Voltammetric and spectroscopic studies on binding of antitumor Morin, Morin–Cu complex and Morin–β-cyclodextrin with DNA

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A B S T R A C T

A systematic comparative study of the binding of antitumor Morin and its complexes with DNA has been investigated in the Britton-Robison (BR) buffer solutions using voltammetric and spectroscopic methods. The results show that Morin molecule, acting as an intercalator, is inserted into the cavity of the β-cyclodextrin (β-CD) as well as into the base stacking domain of the DNA double helix. The interaction of Morin–Cu complex or the inclusion complex of Morin–β-CD with ds-DNA causes hypochromism in the absorption spectra, along with pronounced changes in the electrochemical behavior of the Morin complexes. An isobestic point and a new spectrum band appeared indicating the formation of the new system of Morin–Cu–DNA at λm = 391 nm and Morin–β-CD–DNA at λm = 375 nm. The intercalation of Morin–Cu and Morin–β-CD complexes with DNA produces an electrochemically inactive supramolecular complex. The binding constants were calculated from the increase of the solubility, the strong hypochromism, and the decrease in peak current of Morin and its complexes upon the addition of the host molecules. Calculation of the thermodynamic parameters of the interaction of the inclusion complex of Morin–β-CD with DNA, including Gibbs free energy change, Helmholz free energy and entropy change shows that the complexation is a spontaneous process of association.

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1. Introduction

Recently much attention has been paid to flavonoids because of their antiproliferative effect on various cancer cells [1], their antioxidant potential [2] and inhibitory effect on cytochrome p-450 enzymes [3]. Antitumor promoting activity has also been reported for flavonoids, thus, proving useful as chemopreventive agents in human carcinogenesis [4]. In this context the phenomenon of Morin and its complexes exhibiting different antitumor activities [5] lead us to consider the interaction of Morin (2′,3,4′,5,7-pentahydroxyflavone, Fig. 1) and the complexes of Morin–Cu and Morin–β-CD with ds-DNA.

The interaction of some anticancer drugs with DNA has been studied by variety of techniques, such as gel electrophoresis, X-ray crystallography [6], structural modeling [7], spectroscopy and especially fluorescent spectroscopy [8]. In recent years, there has been a growing interest in the electrochemical investigation of interactions between anticancer drugs and other DNA-targeted molecules and DNA [9,10]. To our knowledge, there are few literatures on the electrochemical behavior of flavonoids, especially on that of Morin [11–15]. However a detailed electrochemical study of four structurally related flavonoids, with identification of their oxidation products at a glassy carbon electrode has been reported by Hendrickson et al. and some interesting results have been obtained [13,16].

In this paper, the binding of antitumor Morin, Morin–Cu complex and the inclusion complex of Morin–β-CD with DNA were investigated using cyclic and square wave voltammetry as well as UV–vis spectroscopy techniques. The aim of the work is to characterize the binding mode and affinity of binding of antitumor Morin and its complexes with DNA.

2. Experimental

2.1. Instrumentation

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were carried out using an EG&G PAR Model 263A potentiostat/galvanostat controlled by an IBM microcomputer with EG&G PAR M 270 software in conjunction with a PAR Model 303A HMDE. The three electrode system was completed by an Ag/AgCl (saturated KCl) reference electrode and a Pt-wire auxiliary electrode. A PAR Model 305 stirrer was also used for SWV. The ultraviolet and visible absorption spectra were obtained using
Perkin Elmer (Lambda 35) spectrophotometer. The pH was measured using Model 3310 pH-meter (Jenway) with accurate to ±0.02.

2.2. Chemicals and reagent

Salamon double strand deoxyribonucleic acid (ds-DNA), β-CD and Morin were obtained from Sigma and were used as received without further purification. Stock standard solution of ds-DNA was prepared by dissolving 0.1 g of ds-DNA in 100 ml autoclaved double distilled water and stored at 4 °C [14] and discarded after no more than 4 days. The concentration of the stock solution of ds-DNA (1.86 × 10^{-3} M in nucleotide phosphate, NP) was determined by UV absorbance at 260 nm using the molar extinction coefficient (ε) at 6600 M^{−1}. Stock solution of β-CD was prepared by dissolving the desired weights in the solution of Britton-Robison (BR) pH 3.2. Stock solution of Morin was prepared by dissolving an appropriate amount of the drug in ethanol. The supporting electrolyte was BR buffer prepared in the usual way, by adding appropriate amount of sodium hydroxide (0.4 M) to an orthophosphoric acid, boric acid buffer prepared in the usual way, by adding appropriate amount of the drug in ethanol. All chemicals were reagent grade (Merck, Darmstadt). Double distilled deionized water was used to prepare the solutions.

2.3. Voltammetric measurements

For voltammetric measurements, the test solution was placed in a polarographic cell of volume 10 ml and deoxygenated by bubbling nitrogen for 15 min. During measurements, a stream of nitrogen was passed over the solution. The square wave voltammetric response was obtained using pulse amplitude, 25 mVpp; frequency, 100 Hz; adsorption time, 60 s; equilibrium time, 15 s. Cyclic voltammetric response was obtained using scan rate 100 mV s^{−1} (unless otherwise stated). Keeping both concentration of the Morin and the total volume of solution constant (5 ml) then electrochemical procedures were carried out, while varying the β-CD or ds-DNA concentration. Typical CV peak potentials were reproducible to better than ±5 mV and SWV peak potentials to ±2 mV. All experiments were carried out at (25 ± 0.05 °C).

2.4. Spectrophotometric measurements

The spectral shift of Morin, Morin–Cu complex and the inclusion complex of Morin–β-CD were studied upon addition of various ds-DNA. A Perkin Elmer (Lambda 35) spectrophotometer was used for all experiments.

2.5. Solubility measurements

The solubilization of Morin in water and aqueous β-CD solution was carried out according to the method of Higuchi and Connors [17]. An excess amount of Morin was mixed with BR buffer solution (10 ml, pH 3.2) containing various concentrations of β-CD (0–9 × 10^{−4} M). The mixture was shaken in a water bath (72 h, 298 K) and then agitated further for 12 h until the equilibrium state was reached. The resulting suspension was filtered through filter paper (0.45 μm). The concentration of Morin in the filtrate was assayed spectrophotometrically at 251 and 351 nm; there was no interference from β-CD in this wavelength. Each of the solubility was determined in triplicate.

3. Results and discussion

3.1. Intercalation of Morin with ds-DNA

Cyclic voltammetric studies of Morin at a HMDE in BR buffer solutions revealed that the direct reduction of the carbonyl group of the γ-pyrene ring at a rather negative potential [13,16]. The peak potential separations ΔE_P = E_{pC} − E_{pa} are beyond 184 mV indicating an irreversible redox process. The decrease in peak current of Morin upon the addition of ds-DNA into the Morin solution is the maximum at pH 3.2. Therefore pH 3.2 was selected as the optimum pH to study the interaction between Morin and ds-DNA. Under these conditions the dramatic changes of CV behavior of Morin (pK_a = 3.46) in the presence of ds-DNA were occurred (Fig. 2). When ds-DNA is added to a solution of Morin, the peak current height decreases and shifts of peak potentials from −580 mV to more negative values −630 mV are observed. The cathodic peak current decreased to ca. 28% of those in the presence of ds-DNA. According to these observations, it seems that the decrease of peak current of Morin after an addition of excess of ds-DNA is caused by the intercalation of Morin to the bulky, slowly diffusing ds-DNA, which results in considerable decrease in the apparent diffusion coefficient. This is emphasized from the decrease in the slope of the linear ip−1/2 plots (R > 0.9989), where the slope values are 0.0265 and 0.0173 μA M^{−1/2} cm^{1/2} in absence and presence of ds-DNA respectively. From these values, the diffusion coefficient (D_p) of the free Morin was found to be 1.24 × 10^{−7} cm^{2} s^{−1}, whereas D_p = 5.31 × 10^{−8} cm^{2} s^{−1} for the bound Morin (in presence

![Fig. 1. The molecular structure of Morin.](Image)

![Fig. 2. Cyclic voltammetry of 9.9 μM Morin at HMDE in BR buffer (pH 3.2) in the absence (1) and presence of (2) 1.46, (3) 2.9, (4) 4.3, (5) 5.7 and (6) 7.08 μM ds-DNA. Scan rate 100 mV s^{−1}.](Image)
of 1.82 μM ds-DNA, NP). The changes in current upon addition of ds-DNA can be explained in terms of diffusion of an equilibrium mixture free and bound Morin to the electrode and which can be used to quantify the binding of Morin to ds-DNA. In this context current titrations were performed by keeping the concentration of DNA using CV and SWV at pH 3.2. The current titration was described by the following equation [18]:

\[ \log \left( \frac{1}{[\text{DNA}]} \right) = \log K + \log \left( \frac{I_{H-G}}{I_{G}} \right) \]  

where \( K \) is the apparent binding constant, \( I_{C} \) and \( I_{H-G} \) are the peak current of the free guest (G) and the complex (H–G) respectively. Under the assumptions of irreversible, diffusion-controlled electron transfer and a 1:1 association complex between the drug and ds-DNA (in nucleotide phosphate), then the plot of log(\( \frac{1}{[\text{DNA}]} \)) versus \( \log \left( \frac{I_{H-G}}{I_{C} - I_{H-G}} \right) \) becomes linear with the intercept of log \( K \). The binding constant of this complex was evaluated according to Eq. (1) and the result is cited in Table 1. The value of \( K \) demonstrated that Morin (pK\(_a\) = 3.46) binds to ds-DNA with a high association constant.

In order to investigate the mode of Morin binding to ds-DNA, the absorption spectra has been also employed in examination of the reaction, as Morin presumably binds to ds-DNA by intercalation. When Morin and ds-DNA are mixed, the spectrum is significantly different from that of Morin or ds-DNA (Fig. 3). The absorption bands at 251 and 351 nm showed decrease in the peak intensities (hypochromicity) on increasing concentration of ds-DNA. Also the band at 351 nm shifted to longer wavelength. Such pronounced hypochromism and bathochromism were suggested to be due to a strong intercalation of the Morin molecule into ds-DNA base pairs [19,20]. This implies a close proximity of Morin chromophore of the ds-DNA base pairs i.e. a strong overlap between the electronic states of the intercalating chromophore and that of the ds-DNA bases occurs.

Assuming the most common host–guest ratio of 1:1, the binding constant \( K \) was determined from the differences in absorbance \( (A) \) due to ds-DNA intercalation using the following equation:

\[ \log \left( \frac{1}{[\text{DNA}]} \right) = \log K + \log \left( \frac{A_{H-G}}{A_{C} - A_{H-G}} \right) \]  

in which \( A_{C} \) and \( A_{H-G} \) are the absorption spectrum at \( \lambda_{m} = 351 \) nm of the free guest (G) and the complex (H–G) respectively. Our experimental data at pH 3.2, which are in good agreement with those obtained from voltammetric technique, are listed in Table 1. It is easily found that the result of absorption spectra is consistent with CV experiments. The interaction occurred between neutral Morin and DNA in, pH 3.2, buffer solution, and the interaction mode of Morin with ds-DNA is intercalation, which is contrast to the interaction in neutral buffer solution [21,22]. At pH 7.1 it was suggested that Morin binds with DNA in a non-intercalation mode in which DNA has negative charge due to phosphate group and its binding is weak. Whereas at pH 5.0, Zhu et al. [23], reported that Morin possesses a greater density of negative charges, which prevent its interaction into ds-DNA. Therefore in consideration the dissociation constant of Morin (pK\(_a\) = 3.46), under our experimental conditions of pH 3.2 the neutral Morin should show a greater affinity to DNA. The obtained results at different pH values show that the binding constant of Morin with DNA decreases in the following sequence 1.58 × 10\(^5\) M\(^{-1}\) (pH 3.2) > 8.12 × 10\(^4\) M\(^{-1}\) (pH 4.2) > 7.65 × 10\(^3\) M\(^{-1}\) (pH 5.2). At the same time the magnitudes of the change in Gibbs free energy values at different pHs decrease in the same order. This indicates that the binding process of Morin with DNA is favorable and spontaneous at pH 3.2 than the other pHs. The obtained results show that the binding mode and the binding affinity of Morin to DNA depend mainly on the pH value of the buffer solution and consequently the interaction of Morin with ds-DNA is correlated to the dissociation constant of Morin.

3.2. Morin–Cu complex binding to DNA

Cyclic voltammetry of Morin in the presence of Cu\(^{2+}\) which is capable of forming complex [21] with the investigated drug is interesting. The addition of Cu\(^{2+}\) to a solution of Morin at pH 3.2 resulted in the disappearance of the cathodic reduction peak of Morin at −580 mV and the appearance of well-defined peak at −332 mV. This new peak corresponds to the reduction of Morin–Cu complex. The effect of the Cu\(^{2+}\) concentration on the sensitivity of the SWV is shown in Fig. 4. By increasing the Cu\(^{2+}\) concentration from 0.56 to 4.33 μM in the presence of 5.6 μM Morin the reduction peak of
Morin–Cu complex at $-355$ mV increases while the reduction peak of Morin at $-580$ mV decreases. Plot of cathodic peak current ($i_p$) for the reduction of Morin and Morin–Cu complex as a function of log $\left[Cu^{2+}\right]$ indicates the stoichiometric ratio of 1:2 between $Cu^{2+}$ and Morin as shown in the insert of Fig. 4.

Recently attention has been paid to Cu-complexes of Morin in presence of DNA due to its higher antitumor activity than that of Morin [21]. Therefore cyclic voltammograms of Morin–Cu complex at pH 3.2 in presence of ds-DNA are shown in Fig. 5. Addition of ds-DNA to the Morin–Cu complex causes the peak current of the reduction of complex to diminish considerably. Additionally, the peak potential shifted to more negative values indicating that the binding of Morin–Cu complex with DNA may be intercalation [20,24,25]. The peak potential separations $\Delta E_p = E_{pc} - E_{pa}$ are beyond 232 mV indicating an irreversible redox process. The plot of $i_p$ of the reduction complex as function of $v^{1/2}$ was linear as expected for a diffusion-controlled process. Important changes in absorption spectra of Morin–Cu complex were also observed upon the addition of ds-DNA (Fig. 6). When the concentration of ds-DNA was increased, the intensity of absorption spectra of Morin–Cu complex at $\lambda_m = 353$ nm decreased (hypochromicity) and a new spectrum band appeared at $\lambda_m = 422$ nm which is the characteristic spectra of the Morin–Cu–DNA adduct. Moreover, an isobestic point appeared at $\lambda_m = 391$ nm, which indicates the formation of the new adduct of the Morin–Cu complex. Comparing these changes in the spectra of Morin with Morin–Cu complex in the presence of DNA, the difference is distinct. This phenomenon also indicates that both the complex Morin–Cu and Morin bind to DNA in different mode. Based on the above mentioned results of CV that on the addition of ds-DNA to Morin–Cu complex produced no new reduction peak while the absorption spectra of Morin–Cu complex show clearly a new band, the conclusion can be drawn that Morin–Cu complex interacting with DNA forms an electrochemically inactive complex, which should be called a supramolecular complex [23] which cannot be reduced on the electrode. The obtained results indicate that apparent diffusion coefficient of Morin–Cu–DNA adduct is smaller than Morin–DNA system. Furthermore the obtained binding constant of Morin–DNA system is lower than Morin–Cu–DNA adduct (Table 1). The stronger binding of Morin–Cu complex to DNA may be largely due to the increase of the molecular planarity of the complex and the decrease of the collision frequency of the solvent molecules with the complex which caused by the planar aromatic group of the complex stacks between adjacent base pairs of DNA. This phenomenon clearly suggests that Morin–Cu complex may fully intercalate into ds-DNA showing the Morin–Cu complex has more antitumor activity than of Morin.

### Table 1

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Slope ($\mu$A mV$^{-1/2}$ s$^{1/2}$)</th>
<th>$D_i$ (cm$^2$ s$^{-1}$)</th>
<th>$D_a$ (cm$^2$ s$^{-1}$)</th>
<th>$\log K$</th>
<th>$\Delta G$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morin–DNA</td>
<td>0.0173</td>
<td>1.24 × 10$^{-7}$</td>
<td>$5.3 \times 10^{-8}$</td>
<td>5.19</td>
<td>5.05</td>
</tr>
<tr>
<td>Morin–Cu–DNA</td>
<td>0.052</td>
<td>3.06 × 10$^{-8}$</td>
<td>$6.6 \times 10^{-9}$</td>
<td>5.36</td>
<td>5.06</td>
</tr>
<tr>
<td>Morin–β-CD</td>
<td>0.0234</td>
<td>1.24 × 10$^{-7}$</td>
<td>$9.7 \times 10^{-8}$</td>
<td>2.45</td>
<td>2.32</td>
</tr>
<tr>
<td>Morin–β-CD–DNA</td>
<td>0.0127</td>
<td>9.7 × 10$^{-8}$</td>
<td>$2.85 \times 10^{-8}$</td>
<td>5.50</td>
<td>5.07</td>
</tr>
</tbody>
</table>

$a$: from the result of voltammetry and $b$: from the result of spectroscopy.

### 3.3. Formation of Morin–β-CD inclusion complex

The square wave voltammograms of Morin in absence and the presence of β-CD were recorded as a function of potential at pH 3.2 (Fig. 7). In the presence of the host molecule, the peak potential, as well as the peak current of Morin is modified. With the increase in the amounts of β-CD, the cathodic peak potentials shifted to a negative direction, and at the same time, the cathodic peak current decreased. The reduction peak shifted to more negative potential by 167 mV in the presence of β-CD revealed that the reduction of Morin molecules became more difficult when they included into the cavity of β-CD to form an inclusion complex [26]. The decrease of the peak current observed upon addition of β-CD is due to the lower diffusion coefficient of Morin–β-CD complex compared to that of the free guest (Table 1). The slope of the linear plot of $i_p$ versus $v^{1/2}$ without β-CD (0.0265 $\mu$A mV$^{-1/2}$ s$^{1/2}$) was more than that with β-CD (0.0234 $\mu$A mV$^{-1/2}$ s$^{1/2}$), indicating...
that the diffusion coefficient of the free form of Morin \((D_t = 1.24 \times 10^{-7} \text{ cm}^2 \text{s}^{-1})\) was larger than that of the complex form of Morin with \(\beta\)-CD \((D_b = 9.7 \times 10^{-8} \text{ cm}^2 \text{s}^{-1})\). At higher concentration of \(\beta\)-CD two peaks are observed, denoted \(P_1\) and \(P_2\) located at ca. \(-533\) and \(-683\) mV, respectively. The intensity of the \(P_1\) peak is always less than \(P_2\) peak. The peak \(P_1\) reflects capacitive changes as reorientation process of the inclusion complex, which occurs before its reduction. In the presence of different concentration of \(\beta\)-CD the cathodic \(P_1\) potentials, as well as the peak currents, were considerably influenced. Thus result reveals that Morin is more difficult to reduce in the presence of \(\beta\)-CD as a result of the Morin entering the hydrophobic cavity with lower polarity relative to the bulk solution. Also, the peak current is diminished due to the decrease of the diffusion coefficient of the host–guest associated compared to that of the free guest, as discussed above. The decrease in the peak current \((P_0)\) enables us to calculate the formation constant of the complex formed between Morin and the studied host molecules. The \(K\) values could therefore be calculated from Eq. (1). According to this equation the plot of \(\log ([1/\beta\text{-CD}])\) versus \(\log ([IC–G]/([IC–G] – [IH–G]))\) is linear with good correlation coefficient \((R ≥ 0.994)\), indicating that 1:1 complex is formed in Morin–\(\beta\)-CD system. The \(K\) value obtained by this approach is shown in Table 1. UV–vis spectra was also used to testify the formation of the inclusion complex. The absorption spectra of Morin upon increasing the concentration of \(\beta\)-CD showed increase in the absorbance intensity but does not cause a shift in \(\lambda_m\) (Fig. 8). These changes in the absorbance values are believed to result from changes in the solvent microenvironment upon inclusion of the solute. The binding constant of Morin–\(\beta\)-CD was determined from spectrophotometric experiments (Table 1). There is fair agreement, within experimental error, between \(K\) values of Morin–\(\beta\)-CD obtained from both voltammetric and spectrophotometric techniques.

The formation of the inclusion complex of Morin with \(\beta\)-CD has also been characterized by the phase solubility. An increase in the solubility of the complex with respect to the free form was observed. In aqueous solution of Morin–\(\beta\)-CD complexes, the free drug molecules are in equilibrium with the drug molecules entrapped within cavity. Thus, on increasing the concentration of \(\beta\)-CD more Morin molecules will transfer from the aqueous solution to hydrophobic cavity of \(\beta\)-CD.

The apparent binding constant \((K = 1.10 \times 10^2 \text{ M}^{-1})\) of the formed complex was calculated from the phase solubility diagram according to the following equation \([17,27]\):

\[
K = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})}
\]

using the slope and the intercept of the phase solubility diagram.

The Gibbs free energies of transfer of Morin from aqueous solution to the cavity of \(\beta\)-CD have been calculated from the following equation:

\[
\Delta G_{\text{trans}} = -RT \ln \left( \frac{S}{S_0} \right)
\]

where \(S\) and \(S_0\) are solubilities of Morin in presence and absence of \(\beta\)-CD, respectively. The \(-\Delta G_{\text{trans}}\) values are 0.0465, 0.1147, 0.1459, 0.233 kJ mol\(^{-1}\) at \(\beta\)-CD concentrations 0.2, 0.4, 0.6, 0.9 mM, respectively. The obtained values are negative and increase negativity with increasing \(\beta\)-CD concentration. This indicates that the transfer process is spontaneous and supports the view that \(\beta\)-CD is more favorable environment than water for Morin.

3.4. Interaction of the inclusion complex of Morin–\(\beta\)-CD with DNA

The aforementioned results indicate that, on increasing the concentration of \(\beta\)-CD more Morin molecules will transfer from the aqueous solution to hydrophobic cavity of \(\beta\)-CD to form inclusion complex. If the groups included by \(\beta\)-CD are just those that interact with or intercalate into DNA, the inclusion action must affect the interaction, which could be embodied in the change of its electrochemical characteristics. Therefore the CV of Morin–\(\beta\)-CD in presence of DNA are shown in Fig. 9. It was observed that, in presence of DNA, the redox potential of Morin–\(\beta\)-CD complex shifted to a more negative value and the peak currents of CVs decrease with increasing concentration of ds-DNA. The peak potential of CV waves shifts to more positive or negative values, indicating that the action of Morin–\(\beta\)-CD complex with DNA may be intercalation \([24,25]\). If the inclusion complex of Morin–\(\beta\)-CD decomposed in the presence of DNA, the redox potential would have shifted to a more positive value at which Morin was oxidized or reduced. So, it is a reasonable interpretation that the inclusion complex does not decompose while it binds to DNA. Accordingly to the experimental data, linear equation of log \((1/[DNA])\) versus log \((IC–G/(IC–G – IH–G))\) was obtained.
The linear correlation coefficient ($r$) was 0.993. According to the $y$ intercept of the linear equation, the apparent binding constant of Morin–β-CD complex with DNA was obtained (Table 1).

The absorption spectra of Morin–β-CD complex upon increasing the concentration of DNA is more interesting. As the concentration of ds-DNA was increased, the absorption spectra of Morin–β-CD complex decreased and a new absorption band appeared at $\Delta m = 425$ nm which is the characteristic spectra of Morin–β-CD–DNA adduct (Fig. 10). Moreover, an isobestic point appeared at $\Delta m = 375$ nm, which indicates the formation of the new adduct of Morin–β-CD–DNA. Therefore UV–vis spectra confirmed the formation of a new adduct of Morin–β-CD with DNA at pH 3.2. Through comparing the changes of voltammetric and spectroscopic characteristics of Morin–β-CD complex upon the addition of DNA, the conclusion can be drawn that the Morin–β-CD complex interacting with DNA via intercalation forms an electrochemically inactive system, which should be called a supramolecular complex. This complex cannot be reduced on the electrode surface. Based on the variation in the absorbance, the binding constant of Morin–β-CD–DNA system was determined (Table 1) according to the Eq. (2), which is in agreement, within the experimental error, with those obtained electrochemically at the HMDE.

The large binding constant ($K$) observed for Morin–β-CD–DNA supracomplex if compared to that of Morin–DNA complex is attributed to a mode of binding that involves a strong stacking interaction between the Morin–β-CD and the base pairs of DNA. The binding affinity increases in the sequence: Morin–β-CD–DNA > Morin–Cu–DNA > Morin–DNA.

The change in Gibbs free energy values ($\Delta G$) for the binding of Morin and its complexes with DNA were calculated from the well-known relationship:

$$\Delta G = -RT \ln K \quad (5)$$

In comparison the $\Delta G$ values (Table 1) for the binding process of Morin and its complexes with DNA, it can be seen that the observed Gibbs energy value of Morin–β-CD–DNA supramolecular complex is more negative. This indicates that the binding process of Morin–β-CD with DNA is more favorable.

The binding constant of Morin–β-CD–DNA supramolecular complex at different temperatures was studied using CV voltammetry. It can be observed that the value of $K$ increases as the temperature increases, revealing the influence of temperature on stability of the complex (Table 2). Gibbs free energy of Morin–β-CD–DNA is negative and increases negatively with increasing the temperature. The results allow other information to be obtained, as the thermodynamical parameters involved in the complex formation. The integrated form of Van’t Hoff equation (Eq. (6)) permits to calculate the values of enthalpy and entropy changes depending on variation of the binding constant with temperature [27].

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (6)$$

The Van’t Hoff plot for the supramolecular complex of Morin–β-CD–DNA shows a linear behavior. The relative thermodynamic parameters were calculated and following results were obtained: $\Delta H = 14.17$ kJ mol$^{-1}$ and $\Delta S = 152.97$ J K$^{-1}$ mol$^{-1}$. The positive value of enthalpy change indicates that the interaction process of Morin–β-CD with ds-DNA is endothermic. The change of entropy is also positive. This behavior indicates that the complexation causes an increase in transitional and rotational degrees of freedom of the complexed molecular.

On comparison of the binding affinity and thermodynamic parameters of antitumor Morin, Morin–Cu and Morin–β-CD

<table>
<thead>
<tr>
<th>System</th>
<th>$K$ ($10^3$ M$^{-1}$) @278 K</th>
<th>$K$ ($10^3$ M$^{-1}$) @298 K</th>
<th>$K$ ($10^3$ M$^{-1}$) @308 K</th>
<th>$-\Delta G$ (kJ mol$^{-1}$) @278 K</th>
<th>$-\Delta G$ (kJ mol$^{-1}$) @298 K</th>
<th>$-\Delta G$ (kJ mol$^{-1}$) @308 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morin–DNA</td>
<td>0.98</td>
<td>1.15</td>
<td>1.58</td>
<td>1.77</td>
<td>26.55</td>
<td>27.88</td>
</tr>
<tr>
<td>Morin–Cu–DNA</td>
<td>1.44</td>
<td>1.69</td>
<td>2.29</td>
<td>2.49</td>
<td>27.44</td>
<td>28.81</td>
</tr>
<tr>
<td>Morin–β-CD–DNA</td>
<td>2.21</td>
<td>2.65</td>
<td>3.2</td>
<td>4.02</td>
<td>28.42</td>
<td>29.88</td>
</tr>
</tbody>
</table>

**Table 2**

Binding constant ($K$) and standard Gibbs free energy ($\Delta G$) of Morin–DNA, Morin–Cu–DNA and Morin–β-CD–DNA systems calculated from the results of cyclic voltammetry at different temperatures.
complex with DNA we observed the following tendencies (Tables 2 and 3). Morin–Cu complex or the inclusion complex of Morin–β-CD with DNA displaced high-binding affinity with the host molecule than that of Morin. At the same time the magnitude of changes in ΔG, ΔH and ΔS indicated that the binding process of Morin complexes with DNA is more favorable and spontaneous than that of Morin. In conclusion the results presented here show that different biological and antitumor activity of Morin and its complexes yield distinct changes in their binding affinity with DNA.

4. Conclusion

In this paper a systematic comparative study of the binding of antitumor Morin, Morin–Cu and Morin–β-CD complexes with DNA has been investigated. Based on our investigation, although both Morin and its complexes can bind to DNA, the nature of binding was found to be different for each of them. Morin molecule, acting as intercalator, is inserted into cavity of the β-CD as well as into the base stacking domain of the DNA double helix. Through comparing the changes of voltammetric and spectroscopic characteristics of Morin complexes upon the addition of DNA, it can be drawn that Morin–Cu and Morin–β-CD complexes interacting with DNA via intercalation forms an electrochemically inactive adduct which should be called a supramolecular complex. The results offer an opportunity to understand how the structure of the molecules affects their binding and affinity of binding to DNA. This will help to design new complexes, which have biological and antitumor activities.

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References