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Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 625–629
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Electrochemical studies of the interaction of the anticancer herbal drug emodin with DNA

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Received 18 February 2006; received in revised form 18 May 2006; accepted 24 May 2006
Available online 7 July 2006

Abstract

The interaction between anticancer herbal drug emodin and DNA was studied with differential pulse voltammetry (DPV) and cyclic voltammetry (CV) at the bare or DNA modified GCE and ultraviolet–visible (UV) spectra. The DPV of emodin showed that peak potentials shifted to more positive value and peak currents decreased with the addition of DNA. UV spectra exhibited that the absorption of emodin at about 440 nm decreased with red shift. The results showed that the herbal drug emodin interacted with DNA by intercalating into the double helix of DNA. Under our experiment conditions, the decrease of peak current was proportional to DNA concentration, which can be applied to determining DNA concentration. The combining constant (β) and combing number (m) of DNA–*m*EM were determined too.

Keywords: Anti-cancer herbal drug; Emodin; DNA; Modified electrode; Determination

1. Introduction

Currently, herbal medicines especially anticancer herbal drugs are gaining more attention from modern pharmaceutical institutes, as scientists become aware that herbal medicine is an almost infinite resource for drug development. Furthermore, the toxicity of the herbal drug is very low and most of them have no side-effect. The exploitation of the natural source makes the difference in the field of the medicine. Emodin (1,3,8-trihydroxy-6-methylanthraquinone; Fig. 1) is one of the active ingredients which is isolated from Chinese herbal medicine *Rheum officinale* Baill (Chinese name: Da-Huang) and has specific in vitro and in vivo anti-tumor activity [1]. This herb is commonly employed as a purgative crude herb in traditional herbal therapy [2].

The investigation based on DNA interactions with small molecular compounds has great importance to understand the reaction mechanisms of some anti-tumor drugs, origins of some diseases, design new DNA-targeted drugs and screen these drugs in vitro. Recent developments of DNA biosensors have attracted substantial research efforts directed toward clinical diagnostics and biomedical as well as forensic applications. Electrochemical DNA biosensors enable us to evaluate and predict drugs–DNA interaction [3]. The immobilization of DNA onto an electrode surface is in many ways the crucial aspect of the developing DNA biosensors for monitoring drug because it dictates the accessibility of the DNA to drugs in solution and hence can influence the affinity of drug binding [4–6].

Due to the existing resemblance between electrochemical and biological reactions it can be assumed that the oxidation mechanisms on the electrode and in the body share similar principles [7]. The electrochemical method is a rapid, high throughput, and low cost technique for studying of the interaction between DNA and drugs. Electrochemical approach could contribute a lot in order to speed up the drug screening process. Observing the electrochemical signal related to DNA–drug interactions can provide evidence for the interaction mechanism, the nature of the complex formed, binding constant, binding site size and the role of free radicals generated during interaction in drug action. Much attention has been paid to the electrochemical investigations of the anticancer drug with DNA [8–13]. To our knowledge, no attention has been paid to the interaction between anticancer herbal drug emodin and DNA.

In this paper, the interaction of emodin with DNA was investigated electrochemically by using differential pulse voltammetry (DPV) and cyclic voltammetry (CV) at bare or DNA modified

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doi:10.1016/j.jpba.2006.05.017

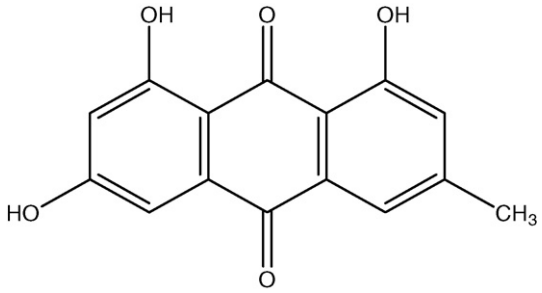

Fig. 1. The structure of emodin.

GCE. The results showed that the herbal drug emodin interfered with DNA by intercalating into the double helix of DNA. The interference of emodin with DNA was further proved by the UV spectra.

2. Experimental

2.1. Instrumentation and materials

Model 650A electrochemical system (CHI Instrument Company, USA) was employed for electrochemical techniques. A standard three-electrode electrochemical cell was used for all electrochemical experiments with glassy carbon electrode (GCE) ($d = 3$ mm) as working electrode, a platinum (Pt) wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The UV spectra were recorded by a UNICO Model UV-2102 spectrophotometer.

Stock solutions 1.00×10^{-3} mol/L of emodin (Checkout Institute of Biology Drugs, China) were prepared with ethanol and stored at 4 °C. Fish tests DNA (Shanghai Sangon Company, China) solutions 1.0 mg/mL was prepared with doubly distilled water. Other reagents used were of analytical grade. Doubly distilled water was used for all preparations. N_2 was employed to deoxygenize and all experiments were carried out at room temperature. All reported potentials are against SCE.

2.2. Procedure

Supporting electrolyte was a 0.05 mol/L NH_4Cl–HCl buffer solution (pH 5.5). In all case, 50% ethanol was added because of the very low solubility of emodin in aqueous solutions.

The ssDNA solution was prepared according to the literature [13]. For preparing the dsDNA modified GCE, dsDNA solution (10 mg/mL) was prepared. The GCE was pretreated by coating with 15 μL dsDNA (10 mg/mL) followed by air-drying, then soaked in doubly distilled water for 4 h, and finally rinsed with water to remove the free dsDNA. In the studies below, all DNA was double-stranded DNA except statement.

3. Results and discussion

3.1. Interaction of emodin with DNA in solution

For considering the acidity of body and the solubility of emodin, pH 5.5, 0.05 mol/L NH_4Cl–HCl buffer solution (50% ethanol) was chosen as supporting electrolyte. Under such condition, the cyclic voltammogram of emodin showed a pair of quasi-reversible peaks in the potential range from −0.2 to −1.0 V with peak potentials of $E_{pa} = -0.557$ V and $E_{pc} = -0.621$ V (Fig. 2). The peak currents i_{pc} (1.277×10^{-5} A) is almost five times as large as i_{pa} (2.635×10^{-6} A), probably because of the decomposition of the reduction product [14]. Considering the sensitivity, we chose the cathodic peak (peak 1) as probe to study the interaction of emodin with DNA by DPV. Fig. 3 shows DPV with and without adding DNA into emodin solution. The peak current decreased and the peak potential shifted to more positive value with the increase of DNA concentration. Probably, this current decrease can be ascribed to the DNA–emodin complexation in the "bulk" solution. Bard et al. reported that positive peak potential shifts of intercalators were observed in the binding form via hydrophobic interactions (intercalation) while electrostatic interactions led to negative shifts [15]. So the shift of peak poten-

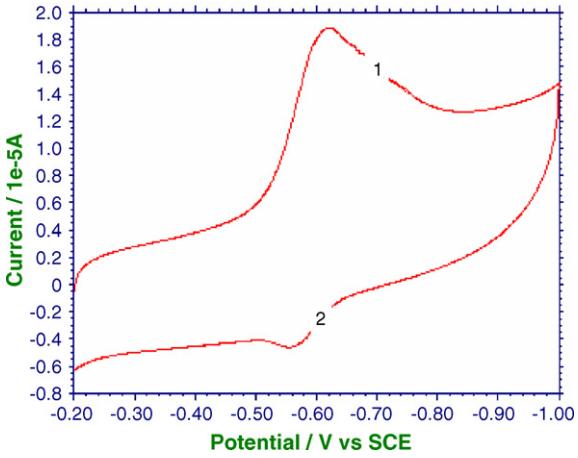

Fig. 2. Cyclic voltammogram of emodin (5.0×10^{-5} mol/L) in 0.05 mol/L NH_4Cl–HCl solution (50% ethanol, pH 5.5). Scan rate: 0.1 V/s.

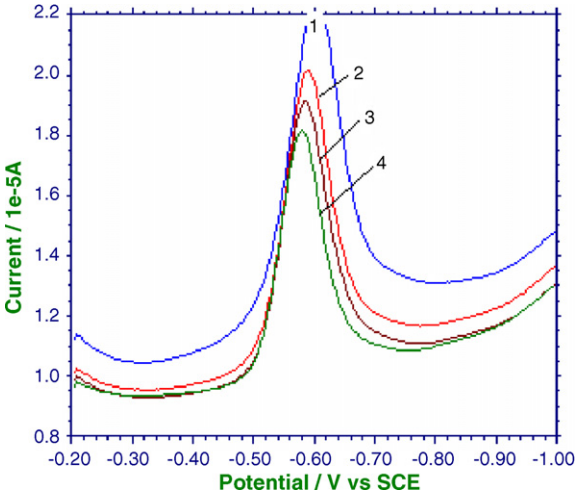

Fig. 3. DPV of emodin with different concentration of DNA at GCE in 0.05 mol/L NH_4Cl–HCl solution (50% ethanol, pH 5.5). Curve (1): 4.00×10^{-5} mol/L emodin; curve (2): (1) + 10 μg/mL DNA; curve (3): (1) + 15 μg/mL DNA; curve (4): (1) + 20 μg/mL DNA.

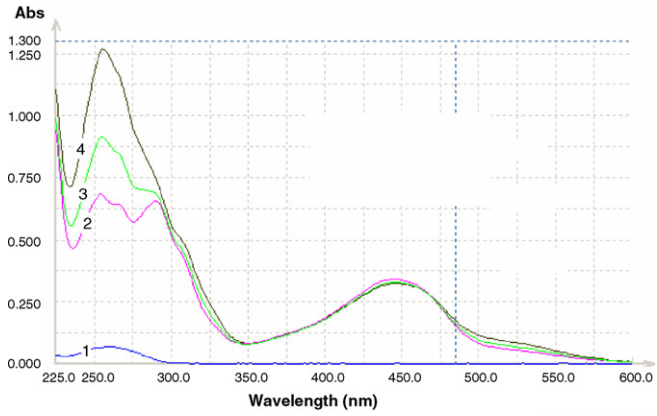

Fig. 4. UV spectra of emodin and DNA–emodin adduct in 0.05 mol/L NH_4Cl–HCl (50% ethanol, pH 5.5). Curve (1): 8 μg/mL DNA; curve (2): 1.00×10^{-5} mol/L emodin; curve (3): (2) + 10 μg/mL DNA; curve (4): (2) + 25 μg/mL DNA.

tial indicated that the planar aromatic ring structure of emodin is expected to facilitate its intercalation into the DNA helix.

Fig. 4 shows the UV spectra of emodin (curve 1), DNA (curve 2) and emodin–DNA (curves 3 and 4). DNA has an absorption peak at about 260 nm. Emodin has three small absorption peaks at about 255, 265 and 288 nm, respectively, and one absorption cingulum at about 445 nm. Having interacted between DNA and emodin, one big absorption peak has been observed at about 260 nm, which ascribes to the combination of DNA and emodin. In wavelength of 350–550 nm, we can find a decrease in the absorbance of emodin with red shift and two isobestic points at about 400 and 475 nm. Based on the literature [16], this result means that the interaction mode of emodin and DNA by emodin intercalating into the double helix of DNA. The result is consistent with that from electrochemistry study.

The effect of temperature was examined over the range of 22–40 °C (Table 1). It was found that the peak current decreased and the peak potential shifted to more positive values at the temperature from 22 to 34 °C. This means that increase temperature makes emodin interposing into DNA easily. Moreover, the peak currents increased and peak potential shifted to positive after 34 °C by increasing temperature. So, the most suitable temperature of emodin interposing into DNA is 34 °C.

3.2. Comparing the interaction of emodin with dsDNA and ssDNA in solution

In order to further study the interaction between emodin and DNA, 20 μg/mL dsDNA and 20 μg/mL ssDNA were added into the NH_4Cl–HCl buffer solution (0.05 mol/L, pH 5.5, 50% ethanol) containing 3.00×10^{-5} mol/L emodin, respectively, at room temperature with stirring for 10 min. Both dsDNA and ssDNA can make the DPV peak currents decrease and the peak potentials shifted positively. But the interaction of dsDNA is very stronger than that of ssDNA. This result supported above viewpoint of emodin interposing into dsDNA because no double helix exists in ssDNA. The interaction of emodin with ssDNA may be simply static action. According to the peak currents decrease of emodin by adding dsDNA, natural DNA may be analyzed quantificationally (Fig. 5).

Table 1
Effect of temperature

T (°C)	E_p (V)	i_p (10^{-5} A)
22	−0.604	1.242
26	−0.600	1.223
30	−0.596	1.161
34	−0.588	1.084
37	−0.584	1.238
40	−0.580	1.392

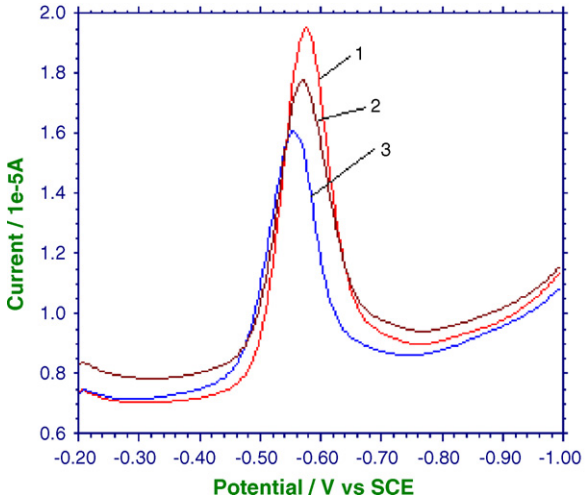

Fig. 5. DPV of emodin at GCE in 0.05 mol/L NH_4Cl–HCl (50% ethanol, pH 5.5). Curve (1): 3.00×10^{-5} mol/L emodin; curve (2): (1) + 20 μg/mL ssDNA; curve (3): (1) + 20 μg/mL dsDNA.

3.3. Interaction of emodin with DNA modified at electrode surface

Cyclic voltammograms were recorded for 1.0×10^{-4} mol/L $K_3[Fe(CN)_6]$ at the bare electrode and the DNA modified electrode (DNA-GCE). We found that the peak currents of $K_3[Fe(CN)_6]$ were decreased markedly at DNA modified electrodes. Because there was repulsion between the negative charged DNA molecules and the negative charged $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$. The result indicated that DNA was immobilized at electrode surface. Then the modified electrodes were used to study the interaction of emodin with DNA by DPV.

Fig. 6 shows the results of comparing emodin at GCE and DNA-GCE by DPV. Obviously, the peak currents are higher at the DNA-GCE than that of at bare GCE. This indicates that the pre-concentration effect of emodin by DNA-GCE took place because of the interaction. The peak potentials are more positive at the DNA-GCE than that of at GCE, indicating that intercalative attractions and stacking interactions of emodin between the base pairs of DNA, in long term, overcome the electrostatic attractions [17].

The effect of the scan rate (υ) on the peak currents of 1.00×10^{-5} mol/L emodin was examined at DNA-GCE by cyclic voltammetry. A linear plot of i_p versus υ is obtained in the scan range of 50–500 mV/s, indicating emodin associ-

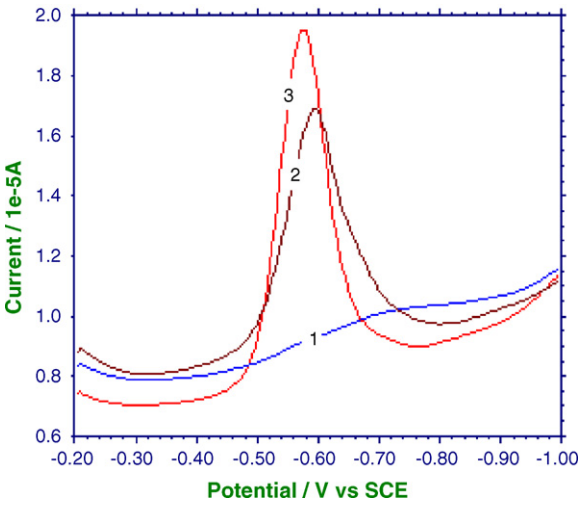

Fig. 6. DPV of emodin (1.00×10^{-5} mol/L) at DNA-GCE in 0.05 mol/L NH_4Cl–HCl (50% ethanol, pH 5.5). Curve (1): DPV of the buffer solution without emodin at DNA-GCE; curve (2): DPV of emodin at GCE; curve (3): DPV of emodin at DNA-GCE.

ated with immobilized DNA. The effect of interaction time of emodin with immobilized DNA was then studied by DPV. The results indicated that the currents increased within 15 min and a levelling-off at longer accumulation time at the DNA-GCE.

Cyclic voltammograms were recorded for 1.00×10^{-4} mol/L $K_3[Fe(CN)_6]$ at the DNA-GCE in the absence and presence 1.00×10^{-5} mol/L emodin. The result showed cyclic voltammograms of $K_3[Fe(CN)_6]$ were same in both case. The result indicated that the negative charges of the immobilized DNA were not changed by emodin and there was still repulsion between the negative charged DNA molecules and the negative charged $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$. So there is no electrostatic interaction of emodin with the negatively charged nucleic acid sugar-phosphate structure or the intercalative attraction overcomes the electrostatic attractions.

3.4. Determination of the combining constant (β) and combing number (m) of DNA-m emodin adduct

Based on the literature [18], the combing constant β and combing number m were investigated. It is assumed that DNA and emodin (EM) only produce a single complex, DNA–mEM:

$$\mathrm{DNA} + m\mathrm{EM} \rightleftharpoons \mathrm{DNA} - m\mathrm{EM}$$

The equilibrium constant is

$$\beta = \frac{[\mathrm{DNA} - m\mathrm{EM}]}{[\mathrm{EM}]^m[\mathrm{DNA}]}$$

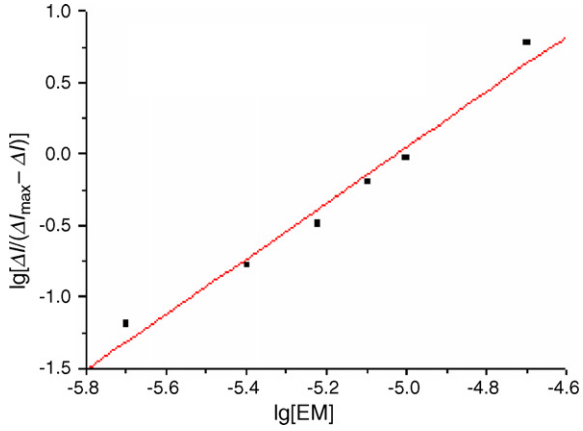

Fig. 7. The relationship between $\log[\Delta I/(\Delta I_{max} - \Delta I)]$ and log[EM] at GCE in the 0.05 mol/L NH_4Cl–HCl (50% ethanol, pH 5.5) buffer solution containing DNA.

and the following equation can be deduced:

$$\log\left[\frac{\Delta I}{\Delta I_{max} - \Delta I}\right] = \log\ \beta + m\ \log[\mathrm{EM}]$$

[DNA] represents the concentration of nucleotide phosphate, which is determined by the UV absorption at 260 nm using the molar extinction coefficient = 6600 M^{-1} cm^{-1} [19]. If DNA and emodin form a single complex, the plot of $\log[\Delta I/(\Delta I_{max} - \Delta I)]$ versus log[EM] becomes linear with slope m (Fig. 7). The results $m = 1.95$ and $\log \beta = 9.82$ were obtained from the experimental data, which means that only one kind of complex is formed. In other words, emodin binding DNA forms a complex of DNA–2EM with $\beta = 6.61 \times 10^9$ or DNA interacting with emodin forms a complex of two molar emodins per molar base pair.

3.5. The relationship between peak currents decrease of emodin and DNA concentrations

After adding DNA into emodin solution, the peak currents of emodin decreased. The decrease of peak currents was proportional to DNA concentrations, which can be applied to DNA concentration determination. So emodin can be used as a new indicator measuring DNA concentration. Under the optimal experiment conditions, the peak currents of 1.00×10^{-5} mol/L emodin decreased linearly with DNA concentrations from 1.5 to 15.0 μg/mL with the detection limit of 1.3 μg/mL. The linear regression equation and correlation coefficient are:

$$\Delta i_p\,(10^{-7}\ \mathrm{A}) = 5.799 + 0.9631 C_{\mathrm{DNA}}\ (\mu\mathrm{g/mL}),$$

$$\gamma = 0.994$$

Table 2
Sample determination

Sample	Added (μg/mL)	Average found (μg/mL)	Average recovery (%)	R.S.D. ($n = 6$, %)	Results of UV (μg/mL)
DNA	4.00	3.82	95.5	2.98	4.06
	8.00	7.67	95.8	2.86	7.89
	12.00	11.86	98.8	2.79	11.94

Based on the literature [13], we regarded the mixed solution containing familiar anion and cation (K^+, Na^+, Cl^-, SO_4^{2-}) as a synthetic sample. The recovery of DNA with the addition of DNA and the relative standard deviation (R.S.D.) were determined, respectively. The result was consistent with the conclusion of UV spectra. Table 2 shows the results of simulation sample determination.

4. Conclusions

The study of the interaction between the antitumor herbal drug and DNA is very important in the development of a new antitumor drug. This paper investigated the interaction of emodin with DNA by electrochemistry and UV spectra. The results indicate that the interaction between DNA and emodin is that of emodin intercalating into the double helix of DNA. There is no electrostatic interaction between emodin and DNA. Emodin binding DNA formed a complex of DNA–2EM with $\beta = 6.61 \times 10^9$ via intercalation. Therefore, emodin can prevent cancerous cells from dividing and producing more cancer cells by inhibiting DNA reproducing and simultaneously exhibits the toxic effect to a certain extent. Furthermore, emodin can be used to determine the DNA concentration as a new electrochemical probe.

Acknowledgement

Financial support provided by National Natural Science Foundation of China (No.20475050) is greatly appreciated.

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