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Spectroscopic studies on the interaction of morin–Eu(III) complex with calf thymus DNA

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ABSTRACT

The interaction between morin–Eu(III) complex and calf thymus DNA in physiological buffer (pH 7.4) was investigated using UV–vis spectrophotometry, fluorescence spectroscopy, viscosity measurements and DNA melting techniques. Hypochromicity and red shift of the absorption spectra of morin–Eu(III) complex were observed in the presence of DNA, and the fluorescence intensity of morin–Eu(III) complex was greatly enhanced with the addition of DNA. Moreover, fluorescence quenching and blue shift of the emission peak were seen in the DNA–ethidium bromide (EB) system when morin–Eu(III) complex was added. The relative viscosity of DNA increased with the addition of morin–Eu(III) complex, whereas the value of melting temperature of DNA–EB system decreased in the presence of morin–Eu(III) complex. All these results indicated that morin–Eu(III) complex can bind to DNA and the major binding mode is intercalative binding. The 3:1 morin:Eu(III) complex (estimated binding constant = 2.36×10^6 L mol^{-1}) is stabilized by intercalation into the DNA. The calculated binding constants of morin–Eu(III) complex with DNA at 292, 301 and 310 K were 7.47×10^4, 8.89×10^4 and 1.13×10^5 L mol^{-1}, respectively. The thermodynamic parameters were also obtained: ΔH^{θ} was 20.14 kJ mol^{-1} > 0 and ΔS^{θ} was 161.70 J mol^{-1} K^{-1} > 0, suggesting that hydrophobic force plays a major role in the binding of morin–Eu(III) complex to DNA.

1. Introduction

The interaction of drug molecules with DNA has become an active research area in recent years [1]. Because the intracellular target for a wide range of anticancer and antibiotic drugs is DNA [2–4], illustrating the binding between small molecules and DNA can greatly help understand drug–DNA interactions and design new and promising drugs for clinical use. Generally, a variety of small molecules interacts reversibly with DNA, primarily through three modes: (i) intercalative binding that small molecules intercalate into the base pairs of nucleic acids [5]; (ii) groove binding in which the small molecules bound on nucleic acids are located in the major or minor groove [5]; (iii) long-range assembly on the molecular surfaces of nucleic acids so that the small molecules are not related to the groove structure of the nucleic acids [6]. The intercalative binding is stronger than other two binding modes because the surface of intercalative molecule is sandwiched between the aromatic, heterocyclic base pairs of DNA [7,8].

Morin(2′,3,4′,5,7-pentahydroxyflavone, Fig. 1), a flavonoid compound, is present in tea, coffee, cereal grains, fruits, vegetables and many traditional Chinese herbal medicines [9]. It has been shown in various biological and pharmacological activities, including anti-inflammatory, antioxidant, anticancer and cardiovascular protection [10–12].

Morin is known to complex with many metal ions to form stable compounds. In recent years, tremendous interest has been drawn to interactions between transition metal complexes of morin and nucleic acids due to potential applications of the metal complexes as anticancer drugs or as complexes with other biological functions [13,14]. Some authors reported that morin, Zn(II) and Cu(II) complexes of morin can bind to DNA, respectively, but the binding mode is different. The complexes bind to DNA mainly by intercalating mode, while morin binds in a non-intercalating mode [15,16]. Moreover, the complexes show higher antitumor activities than that of morin [17]. Rare earth metals not only have more physiological activities, but also their toxicities are decreased after coordinating with a ligand [18]. Zhou et al. found that the antitumour activities of rare earth metal complexes of quercetin with La(III), Eu(III) and Gd(III) are superior to quercetin, the major binding mode of quercetin–La(III) complex with DNA is intercalative binding [19]. Rare earth metals and their complexes as chemical nucleases are superior to transition metals and their complexes because they can bind to nucleic acid more efficiently by hydrogen bonding and hydrolyzed mode [20]. Whereas, so far the interactions between rare earth metal complexes of morin with DNA have seldom been reported.

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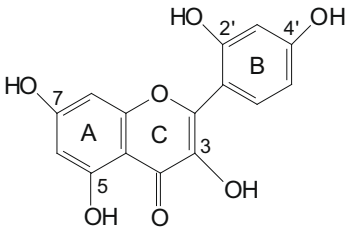

Fig. 1. Molecular structure of morin.

A number of techniques have been employed to study the interaction of drugs with DNA, including fluorescence spectroscopy [21], UV-spectrophotometry [22], electrophoresis [23], nuclear magnetic resonance [24], electrochemical methods [25], etc. UV–vis absorption and fluorescence spectroscopy are regarded as effective methods among these techniques because they are sensitive, rapid and simple [26].

In this work, we used UV–vis absorption, fluorescence spectroscopy, viscosity measurements and DNA melting techniques to explore the interaction between morin–Eu(III) complex and calf thymus DNA. We believe this will be helpful to further understand the mechanism of interactions between DNA and morin's rare earth metal complexes as well as further understand morin's pharmacological effects. The knowledge gained from this study should be useful for the development of potential probes for DNA structure and new therapeutic reagents for tumours and other diseases.

2. Materials and methods

2.1. Apparatus

UV–vis absorption spectra were measured on a Shimadzu UV-2450 spectrophotometer using a 1.0 cm cell. Fluorescence measurements were performed with a Hitachi spectrofluorimeter Model F-4500 equipped with a 150 W Xenon lamp and a thermostat bath, using a 1.0 cm quartz cell. The widths of both the excitation slit and emission slit were set at 5.0 nm, and the scan rate at 1200 nm min^{-1}. pH measurements were carried out with a pHS-3C digital pH-meter (Shanghai Exact Sciences Instrument Co. Ltd., Shanghai, China) with a combined glass-calomel electrode. The viscosity measurements were carried out using an NDJ-79 viscosity meter (Yinhua Flowmeter Co. Ltd., Hangzhou, China). An electronic thermostat water-bath (Shanghai Yuejin Medical Instrument Company, Shanghai, China) was used for controlling the temperature. All experiments, unless specified otherwise, were carried out at room temperature (25 ± 1 °C).

2.2. Materials

A stock solution (3.0×10^{-3} mol L^{-1}) of morin (Sigma Chem. Co., USA) was dissolved in anhydrous methanol. Calf thymus DNA (Sino-American Biotechnology Company, Beijing, China) was used without further purification, and its stock solution was prepared by dissolving appropriate solid DNA into doubly distilled water and stored at 4 °C. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient ε_{260} = 6600 L mol^{-1} cm^{-1} [27]. Purity of the DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of >1.8 at A_{260}/A_{280}, indicating that DNA was sufficiently free from protein [28]. The stock solution of Eu(III) (1.0×10^{-3} mol L^{-1}) was prepared by dissolving its oxide, Eu_2O_3 (99.95%, Fourth Reagent Factory, Shanghai, China) in a minimum amount of hydrochloric acid and diluted by doubly distilled water. Ethidium bromide (EB) stock solution (1.34×10^{-3} mol L^{-1}) was prepared by dissolving its crystals (Sigma Chem. Co., USA) in doubly distilled water and stored in a cool and dark place. All the solutions were adjusted with the Tris–HCl buffer solution (0.05 mol L^{-1}, pH 7.4), which was prepared by mixing and diluting 25 mL of Tris solution (0.2 mol L^{-1}) with 45 mL of HCl (0.1 mol L^{-1}) to 100 mL. All chemicals were of analytical reagent grade, and doubly distilled water was used for the preparation of the test solutions.

2.3. Procedures

2.3.1. Interaction between morin and Eu(III)

The molar ratio method introduced by Yoe and Jones [29] allows us to deduce the composition of complexes in solution from the spectrophotometric spectra. For this purpose, solutions containing a constant concentration (4.2×10^{-5} mol L^{-1}) of morin and variable concentration of Eu(III) (from 1.5×10^{-6} mol L^{-1} to 8.4×10^{-5} mol L^{-1}) were prepared.

2.3.2. Binding of morin–Eu(III) complex with DNA in the presence of EB

An aliquot from each of the DNA solutions in a concentration series, was added to a 2.0 mL solution mixture consisting of morin at a given concentration and Eu(III) in a 3:1 ratio adjusted by the Tris–HCl buffer (pH 7.4). Each sample mixture was combined in a 10 mL volumetric flask, and diluted to the mark with doubly distilled water. After these solutions were allowed to stand for 10 min to equilibrate, the UV–vis measurement was then made.

The competitive interaction between EB and morin–Eu(III) complex with DNA was carried out as follows: fixed amounts of the EB and DNA were titrated with increasing amounts of morin–Eu(III) complex solution. The changes in fluorescence intensities, as appropriate, were monitored against a blank after each addition of the morin–Eu(III) complex.

2.3.3. Viscosity measurements

Viscosity measurements were performed using a viscometer, which was immersed in a thermostat water-bath at 25 ± 0.1 °C. Typically, 10.0 mL of buffer and 10.0 mL of DNA solution were transferred into the viscometer separately. An appropriate amount of morin–Eu(III) complex was then added into the viscometer to give a certain r(r = [drug]/[DNA]) value while keeping the DNA concentration constant. After a thermal equilibrium was achieved (15 min), the flow times of the samples were repeatedly measured with an accuracy of ±0.2 s by using a digital stopwatch. Flow times were above 200 s, and each point measured was the average of at least five readings. The data were presented as $(\eta/\eta_0)^{1/3}$ versus r, where η and η_0 are the viscosity of DNA in the presence and absence of the complex, respectively.

2.3.4. DNA melting studies

DNA melting experiments were carried out by monitoring the fluorescence intensities of the sample at different temperatures in the absence and presence of the morin–Eu(III) complex. The temperature of the sample was continuously monitored with a thermocouple attached to the sample holder. The fluorescence intensities were then plotted as a function of temperature ranging from 20 to 100 °C. The melting temperature (T_m) of DNA was determined as the transition midpoint.

3. Results and discussion

3.1. Interaction between morin and Eu(III)

The changes in UV–vis absorption of morin in the presence of Eu(III) (with increasing concentration) were examined in the

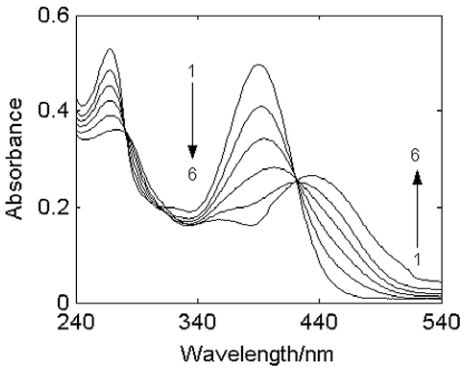

Fig. 2. Absorption spectra of morin in the presence of Eu(III) at pH 7.4. $c_{Eu(III)}$ = 0, 0.3, 0.6, 0.9, 1.2 and 2.1 × 10^{-5} mol L^{-1} for curves 1–6; c_{morin} = 4.2 × 10^{-5} mol L^{-1}.

Tris–HCl buffer solution (pH 7.4) (Fig. 2). The UV–vis spectra of morin showed an intense absorbance at 390 nm (band I) and 265 nm (band II) (curve 1). Band I located in the wavelength range of 300–400 nm is related to conjugated system between ring B and carbonyl of ring C, and band II located in the wavelength range of 240–300 nm is related to conjugated system between ring A and carbonyl of ring C (Fig. 1) [30,31]. When the solution of Eu(III) was added, band I gradually shifted to longer wavelengths, accompanied with decrease in absorption. Simultaneously, a new stronger absorbance peak appeared at 431 nm (curve 6). The results indicated formation of a complex between morin and Eu(III). The observation of an isobestic point at 422 nm suggested the existence of a fairly simple equilibrium in solution that involved only two dominant species: the free and complexed molecules. There are two possible chelating sites on morin that can interact with Eu(III): the 3- or 5-hydroxyls, and the 4-carbonyl. Presence of the peak at 265 nm demonstrated that ring A remained unchanged. The appeared new peak at 431 nm suggested that Eu(III) had bonded to 3-hydroxyl and 4-carbonyl of ring C [32]. In deed, a great number of proofs clearly indicated that this complex involves the 3-hydroxyl-4-keto group: (i) among 3-hydroxyl and 5-hydroxyl molecules, 3-hydroxyl has greater chelation power; (ii) the delocalization of the oxygen electrons of 3-hydroxyl group is higher than 5-hydroxyl, which facilitates the delocalization of the π electrons [33,34]. Band I bathochromic shift can be explained by the interaction of Eu(III) with the 3-hydroxyl group of morin resulting in electronic redistribution between the morin molecule and Eu(III) to become a big extended π bond system. Electron distribution in morin changed from n–π transition to π–π* transition and the energy of this electron transition is decreased. Morin can attract p bond electron cloud to orientate by itself and make p bond electron cloud excurse and polarize, which lead to difference of π–π* energy level decreasing and absorbance bond red shifting. The effect of hauling electrons caused by the bond of Eu(III) with morin results in a lower absorbance at 265 nm. This rationale is also in accordance with previous work, which demonstrated that the 3-hydroxyl and 4-carbonyl structure in ring C of morin is important for rare earth metal chelating [19,35]. The curves of absorbance versus [Eu(III)]/[morin] molar ratio plotted at 390 and 431 nm allow us to find a stoichiometry of 1:3 for the complex (Fig. 3). A threefold excess of Eu(III) is necessary to obtain a full complexation of morin, more important excess of Eu(III) has no effect on the absorbance value. According to Job's method [36], the binding constant of morin–Eu(III) complex is calculated to be 2.36 × 10^6 L mol^{-1}.

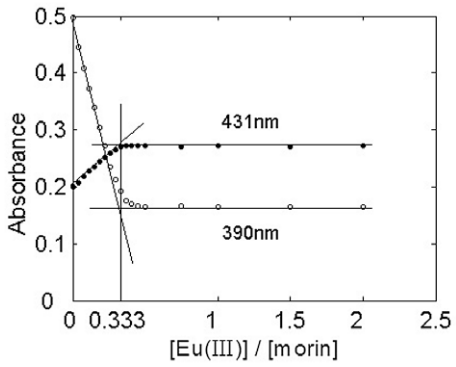

Fig. 3. Absorbance versus [Eu(III)]/[morin] molar ratio plot at 390 and 431 nm.

3.2. Binding characteristics of morin–Eu(III) complex with DNA

3.2.1. UV–vis absorption spectroscopy

In general, if a small molecule interacts with DNA, changes in absorbance (hypochromism) and in the position of the band (red shift) should occur. This phenomenon indicates that the small molecule has intercalated into DNA base pairs, and is involved in a strong interaction in the molecular stack between the aromatic chromophore and the base pairs. The spectral effects have been rationalized as follows [37,38]: the empty π*-orbital of the small molecule couples with the π*-orbital of the DNA base pairs, which causes an energy decrease, and a decrease of the π–π* transition energy. Therefore, the absorption of the small molecule should exhibit a red shift. At the same time, the empty π*-orbital is partially filled with electrons to reduce the transition probability, which leads to hypochromism.

In this work, UV–vis absorption spectra were obtained by titration of 3.5 × 10^{-5} mol L^{-1} morin–Eu(III) complex solution with increasing concentration of DNA (Fig. 4). In the absence of DNA, the spectra of the morin–Eu(III) complex (curve 1) was characterized by a peak at 431 nm. With the increase of DNA concentration, the absorption spectra showed clear hypochromicity at the maximum of 431 nm together with a red shift of $\Delta\lambda$ = 8 nm. The hypochromicity and red shift in the absorption spectra of morin–Eu(III) complex indicated the intercalative binding of the morin–Eu(III) complex to DNA bases.

3.2.2. Fluorescence studies

The fluorescence spectra of morin–Eu(III) complex is shown in Fig. 5. As can be seen from Fig. 5, the fluorescence intensity of morin–Eu(III) complex was dramatically enhanced when the DNA was added, which indicated morin–Eu(III) complex could bind to DNA. The observation reflected that the stronger enhancement for morin–Eu(III) complex may be largely due to the increase of the

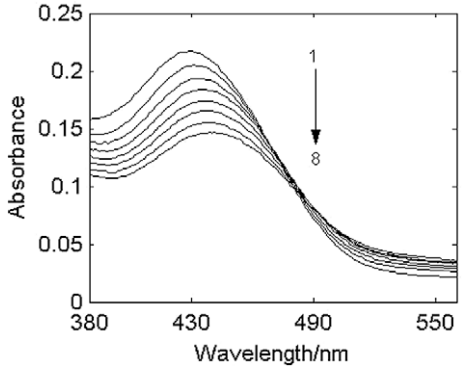

Fig. 4. Absorption spectra of morin–Eu(III) complex in the presence of DNA at pH 7.4. c_{DNA} = 0, 0.71, 1.42, 2.13, 2.84, 3.55, 4.26 and 4.97 × 10^{-5} mol L^{-1} for curves 1–8; $c_{morin-Eu(III)}$ = 3.5 × 10^{-5} mol L^{-1}.

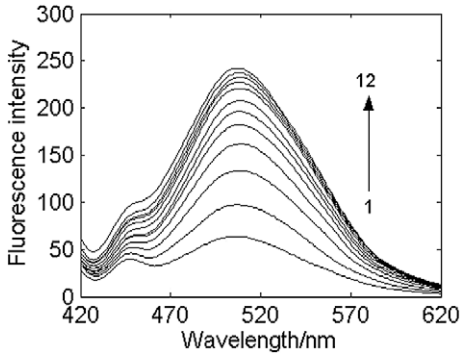

Fig. 5. Fluorescence spectra of morin–Eu(III) complex in the presence of DNA (pH 7.4, T = 292 K, λ_{ex} = 385 nm, λ_{em} = 509 nm). c_{DNA} = 0, 0.71, 1.42, 2.13, 2.84, 3.55, 4.26, 4.97, 5.68, 6.39, 7.10 and 7.81×10^{-5} mol L^{-1} for curves 1–12; $c_{morin-Eu(III)}$ = 3.5×10^{-5} mol L^{-1}.

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. The increase in the molecular planarity and the decrease of the collision frequency between solvent molecules with the complexes usually lead to emission enhancement [39]. The binding of morin–Eu(III) complex to DNA leading to marked increase in emission intensity also agrees with those observed for other intercalators [40].

In order to see the interaction between morin–Eu(III) complex and DNA, the binding constant was determined from the fluorescence intensity based on the following equation [41]:

$$1/\Delta F = 1/\Delta F_{max} + (1/K[L])(1/\Delta F_{max}) \quad (1)$$

where $\Delta F = F_x - F_0$ and $\Delta F_{max} = F_\infty - F_0$. F_0, F_x and F_∞ are the fluorescence intensities of morin–Eu(III) complex in the absence of DNA, at an intermediate concentration of DNA, and at the saturation of interaction, respectively; K is the binding constant and [L] refers to the DNA concentration. The linearity in the plot of $1/(F_x - F_0)$ against 1/[L] confirmed a one-to-one interaction between the two partners (Fig. 6). According to the above equation, the values of $K\Delta F_{max}$ and ΔF_{max} are obtained from the slope and intercept of the linear plot, and the value of K can be calculated by dividing intercept to slope. The values of K at three temperatures (292, 301, 310 K) are shown in Table 1.

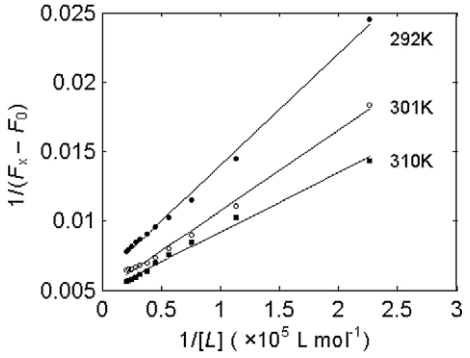

Fig. 6. The plots of $1/(F_x - F_0)$ vs 1/[L]. $c_{morin-Eu(III)} = 3.5 \times 10^{-5}$ mol L^{-1}.

Table 1
Binding constants and thermodynamic parameters of the interaction of morin–Eu(III) complex with DNA at different temperatures.

T (K)	K (L mol^{-1})	R[a]	SD[b]	ΔH^θ (kJ mol^{-1})	ΔS^θ (J mol^{-1} K^{-1})	ΔG^θ (kJ mol^{-1})
292	7.47×10^4	0.9955	0.024			−27.08
301	8.89×10^4	0.9975	0.028	20.14	161.70	−28.53
310	1.13×10^5	0.9981	0.027			−29.99

[a] R is correlation coefficient.
[b] SD is standard deviation.

3.2.3. Competitive interaction of morin–Eu(III) complex and EB with DNA

Further support for the mode of binding between morin–Eu(III) complex and DNA is given through the competitive experiment. Here ethidium bromide (EB) has been employed in the examination of the reaction, as EB presumably binds initially to DNA by intercalation [13,42]. The experiment was carried out in 3 mL of 6.0×10^{-6} mol L^{-1} EB and 7.0×10^{-5} mol L^{-1} DNA (at saturating binding levels [43]) titrated with 6.0×10^{-4} mol L^{-1} morin–Eu(III) complex solution. Fig. 7 shows the emission spectra of the DNA–EB system in the absence and presence of morin–Eu(III) complex. When the concentration of morin–Eu(III) complex was added, a remarkable fluorescence decrease of DNA–EB system was observed at the maximum of 598 nm and blue shift of $\Delta\lambda$ = 5 nm. This phenomenon suggested that morin–Eu(III) complex substituted for EB in the DNA–EB system which led to a large decrease in the emission intensity of the DNA–EB system. These changes in the state of intercalation have been observed in several instances [44,45].

3.2.4. Viscosity measurements

Viscosity experiment is an effective tool to determine the binding mode of small molecules and DNA. A classical intercalation binding demands the space of adjacent base pairs to be large en-

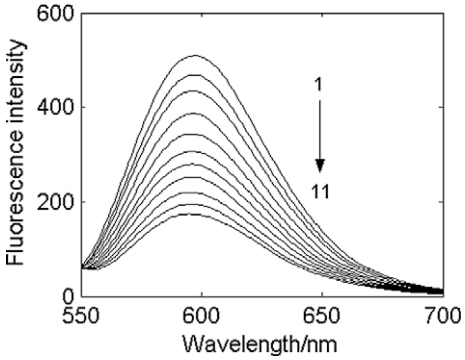

Fig. 7. Fluorescence spectra of DNA–EB system in the presence of morin–Eu(III) (pH 7.4, T = 292 K, λ_{ex} = 528 nm, λ_{em} = 598 nm). $c_{morin-Eu(III)}$ = 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0×10^{-6} mol L^{-1} for curves 1–11; $c_{EB} = 6.0 \times 10^{-6}$ mol L^{-1}, and $c_{DNA} = 7.0 \times 10^{-5}$ mol L^{-1}.

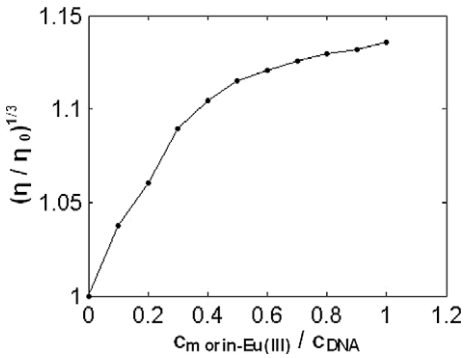

Fig. 8. Effect of increasing amount of bound ligand on the relatively viscosity of DNA.

ough to accommodate the bound ligand and to elongate the double helix, resulting in an increase of DNA viscosity [46]. A series of solutions was made which contained a fixed concentration of DNA and various concentrations of morin–Eu(III) complex. Then, the viscosity measurements were conducted at room temperature (avoiding from light after a 48 h reaction). The changes in relative viscosity of DNA with increasing concentrations of morin–Eu(III) complex are shown in Fig. 8. It can be seen that the relative viscosity of DNA increased steadily with increasing the amounts of the morin–Eu(III) complex. Such behavior further suggested that an intercalation binding should be the interaction mode of the morin–Eu(III) complex with DNA.

3.2.5. Melting studies

UV–vis absorption spectroscopy is a general method for determining the melting temperatures (T_m) [47,48]. Considering the higher sensitivity, the fluorescence technique is used to determine T_m in this experiment. With the increasing temperature, DNA denatures and the double helixes unfold. The interaction of classical intercalators with DNA, such as EB, can increase the stability of helix of DNA, and cause the T_m of DNA to increase [49,50].

The T_m of the complexes of DNA-EB and morin–Eu(III)–DNA–EB system were determined, respectively, by monitoring the maximum fluorescence of the system as a function of temperature ranging from 20 to 100 °C. For each monitored transition, the T_m of tested solution was determined as the transition midpoint of the melting curve. The melting curves are shown in Fig. 9. It can be seen that the T_m of DNA–EB system in the absence of morin–Eu(III) complex is 88 ± 1 °C under the experimental conditions. The observed melting temperature of DNA–EB system in the presence of morin–Eu(III) complex is 82 ± 1 °C. Heat and alkali can destroy the double helix structure of DNA and change it into single helix at the melting temperature (T_m). Interaction of small molecules with double stranded DNA can influence T_m. The interaction of morin–Eu(III) complex with DNA may cause the T_m to be decreased. The change in T_m of DNA–EB complex after the addition of morin–Eu(III) complex reveals that morin–Eu(III) complex substituted for EB in the DNA–EB system to affect the binding of EB to DNA and to make the stability of DNA decrease [16]. The results reconfirmed that the binding mode of the morin–Eu(III) complex with DNA is intercalative binding.

3.2.6. Determination of the thermodynamic parameters

Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of a complex. Therefore, the thermodynamic parameters dependent on temperatures were analyzed in order to further characterize the interaction forces between morin–Eu(III) complex and DNA. The interaction forces between a small molecule and macromolecule mainly include hydrogen bonds, van der Waals force, electrostatic force and hydrophobic interaction force. The thermodynamic parameters of binding reaction are the main evidence for confirming the binding force.

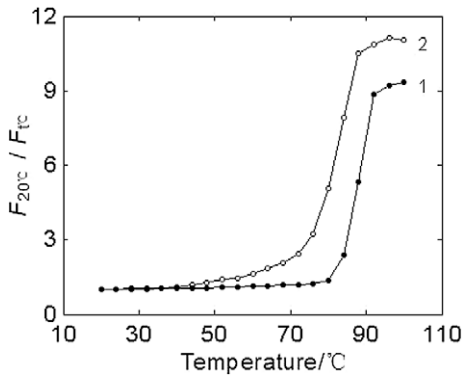

Fig. 9. Melting curves of DNA–EB in the absence (1) and presence (2) of morin–Eu(III) complex at pH 7.4. $c_{DNA} = 8.04 \times 10^{-5}$ mol L^{-1}, $c_{EB} = 6.70 \times 10^{-6}$ mol L^{-1} and $c_{morin-Eu(III)} = 6.70 \times 10^{-6}$ mol L^{-1}.

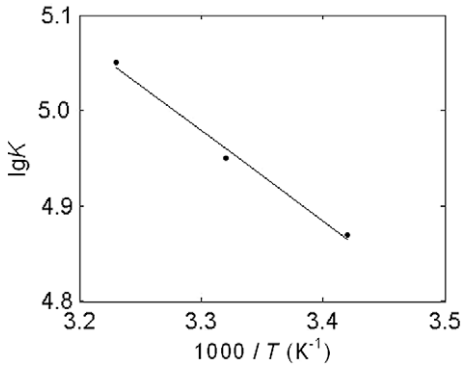

Fig. 10. The van't Hoff plot for the interaction of morin–Eu(III) complex with DNA.

If the enthalpy change (ΔH^θ) does not vary significantly over the temperature range studied, then its value and that of entropy change (ΔS^θ) can be determined from the van't Hoff equation:

$$\log K = -\frac{\Delta H^\theta}{2.303RT} + \frac{\Delta S^\theta}{2.303R} \quad (2)$$

where K and R are binding constant and gas constant, respectively. The temperatures used were 292, 301 and 310 K. The enthalpy change (ΔH^θ) and entropy change (ΔS^θ) were obtained from the slope and intercept of the linear van't Hoff plot based on log$K$ versus $1/T$ (Fig. 10). The free energy change (ΔG^θ) is estimated from the following relationship:

$$\Delta G^\theta = \Delta H^\theta - T\Delta S^\theta \quad (3)$$

The values of ΔH^θ, ΔS^θ and ΔG^θ are listed in Table 1. From Table 1, it can be seen that the negative value of ΔG^θ revealed the interaction process is spontaneous, while the positive ΔH^θ and ΔS^θ values associated with the interaction of morin–Eu(III) complex with DNA indicated that the binding is mainly entropy driven and the enthalpy is unfavorable for it. In other words, the hydrophobic interaction plays a major role in the binding [51].

4. Conclusion

The binding interactions of morin–Eu(III) complex with DNA in physiological buffer were illustrated with UV–vis and fluorescence spectroscopic techniques. The binding constants of morin–Eu(III) complex with DNA were measured at different temperatures and the thermodynamic parameters were calculated as well. The intercalative binding of morin–Eu(III) complex with DNA was deduced by taking account of relevant UV–vis absorption spectra, fluorescence spectra, viscosity measurements and melting temperature determinations. It was found that hydrophobic force plays a major role in the binding of morin–Eu(III) complex to DNA. We believe that the binding mode of morin–Eu(III) complex with DNA studied here will provide useful information on the mechanism of anticancer drugs binding to DNA and thus will be beneficial to new drug design.

Acknowledgements

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References

[1] S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman, M.A. Anwar, A.M. Khalid, J. Pharm. Biomed. Anal. 37 (2005) 205–217.
[2] L.H. Hurley, Nat. Rev. Cancer 2 (2002) 188–200.
[3] H. Mansouri-Torshizi, M. Islami-Moghaddam, A. Divsalar, A.A. Saboury, Bioorg. Med. Chem. 16 (2008) 9616–9625.
[4] B.K. Sahoo, K.S. Ghosh, R. Bera, S. Dasgupta, Chem. Phys. 351 (2008) 163–169.
[5] R.F. Pasternack, E.J. Gibbs, J.J. Villafranca, Biochemistry 22 (1983) 2406–2414.
[6] K.E. Erkkila, D.T. Odom, J.K. Barton, Chem. Rev. 99 (1999) 2777–2795.
[7] L.S. Ling, Z.K. He, F. Chen, Y.E. Zeng, Talanta 59 (2003) 269–275.
[8] X.Q. He, Q.Y. Lin, R.D. Hu, X.H. Lu, Spectrochim. Acta A 68 (2007) 84–190.
[9] J. Kuhnau, World Rev. Nutr. Diet 24 (1976) 117–191.
[10] S.H. Fang, Y.C. Hou, W.C. Chang, S.L. Hsiu, P.D. Chao, B.L. Chiang, Life Sci. 74 (2003) 743–756.
[11] D.P. Makris, J.T. Rossiter, J. Food Compos. Anal. 15 (2002) 103–113.
[12] Y. Iwase, Y. Takemura, M. Ju-ichi, T. Mukainaka, E. Ichiishi, C. Ito, H. Furukawa, M. Yano, H. Tokuda, H. Nishino, Cancer Lett. 173 (2001) 105–109.
[13] M.M. Hassanien, K.S. Abou-El-Sherbini, Talanta 68 (2006) 1550–1559.
[14] Z.H. Xu, F.J. Chen, P.X. Xi, X.H. Liu, Z.Z. Zeng, J. Photochem. Photobiol. A: Chem. 196 (2008) 77–83.
[15] Y.M. Song, J.W. Kang, J. Zhou, Z.H. Wang, X.Q. Lu, L.F. Wang, J.Z. Gao, Spectrochim. Acta A 56 (2000) 2491–2497.
[16] Y.M. Song, J.W. Kang, Z.H. Wang, X.Q. Lu, J.Z. Gao, L.F. Wang, J. Inorg. Biochem. 91 (2002) 470–474.
[17] Z. Qi, W.L. Fang, L. Xiang, J. Transit. Met. Chim. 21 (1996) 23–27.
[18] X.A. Chen, Q.C. He, T. Guan, Y.E. Cheng, H.F. Chen, B.k. Xiong, Chin. J. Rare Earths 1 (1995) 70–73.
[19] J. Zhou, L.F. Wang, J.Y. Wang, N. Tang, J. Inorg. Biochem. 83 (2001) 41–48.
[20] R.D. Hu, Q.Y. Lin, W. Huang, Q. Yu, J. Rare Earths 23 (2005) 372–376.
[21] J. Li, Y.L. Wei, L.M. Guo, C.H. Zhang, Y. Jiao, S.M. Shuang, C. Dong, Talanta 76 (2008) 34–39.
[22] Y.N. Ni, D.Q. Lin, S. Kokot, Anal. Chim. Acta 606 (2008) 9–25.
[23] F. Araya, G. Huchet, I. McGroarty, G.G. Skellern, R.D. Waigh, Methods 42 (2007) 141–149.
[24] K. Sandstrom, S. Warmlander, M. Leijon, A. Graslund, Biochem. Biophys. Res. Commun. 304 (2003) 55–59.
[25] L.P. Wang, L. Lin, B.X. Ye, J. Pharm. Biomed. Anal. 42 (2006) 625–629.
[26] S.Y. Bi, H.Q. Zhang, C.Y. Qiao, Y. Sun, C.M. Liu, Spectrochim. Acta A 69 (2008) 23–129.
[27] C.Z. Huang, Y.F. Li, P. Feng, Talanta 55 (2001) 321–328.
[28] J. Marmur, J. Mol. Biol. 3 (1961) 208–218.
[29] J.H. Yoe, A.L. Joens, Ind. Eng. Chem. (Anal. Ed.) 16 (1944) 111–115.
[30] J. Kang, Y. Liu, M.X. Xie, S. Li, M. Jiang, Y.D. Wang, Biochim. Biophys. Acta 1674 (2004) 205–214.
[31] L. Huang, D.Q. Yu, Application of Ultraviolet Spectroscopy in Organic Chemistry, Science Press, Beijing, 2000. p. 281.
[32] J.W. Kang, L. Zhuo, X.Q. Lu, H.D. Liu, M. Zhang, H.X. Wu, J. Inorg. Biochem. 98 (2004) 79–86.
[33] J.P. Cornard, J.C. Merlin, J. Inorg. Biochem. 92 (2002) 19–27.
[34] A.C. Boudet, J.P. Cornard, J.C. Merlin, Spectrochim. Acta A 56 (2000) 829–839.
[35] E. Woźnicka, M. Kopacz, M. Umbreit, J. Kłos, J. Inorg. Biochem. 101 (2007) 774–782.
[36] P. Job, Ann. Chim. Phys. 9 (1928) 113–203.
[37] E.C. Long, J.K. Barton, Acc. Chem. Res. 23 (1990) 271–273.
[38] S.A. Tysoe, R.J. Morgan, A.D. Baker, T.C. Strekas, J. Phys. Chem. 97 (1993) 1707–1711.
[39] X. Wu, C.Y. Guo, F. Wang, J.H. Yang, D.H. Ran, J.H. Zheng, J.B. Wu, Spectrochim. Acta A 65 (2006) 543–548.
[40] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Biochemistry 31 (1992) 9319–9324.
[41] J. Bhattacharya, M. Bhattacharya, A. Chakraborty, U. Chowdhury, R.K. Podder, Biochem. Pharmacol. 47 (1994) 2049–2052.
[42] S. Nafisi, A.A. Saboury, N. Keramat, J.F. Neault, H.A. Tajmir-Riahi, J. Mol. Struct. 827 (2007) 35–43.
[43] M. Selim, S.R. Chowdhury, K.K. Mukherjea, Int. J. Biol. Macromol. 41 (2007) 579–583.
[44] J.P. Rehman, J.K. Barton, Biochemistry 29 (1990) 1701–1710.
[45] J. Lan, Y. Pin, L.Q. Shan, Chem. J. Chin. Univ. 17 (1996) 1345–1348.
[46] L.S. Lerman, J. Mol. Biol. 3 (1961) 8–30.
[47] P. Nagababu, S. Satyanarayana, Polyhedron 26 (2007) 1686–1692.
[48] T.R. Li, Z.Y. Yang, B.D. Wang, D.D. Qin, Eur. J. Med. Chem. 10 (2007) 1–8.
[49] J.B. Chaires, Biochemistry 25 (1986) 8436–8439.
[50] C.V. Kumar, E.H. Asuncion, J. Am. Chem. Soc. 115 (1993) 8547–8553.
[51] D.P. Ross, S. Subramanian, Biochemistry 20 (1981) 3096–3102.