concluded that, no matter whether DNA is present or not, the carbonyl moiety of LMF is reduced, and the electrode reaction processes are both two-electron transfer with one-proton uptake.

3.3. Analytical application

Adding DNA in LMF–Mg(II) solution results in a new peak of LMF–Mg(II)–DNA. This can be applied to DNA concentration. When DNA was adding 5.00×10^{-6} mol l^{-1} LMF–Mg(II) solution, the I_p of LMF–Mg(II)–DNA increased linearly with DNA concentration from 4.00×10^{-7} to 2.60×10^{-6} g ml^{-1} linear regression equation $I_p=-0.389+0.0016C\times10^6$ (I_p μA, C 10^{-7} g ml^{-1}), correlation coefficient $r=0.9989$. The interference of foreign substances is tested. It is found that small amounts of various ions ($<1\times10^{-5}$ mol/l), and amino acids ($<1\times10^{-4}$ mol/l) have little effect on the determination of nucleic acids.

The standard addition method is used for the determination of synthetic samples of calf thymus DNA containing metal ions, amino acids, etc., and the results are given in Table 1. It can be seen that nucleic acid in synthetic samples can be determined with satisfactory results.

3.4. Interaction of LMF–Mg(II) with DNA

Several binding models of quinolone with various DNA have been reported. Shen et al. [11] proposed a cooperative quinolone–DNA binding model for the inhibition of DNA. In this model, norfloxacins were bound in the specific single-

Table 1
Recoveries of ct DNA from synthetic samples

Sample	DNA (μg ml^{-1})	DNA added (μg ml^{-1})	DNA found (μg ml^{-1})	R.S.D. (%)	Recovery (%)
1	0.60	1.0	1.61 1.58 1.59 1.61 1.57	1.26	99.50
2	1.00	0.5	1.52 1.47 1.49 1.51 1.54	1.85	100.4

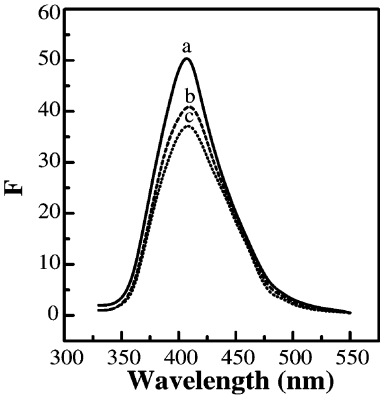


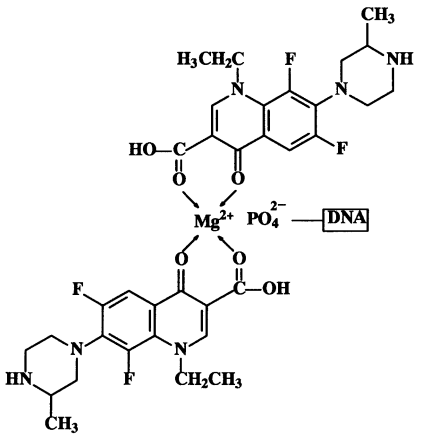
Fig. 6. Fluorescence emission spectra: (a) 5.00×10^{-6} mol l^{-1} LMF, (b) $a+6.00\times10^{-7}$ g ml^{-1} DNA, (c) $b+1.25\times10^{-5}$ mol l^{-1} Mg(II).

stranded DNA pocket which was induced by gyrase and were stabilized by the hydrogen bonding, the π−π stacking of the norfloxacin rings, and the tail-to-tail hydrophobic interactions. But, Lee et al. [12] suggest that norfloxacin binds in the minor groove of B-form DNA in a nonclassic manner. One of the more recent models also suggests that Mg(II) plays an important role in the drug binding to DNA–gyrase complex [13,14]. Palù et al. [13] suggest that Mg(II) acts as a bridge between the phosphate groups of the nucleic acid and the carbonyl and carboxyl moieties of norfloxacin, which contradicts the classical intercalation.

The electrochemical study of LMF–Mg(II) with DNA also demonstrated that the intercalation was impossible. If the drug intercalate into the base pairs of DNA, they will form electrochemically non-active complex for the steric hindrance. In our experiment, a new peak at −1.63 V was obtained, which illustrated the formation of a new binding mode. And on the fluorescence (in Fig. 6) and absorption spectra (in Fig. 7), there is hypochromism, but no band shift, so intercalation was ruled out.

In order to study the binding mode of LMF–Mg(II) with DNA, we did an extensive investigation. On the CV of LMF–Mg(II) in the presence of fsDNA or single-stranded yRNA, there is also a new reduction peak at approximately −1.63 V, and fsDNA, yRNA more easily interacted with LMF-Mg(II) than ctDNA. We investigated the binding process with increasing of the Mg(II). When Mg(II) concentration was lower than 5.00×10^{-6} mol l^{-1} or higher than 2.25×10^{-5} mol l^{-1}, there was no obvious binding, which indicated that Mg(II) played an important role in the interaction of LMF with DNA. From the above experiments, we suggest that the positively charged complex LMF–Mg(II) should be electrostatically interacted with phosphate groups [15], at the same time form stacking interactions with the bases in a single-stranded region of nucleic acid, which is in agreement with the proposal of Palù et al. [13].

Determining the decrease of c current with reference to the method of Li [6], combine number and conditional binding constants, were estimated as $m=7$, $\lg\beta_s=39.02$, which means that only one compound (I) was formed when the concentration of DNA was fixed at 5.00×10^{-6} g ml^{-1}.



(I)

The structure of the complex of DNA-[LMF-Mg(II)]

4. Conclusion

In this paper, we reported that the interaction of the LMF–Mg(II) complex with DNA and its analytical application by voltammetric methods. In NH_3–NH_4Cl buffer (pH=9.10), LMF–Mg(II) complex with DNA forms an electrochemical active complex, which reduced at −1.63 V (vs. SCE). The increase of its peak current is proportional to the concentration of DNA, and the detec-

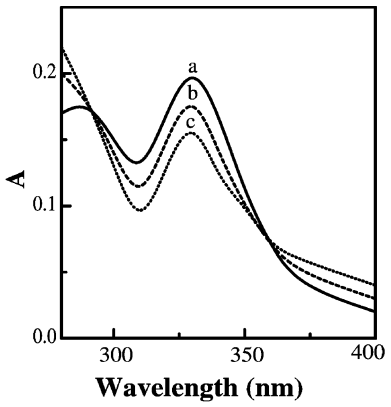


Fig. 7. UV–VIS absorption spectra: (a) 5.00×10^{-6} mol l^{-1} LMF, (b) $a+6.00\times10^{-7}$ g ml^{-1} DNA, (c) $b+1.25\times10^{-5}$ mol l^{-1} Mg(II).

tion limit is 2.33×10^{-7} g ml^{-1}. The electrostatic interaction between the LMF–Mg(II) complex and DNA was suggested by comparing the electrochemical methods with spectroscopic methods.

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