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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 1166–1171
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Electrochemical study on the behavior of Morin and its interaction with DNA

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Received 28 June 2005; received in revised form 30 August 2005; accepted 30 August 2005
Available online 26 October 2005

Abstract

Voltammetric behavior of Morin was studied in 0.1 M HAc–NaAc + 50 mM KCl (pH 3.4) solution at glassy carbon electrode (GCE) using cyclic voltammetry (CV). Morin showed an irreversible anodic peak at 0.720 V in CV which was involving two electrons and two protons. Also, the interaction of Morin with double-stranded calf thymus DNA (ctDNA) was studied by CV at GCE with an irreversible electrochemical equation. As a result of reaction with ctDNA, the voltammetric peak of Morin was a position shift and the peak current decreased. The diffusion coefficients of both free and binding Morin ($D_f = 1.1086 \times 10^{-7}$ cm^2 s^{-1} and $D_b = 8.2544 \times 10^{-9}$ cm^2 s^{-1}), binding constant ($K = 1.7765 \times 10^7$ cm^3 mol^{-1}), and binding site size ($s = 0.8510$) of the Morin–DNA complex were obtained simultaneously by non-linear fit analysis. The results demonstrate that Morin can bind to ctDNA in 0.1 M HAc–NaAc + 50 mM KCl (pH 3.4) solution and the ring B of Morin intercalates between the DNA base pairs.

Keywords: Behavior; Morin; DNA; Interaction; Electrochemical method

1. Introduction

Flavonoids have recently attracted a great interest as potential therapeutic agents against a large variety of diseases, such as anti-viral, anti-allergic, anti-platelet and anti-inflammatory, and possibly protective effects against chronic diseases [1–3]. Anti-tumour promoting activity has also been reported for flavonoids, thus, proving useful as chemopreventive agents in human carcinogenesis [4–7]. These polyphenolic compounds, ubiquitous in higher plants, are also frequent components of major dietary constituents. To our knowledge, there are few literatures on the electrochemical behavior of flavonoids, especially on that of Morin [8,9]. However, a detailed electrochemical study of four structurally related flavonoids, with identification of their oxidation products at a glassy carbon electrode had been reported by Hendrickson et al. and some interesting results have been obtained [9,10]. The response of 3′,4′-adjacent hydroxyl groups is a two-electron and two-proton electrode reaction.

The interaction of DNA with small molecules has been an intensive topic that fascinated scientists for decades because it provides insights into rational design of drugs targeting to DNA. In our group, interaction of Morin and its transition metal(II) complexes have been studied in neutral solution [11,12]. They can bind to DNA, but the binding mode is different and the Morin binds in a weak non-intercalation mode. Also, Zhu et al. [13] draw a similar conclusion because it not only contains 2′,4′-adjacent hydroxyl groups that can easily form intermolecular hydrogen bonds' hydrogen with higher steric hindrance but also possesses a greater density of negative charges which prevent its interaction into DNA.

Also, a variety of methods, such as gel electrophoresis, footprinting technique, X-ray crystallography [14,15], structural modeling [16], spectroscopy and especially fluorescent spectroscopy [17,18], have been used to study these interactions. Electrochemical methods have also been used to explore the interaction of DNA with redoxactive molecules [19–21], because they show many advantages, such as direct monitoring, high sensitivity and simplicity. However, most of them are focused on small molecules with good reversibility in electrochemical reaction while many DNA-targeting drugs show irreversibility in electrochemical reaction. In many cases, irreversible electrode process must be assumed to reversible which can arouse some errors because the electron transfer coefficients of irreversible electroactive compounds play an important

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doi:10.1016/j.jpba.2005.08.030

role in electrochemical reaction, otherwise it cannot deal with electrochemical methods. Recently, Wang et al. developed a novel equation, which is suited for investigating the interaction between DNA and irreversible redox compounds using electrochemical method [22].

In this contribution, the electrochemical method used to study the Morin–DNA interaction and determine the affinity constant and kinetic parameters is described. The irreversible electrochemical property of Morin and interaction with calf thymus DNA (ctDNA) was studied by cyclic voltammetry (CV) for the first time. The diffusion coefficients for the free and binding Morin (D_f and D_b), the binding constant (K) and the binding site size (s) of Morin with DNA were determined. Also, the reaction mechanism of Morin at glassy carbon electrode (GCE) is forecasted.

2. Experimental

Morin was obtained from Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou). Morin was dissolved in ethanol and doubly distilled water as stock solution. Calf thymus DNA was purchased from Sino-American Biotechnology Co. (Beijing, China) and used as received. Native double-stranded DNA was dissolved in doubly distilled water. Its stock solutions were stored at 4 °C. Ratios of UV absorbance of DNA at 260 and 280 nm, A_{260}/A_{280}, of ca. 1.8–1.9, indicate that the DNA was sufficiently free of protein. Other reagents were of analytical reagent grade. Experiments were conducted in 0.1 M HAc–NaAc buffer solution, pH 3.4, containing 50 mM KCl.

Electrochemical studies were carried out using a CHI-832 electrochemical Analyzer (CH Instruments Ltd. Co., USA) with a three-electrode system, including a bare GCE as the working electrode, a platinum wire as a counter electrode, and a saturated calomel reference electrode (SCE). The bare GCE is prepared according to literature [23]. All potentials were referred to the SCE. The bare GCE area is 0.0137 cm^2. A Branson 200 Ultrasonic cleaner (USA) was used to clean the working electrode. UV–vis spectra were recorded in solutions on UV-3400 spectrophometer (Hitachi, Japan).

3. Results and discussion

3.1. Electrochemical behaviors of Morin

Fig. 1 shows the molecular structure of Morin. The electrochemical behaviors of Morin in 0.1 M HAc–NaAc + 50 mM KCl solution with various pH from 3.4 to 5.7 on the GCE were examined, respectively. Only an anodic peak was obtained in the cyclic voltammograms. This suggests that the redox reaction of Morin is an irreversible process. What is more, the oxidation peak current increases with the pH decreasing and the relationship between E_{pa} and pH is linear, the equation is E_{pa} = 0.93126–0.05655pH (E_p, V; correlation coefficient r = 0.99786) were obtained (Fig. 2). The curve slop is −0.05655, indicating that this process involves proton, and the number of protons participating in the electrode reactions is the same as that of electrons. Further, the dissociation constant (pK_1) is 3.46

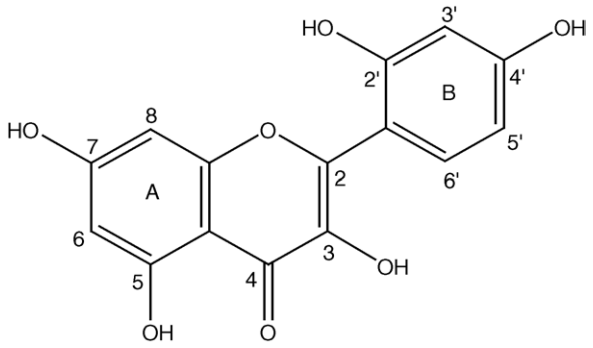

Fig. 1. The molecular structures of Morin.

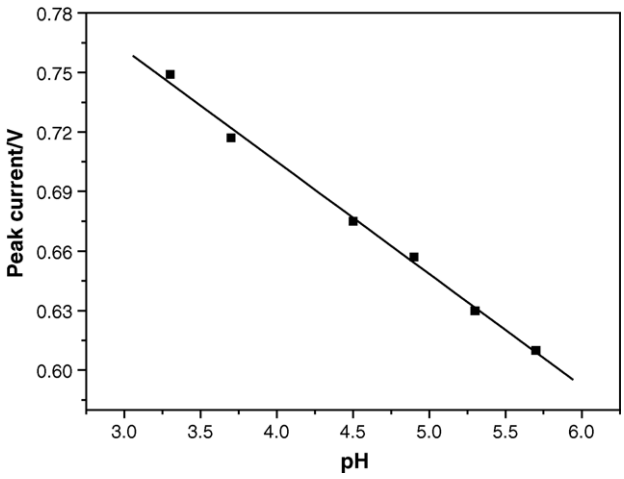

Fig. 2. Effect of pH on CV anodic potential E_{pa} for 4×10^{-5} M Morin at GCE in 0.1 M HAc–NaAc + 50 mM KCl buffer solution (pH 3.4).

[24]. In order to explore the Morin's negative ionization in neutral solution how to effect the interaction between Morin and DNA, a pH 3.4 HAc–NaAc buffer was chosen as the supporting electrolyte and the peak potential was 0.720 V (Fig. 3(a)).

We also investigated the relationships between peak current and scan rate. The results show that the oxidation peak current

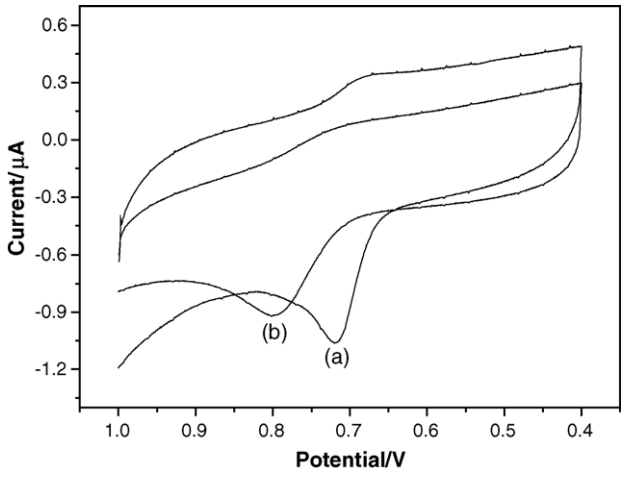

Fig. 3. Cyclic voltammograms: (a) 4.0×10^{-5} M Morin and (b) Morin + DNA (1.2×10^{-4} M). Supporting electrolyte: 50 mM KCl, 0.1 M HAc–NaAc, pH 3.4; sweep rate: 80 mV s^{-1}.

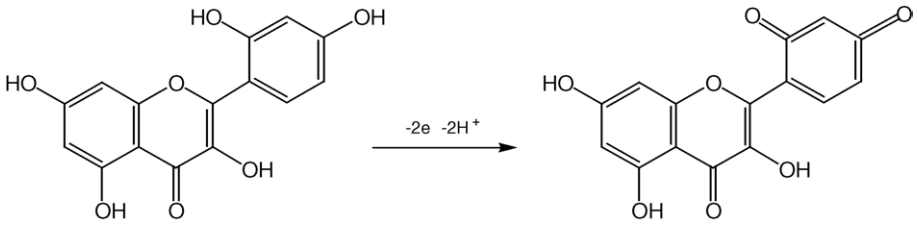

Fig. 4. Proposed mechanism for the oxidation of Morin.

is proportional to the square root of scan rate from 20 to 180 mV s^{-1}. This indicates that the electrochemical process is controlled by diffusion. In this work, the scan rate is chosen 80 mV s^{-1}.

For irreversible electrode reaction process, we may use equations [25]:

$$E_{pa} = E^{o\prime} + \frac{RT}{\alpha n F}\left\{0.780 + 0.5\ \ln\frac{\alpha n D F v}{RT} - \ln k_s\right\} \quad (1)$$

$$|E_p - E_{p/2}| = \frac{1.857RT}{\alpha n F}\ \text{mV} \quad (2)$$

where $E^{o\prime}$ is formal potential, k_s the standard heterogeneous rate constant, D the diffusion coefficient, α the transfer coefficient of the oxidation of Morin and $E_{p/2}$ is the potential, where $i = i_{p/2}$ in cyclic voltammograms. Other symbols have their usual significance. Also, in cyclic voltammograms, the average value of $|E_p - E_{p/2}|$ is 44 mV. The average value αn, calculated from Eq. (2), is 1.08. The value of αn, calculated from Eq. (1), is 1.1305. So, the average of αn is 1.105. Generally, α in the totally irreversible electrode process is assumed as 0.5 [26]. Hence, two electrons are involved in the oxidation process of Morin and the value of α is 0.54. Further, the protons involved in process were 2. It accords with the literature [10]. Being electron richer than ring A, the B ring of Morin is an apparent target of any oxidant [24]. A possible mechanism for the oxidation of Morin in 0.1 M HAc–NaAc + 50 mM KCl (pH 3.4) is shown in Fig. 4. The 2′,4′-OH were oxidized to quinones, which is similar to that of other flavonoids, such as quercetin and rutin [9].

3.2. The interaction of the Morin with ctDNA

Zhu et al. [13] pointed out that interaction of Morin with DNA was strongly limited in, pH 5.0, buffer solution. We obtained similar results in pH 6.0 HAc–NaAc buffer. The peak current and potential were nearly unchanged (Fig. 5). Also, as shown in Fig. 6, on mixing Morin and DNA, the ultraviolet spectrum is no obvious different from that of Morin or DNA, even at a presenting high concentration of DNA. It suggests that Morin binds in a non-intercalation mode with DNA at pH 7.1 solution [11,12] and its binding is weak. But in 0.1 M HAc–NaAc + 50 mM KCl, pH 3.4, buffer solution, the dramatic changes were occurred. Typical CV behavior of Morin in the presence of DNA is shown in Fig. 3(b). When ctDNA is added to a solution of Morin, marked decreases in the peak current heights and shifts of peak potentials from 0.720 V to more positive values 0.785 V are observed. To show that the decrease in current is due to the diffusion of

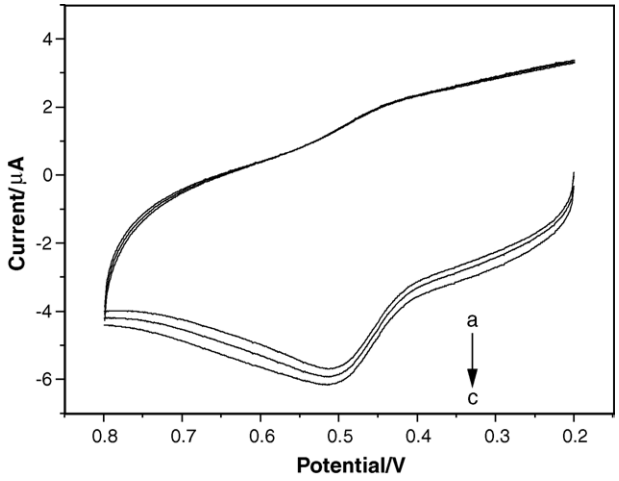

Fig. 5. Cyclic voltammograms of 8.0×10^{-5} M Morin mixed with different concentrations of DNA in 0.1 M HAc–NaAc + 50 mM KCl buffer, pH 6.0. DNA concentrations: (a) 0 M; (b) 1.5×10^{-5} M; (c) 3.0×10^{-5} M. Accumulation time, 2 s; scan rate, 80 mV s^{-1}.

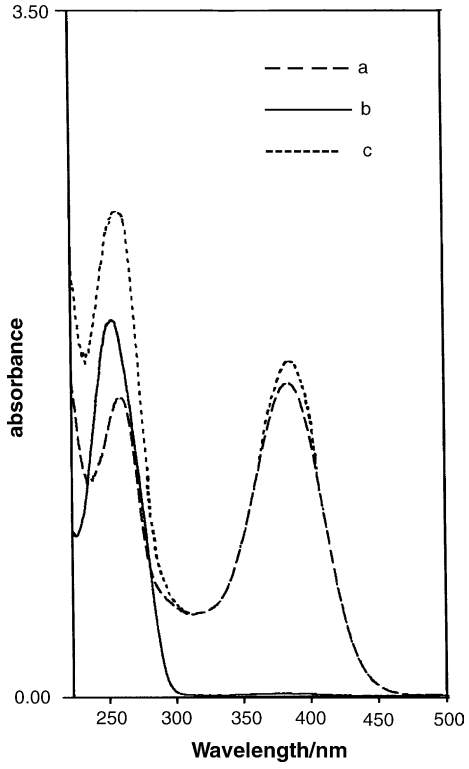

Fig. 6. Absorption spectra of: (a) 8.0×10^{-5} M Morin; (b) 1.5×10^{-5} M DNA; (c) a mixture of 8.0×10^{-5} M Morin and 1.5×10^{-5} M DNA in 0.1 M HAc–NaAc + 50 mM KCl buffer, pH 6.0.

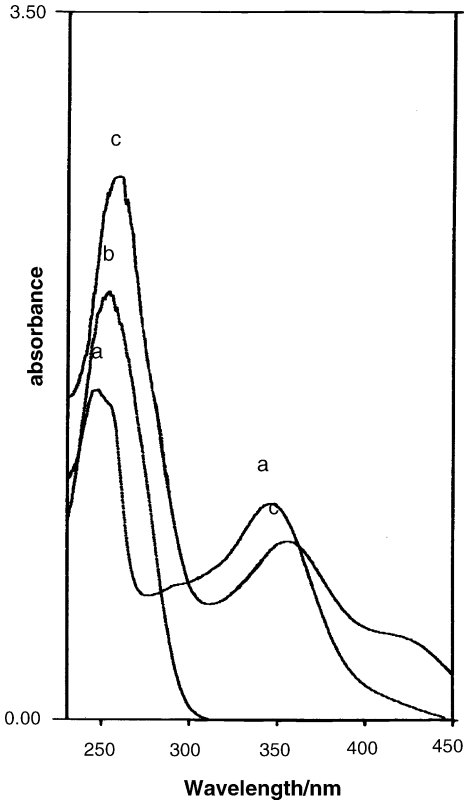

Fig. 7. Absorption spectra of: (a) 8.0×10^{-5} M Morin; (b) 1.5×10^{-5} M DNA; (c) a mixture of 8.0×10^{-5} M Morin and 1.5×10^{-5} M DNA in 0.1 M HAc–NaAc + 50 mM KCl buffer, pH 3.4.

Morin–DNA complex, not due to the increased viscosity of the solution or the blockage of the electrode surface by DNA adsorption, a special CV experiment was designed in a $K_4Fe(CN)_6$ solution in the absence and presence of DNA. In these solutions, the ions of $Fe(CN)_6^{4-}$ did not interact with DNA because of coulombic repulsion between their negative charges. In the absence of DNA, a normal voltammetric peak of $Fe(CN)_6^{4-}$ was observed ($E_{pa} = 0.59$ V and $I_{pa} = 0.64$ μA). Upon the addition of DNA, the peak current (I_{pa}) decreased only a little (0.58 μA). This showed that the addition of DNA affected the current only slightly and there was no shift of peak potential. Thus, there was no obvious effect on the diffusion from the changed viscosity of the solution and the DNA adsorption. Therefore, the great decrease in current in CV experiments could be attributed to the diffusion of Morin bound to the large, slowly diffusing DNA with large molecular weight. The changes in current upon addition of DNA can be explained in terms of diffusion of an equilibrium mixture of free and bound Morin to the electrode and which can be used to quantify the binding of Morin to DNA. Also, as shown in Fig. 7, the ultraviolet spectra of Morin have an intensive band II at $\lambda_{max} = 247.9$ nm and a less intensive band I at $\lambda_{max} = 346.2$ nm. The band I is related to the absorbance of the B ring (cinnamoyl system), whereas the band II is related to the π–π^* transition absorbance in the A ring (benzoyl system). When Morin and DNA are mixed, the spectrum is significantly different from that of Morin or DNA. Band II shows a stronger intense absorbance at higher wavelength. Band

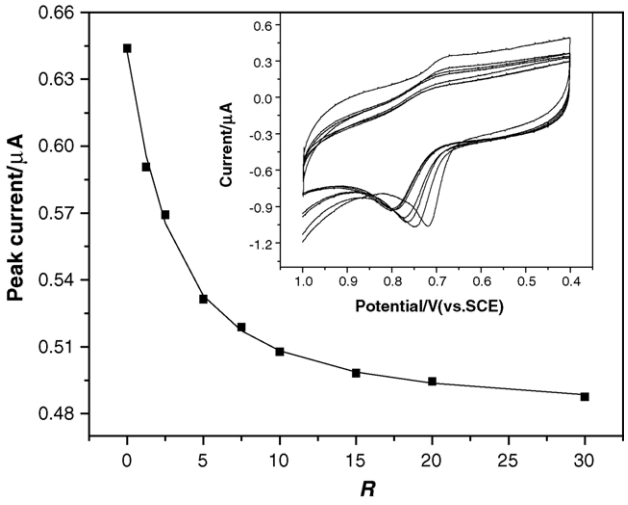

Fig. 8. Binding curve of 4.0×10^{-5} M Morin with DNA, other conditions are the same as in Fig. 2. Inset: cyclic voltammograms of titration Morin with DNA.

I red shifts towards 351.8 nm and displays hypochromicity. The results indicated that Morin and DNA could form Morin–DNA complex. Band I red shifts by ca. 5.6 nm and bring a new shoulder peak at higher wavelength (about 430 nm), suggesting ctDNA have bond in B ring. And, as shown in Fig. 8, it is noteworthy that the current peak is not disappears but is unchanged while the saturated concentration of DNA is reached in solution. It indicates that the ring B of Morin, electrochemical active site, is partly bound with DNA or its binding is not especially tight. It is easily found that the result of ultraviolet spectrum is consistent with CV experiments. The interaction occurred between Morin and DNA in, pH 3.4, buffer solution, and the interaction mode of Morin with DNA is intercalation [27,28]. Contrast to the interaction in neutral solution, it may draw a conclusion that the coulombic repulsion is a main factor that effects the mode of interaction between Morin and DNA because the dissociation constant (pK_1) of Morin is 3.46 [24] and it is a neutral un-ionized non-negative in the pH 3.4 HAc–NaAc solution.

3.3. Determination of the binding constant (K) and the binding site size (s) of Morin–DNA complex

According to the method [22], when measured the total anodic peak current as a function of R, a titration of 4.0×10^{-5} M Morin with different amounts of DNA gave the results shown in Fig. 8.

When the non-specific binding of an electroactive molecule, EM, to a binding site, S, composed of s base pairs, on a DNA duplex, produces a bound species, EM–S:

$$EM + S = EM\text{–}S \tag{3}$$

The microscopic equilibrium constant for binding is

$$K = \frac{C_b}{C_f C_S} \tag{4}$$

where C_b, C_f and C_s represent the equilibrium concentration of EM in EM–S, free EM and free S, respectively. The total

Table 1
Effect of added DNA on peak current

No.	Morin [μM]	C_{NP} [μM]	R value	I_{pa} [μA]
1	0.4	0	0	0.6439
2	0.4	0.5	1.25	0.5907
3	0.4	1	2.5	0.5692
4	0.4	2	5	0.5313
5	0.4	3	7.5	0.5189
6	0.4	4	10	0.5078
7	0.4	6	15	0.4982
8	0.4	8	20	0.4944
9	0.4	12	30	0.4876

concentration of the electroactive molecule, C_t, is

$$C_t = C_b + C_f \tag{5}$$

and the average of number of binding sites (x) along a DNA duplex molecule with an average total number of base pairs L is

$$x = \frac{L}{s} \tag{6}$$

Thus, the total concentration of binding sites can be represented as xC_{DNA}:

$$xC_{DNA} = C_b + C_s \tag{7}$$

where

$$C_{DNA} = \frac{C_{NP}}{2L} \tag{8}$$

Here, C_{NP} is the nucleotide phosphate concentration. The ratio of the nucleotide phosphate concentration and the total concentration of electroactive molecule can be defined as R:

$$R = \frac{C_{NP}}{C_t} \tag{9}$$

For an irreversible reaction in CV at 25 °C, the total anodic current (I_{pa}) with any R can be calculated by

$$I_{pa} = B\left[(\alpha n)_f^{1/2} D_f^{1/2} C_f + (\alpha n)_b^{1/2} D_b^{1/2} C_b\right] \tag{10}$$

where $B = 2.99 \times 10^5 n A v^{1/2}$ [25]. Making appropriate substitutions and elimination C_{DNA} in Eqs. (4)–(10), an equation for I_{pa} is obtained:

$$I_{pa} = B\left\{(\alpha n)_f^{1/2} D_f^{1/2} C_f + \frac{[(\alpha n)_b^{1/2} D_b^{1/2} - (\alpha n)_f^{1/2} D_f^{1/2}]\left[b - \left(\frac{b^2 - 2K^2 C_t^2 R}{s}\right)^{1/2}\right]}{2K}\right\} \tag{11}$$

where $b = 1 + KC_t + \frac{KRC_t}{2s}$.

Eq. (11) is valid for the assumption of non-cooperative, non-specific binding to DNA with the existence of one type of discreet binding site. The diffusion coefficients of EM and EM–DNA (D_f and D_b), the binding constant (K) and the binding site size (s) of EM–DNA could be obtained by non-linear fit analysis of the experimental data (I_{pa} and R) according to the equation. The experimental data are shown in Table 1. With addition of DNA, the peak currents decreased sharply, when R was 20 the decrease in peak current became slight, then remained independent with the concentration of DNA, which showed that Morin interacted with DNA quantitatively, and the interactive balance has been established when R was 20. A non-linear fit analysis of the data to Eq. (11) yielded the binding curve shown in Fig. 8 and following results were obtained: $D_f = 1.1086 \times 10^{-7}$ cm^2 s^{-1}, $D_b = 8.2544 \times 10^{-9}$ cm^2 s^{-1}, $K = 1.7765 \times 10^7$ cm^3 mol^{-1} and $s = 0.8510$. The overall results demonstrate that Morin binds to DNA with a high association constant and covers one base pair. Here, the actual meaning of s is the number of DNA base pairs covered (or made inaccessible to another molecule) by a binding molecule [28]. It can be seen that the apparent diffusion coefficient of Morin–DNA adduct is much smaller than that of Morin, showing that dramatic decrease in the peak current of Morin–DNA adduct is surely caused by the decrease in the apparent diffusion coefficient of Morin after binding to large DNA.

4. Conclusions

In 0.1 M HAc–NaAc + 50 mM KCl (pH 3.4) buffer solution, Morin undergoes a process of two-electron and two-proton electrode reaction result in the 2′,4′-hydroxyl groups be oxidized to 2′,4′-quinone groups. Also, Morin can bind with DNA by intercalating mode at pH 3.4 in which Morin is lack of negative charges and easily interaction with DNA. In addition, the diffusion coefficients of free and binding Morin (D_f and D_b), binding constant (K) and binding site size (s) of the Morin–DNA complex were obtained simultaneously by non-linear fit analysis according to the equation which is suitable to study interaction of DNA with irreversibly electroactive molecules.

Acknowledgements

The present work was supported by a grant from the National Natural Science foundation of China (Nos. 20275031 and 20335030) and KJCXGC-01 from Northwest Normal University.

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