DNA-Binding Properties Studies and Spectra of a Novel Cu(II) Complex with a New Coumarin Derivative

Gao-fei QI, Zheng-yin YANG,* Dong-dong QIN, Bao-dui WANG, and Tian-rong LI

College of Chemistry and Chemical Engineering and State Key Laboratory of Applied Organic Chemistry, Lanzhou University; Lanzhou 730000, P. R. China.

Received October 7, 2007; accepted January 17, 2008; published online January 23, 2008

A new coumarin derivative (8-methylcoumaro-4a,10a-pyrone-3-carbaldehyde benzoyl hydrazone) ligand and its novel Cu(II) complex have been synthesized and characterized on the basis of elemental analyses, molar conductivities, ^{1}H-NMR, IR spectra, UV–visible spectroscopy and thermal analyses. In addition, the interactions of the Cu(II) complex and the ligand with calf-thymus DNA were investigated by spectrometric titrations, ethidium bromide displacement experiments and viscosity measurements. It was found that both the two compounds, specially the Cu(II) complex, strongly bind with calf-thymus DNA, presumably *via* an intercalation mechanism.

Key words Cu(II) complex; coumarin; interaction; calf-thymus DNA; intercalation mechanism

Binding studies of small molecules to DNA are very important in the development of DNA molecular probes and new therapeutic reagents.[1—3] Over the past decades, the DNA-binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron transfer probes, DNA footprinting and sequence-specific cleaving agents and potential anticancer drugs.[4—6] The interaction of metal complexes with DNA has been an area of intense interest to both inorganic chemists and biochemists. Many transition metal complexes, especially copper(II) complexes have been used as probes of DNA structure in solution.[7,8] So design of small copper(II) complexes that bind and react at specific sequences of DNA becomes important. Basically, metal complexes interact with the double helix DNA in either a non-covalent or a covalent way. The former way includes three binding modes: intercalation, groove binding, and external static electronic effects. Among these interactions, intercalation is one of the most important DNA binding modes as it invariably leads to cellular degradation. Intercalators usually have planar aromatic ring systems that occupy the space between two adjacent DNA base pairs. Examples of intercalators include ethidium bromide (EB), acridine orange, and doxorubicin.[9] Moreover, it was reported that the intercalating ability increases with the planarity of intercalators.[10,11] As a result, the elucidation of non-covalent interactions with DNA by small natural products and their synthetic derivatives have drawn a lot of attention from many researchers.[12—15]

In this paper, our work stems from our interest in synthesizing and evaluating the key DNA-binding interactions of a new coumarin derivative containing a large planar aromatic ring systems and its novel copper(II) complex. In our studies, the interaction of the two compounds with calf-thymus (CT) DNA was investigated using a host of physical methods like spectrometric titrations, ethidium bromide displacement experiments and viscosity measurements.

Experimental

Instrumentation Melting points were determined on a Beijing XT4-100X microscopic melting point apparatus. Elemental analyses (C, H, N) were carried out on an Elemental Vario EL analyzer. IR spectra were obtained in KBr discs on a Therrno Mattson FTIR spectrophotometer in the 4000—400 cm^{-1} region. ^{1}H-NMR spectra were recorded on a Varian VR 300-MHz spectrometer in DMSO-d_6 (dimethyl sulfoxide) with TMS (tetramethyl silane) as internal standard. Conductivity measurements were performed in DMF (*N*,*N*-dimethylformamide) with a DDS-11A conductometer at 25.0 °C. UV–visible spectra were recorded on a Shimadzu UV-240 spectrophotometer. Thermal behavior was monitored on a PCT-2 differential thermal analyzer. FAB-MS (fast atom bombardment mass spectrometry) was obtained on a VG ZAB-HS mass spectrometer. The fluorescence spectra were recorded on a Hitachi RF-4500 spectrofluorophotometer.

Materials and Methods Calf thymus DNA (CT-DNA) and EB (ethidium bromide) were purchased from Sigma Chemical Co. (Saint Louis, MO, U.S.A.). EDTA and $CuCl_2 \cdot 2H_2O$ were produced in China. All chemicals used were of analytical grade. All the experiments involving the interaction of the complexes with CT-DNA were carried out in doubly distilled water buffer containing 5 mM Tris [Tris(hydroxymethyl)-aminomethane] and 50 mM NaCl and adjusted to pH 7.1 with HCl. The solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8—1.9 : 1 at 260 and 280 nm, indicating that the CT-DNA was sufficiently free of protein.[16] The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 $M^{-1} cm^{-1}$ at 260 nm.[17] The compounds were dissolved in a mixture solvent of 1% DMF and 99% Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.1) at the concentration 1.0×10^{-5} M. Absorption titration experiments were performed with fixed concentrations drugs (10 μM) while gradually increasing the concentration of CT-DNA. While measuring the absorption spectra, an equal amount of CT-DNA was added to both the compounds solution and the reference solution to eliminate the absorbance of CT-DNA itself. Viscosity experiments were conducted on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained to 25.0 °C. Titrations were performed for the Cu(II) and the ligand (0.5—3 μM), and each compound was introduced into a CT-DNA solution (5 μM) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the concentration of the compound and CT-DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA containing solution corrected from the flow time of buffer alone (t_0), $\eta = t - t_0$.[18,19]

To compare the binding affinity of the two compounds bound to DNA, fluorescence titration method was used. Fixed amounts of compound were titrated with increasing amounts of DNA, over a range of DNA concentrations from 2.5 to 20 μM. An excitation wavelength of 330 nm was used.

Further support for the Cu(II) complexes and the ligand binding to DNA *via* intercalation is given through the emission quenching experiment. EB is a common fluorescent probe for DNA structure and has been employed in examinations of the mode and process of metal complex binding to DNA.[20] A 2 ml solution of 10 μM DNA and 0.33 μM EB (at saturating binding levels) was titrated by 5—25 μM the Cu(II) and ligand (λ_{ex}=500 nm, λ_{em}=520.0—650.0 nm). According to the classical Stern–Volmer equation[21]:

$$F_0/F = K_q[Q] + 1$$

where F_0 is the emission intensity in the absence of quencher, F is the emission intensity in the presence of quencher, K_q is the quenching constant, and [Q] is the quencher concentration. The shape of Stern–Volmer plots can be

* To whom correspondence should be addressed. e-mail: yangzy@lzu.edu.cn

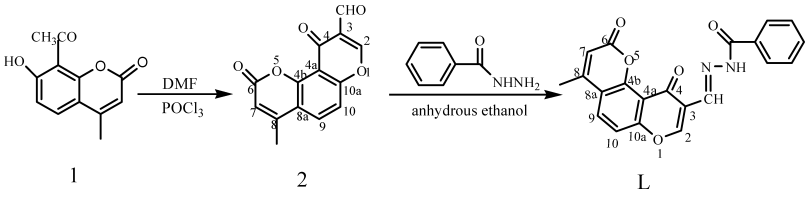

Fig. 1. Scheme of the Synthesis of the Ligand (L)

used to characterize the quenching as being predominantly dynamic or static. Plots of F_0/F *versus* [Q] appear to be linear and K_q depends on temperature.

Preparation of Two Compounds Synthesis of the Ligand: The compounds of **1** and **2** (Fig. 1) were prepared according to the literature.[22,23] Synthesis of the ligand L was in accordance with the following method: an ethanol solution (10 ml) containing benzoyl hydrazine (1.36 g, 10 mmol) was added dropwise to another ethanol solution (10 ml) containing the compound **2** (2.56 g, 10 mmol). After stirring at room temperature for 4 h, the mixture became clear, and soon the yellow precipitate solid was formed after continuing stirring. The precipitate was collected by filtration and washed with ethanol. Recrystallization from anhydrous ethanol gave the ligand L, which was dried in a vacuum. Yield, 86.5%. mp 163—165 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 11.00 (1H, s, NH), 8.83 (1H, s, CH=N), 8.33 (1H, s, 2-H), 8.03 (1H, d, J=8.6 Hz, H-10), 6.95 (1H, d, J=8.6 Hz, H-9), 7.53—7.76 (4H, t, J=8.8 Hz, ph-H), 8.0 (1H, d, J=8.8 Hz, ph-H(p)), 6.18 (1H, s, 7-H), 2.40—2.47 (3H, s, CH_3). FAB-MS: m/z=375 $[M+H]^+$. IR ν_{max} (cm^{-1}) 1714 (C=O of ring b), 1657 (O–C=O of ring a), 1617 (CH=N), 1600 (C=O of phCO).

Synthesis of the Cu(II) Complex: The ligand (1 mmol, 0.37 g) was dissolved in anhydrous ethanol (10 ml) and a solution of $CuCl_2\cdot 2H_2O$ (1 mmol, 0.17 g) in anhydrous ethanol (10 ml) was then added dropwise with stirring. Then the mixture solution was refluxed on an oil-bath at 80 °C for 4 h with stirring. After cooling to room temperature, a large amount of green precipitate appeared. It was separated from the solution by suction filtration, purified by washing several times with ethanol, and dried for 24 h in vacuum. $[CuL\cdot Cl_2\cdot H_2O]$: green. Yield: 83%. IR ν_{max} (cm^{-1}) 3259 (H_2O), 1691 (C=O of ring b), 1659 (O–C=O of ring a), 1598 (CH=N), 1544 (C=O of phCO). Λ_m (S $cm^2\,mol^{-1}$): 40. *Anal.* Calcd for $C_{21}H_{16}N_2O_6Cl_2Cu$: C, 47.91, H, 3.06, N, 5.32. Found: C, 47.96, H, 3.23, N, 5.78.

Results and Discussion

Characterization of the Compounds Properties of the Compounds and Structure of the Cu(II) Complex: The ligand is soluble in methanol and ethanol, while the Cu(II) complex is slightly soluble in methanol, insoluble ethanol. The two compounds are soluble in DMF; DMSO; insoluble in water; benzene and diethyl ether. But they are air stable for extended periods. Since the crystal structure of the Cu(II) complex has not been obtained yet, we characterized the complex and determined its possible structure by elemental analyses, molar conductivities, IR data, thermogravimetry-differential thermal analysis (TG–DTA), and UV–vis measurements. The likely structure of the Cu(II) complex is shown in Fig. 2.

Stability and Molar Conductivity of the Cu(II) Complex in Solution: The stability of Cu(II) complex in an aqueous solution has been studied by observing the UV–vis spectrums and estimating the molar conductivities at different time intervals for any possible change. The tested Cu(II) complex was prepared in DMF and for experiments freshly diluted in phosphate buffer system (at pH 7.4, 7.8). Then, the UV–vis spectrums and molar conductivities were researched at different time intervals. The investigations reveals that the UV–vis spectra have remained unaltered for the solutions and its molar conductance values have no obvious change for very freshly prepared and for over the whole experiment (12 h). It indicates that the Cu(II) complex is quite stable in solution. The molar conductivity of the Cu (II) complex is 39.5—40 (S $cm^2\,mol^{-1}$) in DMF, showing that it is non-electrolytes in DMF.[24] This means that the chloridion takes part in co-ordination in coordinative bond.

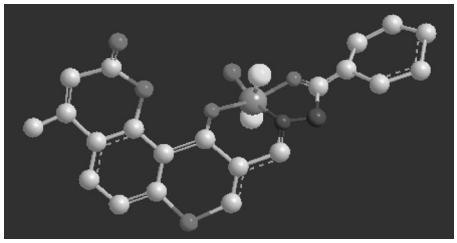

Fig. 2. The Suggested Structure of the Complex

IR Spectra: The IR of the ligand and its Cu(II) complex is presented in the experimental section. The IR spectra of the ligand exhibit bands of the (C=O of ring b) and (C=O of phCO) vibrations at the 1714 and 1600 cm^{-1}, but in its Cu(II) complex they move to the 1691 and 1544 cm^{-1} respectively, Δ (ligand-complex) is equal to 23—56 cm^{-1}. These shifts indicate that the group loses its original characteristics and forms coordinative bonds with metal. The IR spectra of the Cu(II) complex indicates that the ν (C=N) band of the ligand at 1617 cm^{-1} due to the azomethine linkage is shifted towards lower frequency 1598 cm^{-1} (19 cm^{-1}), indicating that the ligand coordinates to metal ions *via* the azomethine nitrogen.[25] The aqueous ν (OH) band of the Cu(II) complex appears at 3259 cm^{-1}, showing that there is some water in the complex. In the two compounds, the ν (O–C=O of ring a) appears at the 1657 and 1659 cm^{-1} respectively, which means that (–O–C=O) of ring 'a' does not take part in co-ordination.

UV–Vis Spectra: The study of the electronic spectra in the ultraviolet and visible ranges for the ligand and its Cu(II) complex was carried out in a buffer solution. The electronic spectra of ligand has a strong band at λ_{max}=361 nm, a medium band at λ_{max}=338 nm. The complex also yields two bands, but the two bands at 338 and 361 nm in the ligand are shifted to 340 and 364 nm or so in its Cu(II) complex. These indicate that the Cu(II) complex has been formed.

Thermal Analyses: The Cu(II) complex begins to decompose at 278 °C and there are two exothermic peaks appearing around 278 and 474 °C. The corresponding TG curves show a series of weight loss. Under 278 °C, there are no endothermic peak and no weight loss on corresponding TG curves. It indicates that there are no crystal solvent molecules. While being heated to 600 °C, the complex becomes its corresponding oxide and maintains a constant weight.

DNA-Binding Mode and Affinity Electronic Absorption Titration: Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with metal

complex.[20,26,27] If the binding mode was intercalation, the π^* orbital of the intercalated ligand can couple with the π orbital of the base pairs, thus, decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in the bathochromism. On the other hand, the coupling π orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism.[28] Figure 3 shows the absorption spectra variations of the ligand and its Cu(II) complex in the absence and presence of the CT-DNA (at a constant concentration of the compounds). The electronic spectra of ligand has a strong band at 361 nm, a medium band at 338 nm. While in the spectra of the Cu(II) complex, the strong band and the medium band were shifted to 364 and 340 nm respectively. In the presence of CT-DNA, the absorption bands of Cu(II) complex at about 340 nm and 364 nm exhibits hypochromism of about 8.81% and 14.9%, and bathochromism of about 2 and 3 nm, respectively. The ligand at 338 nm and 361 nm exhibits hypochromism of about 4.61% and 6.89%, and bathochromism of about 1 and 3 nm. It is noteworthy that the hypochromicity of the complex is greater than that of the present ligand.

The degree of hypochromism generally correlates well also with overall binding strength.[28] Figure 4 shows absorption titration data for the ligand at 361 nm and its Cu(II) complex at 364 nm as a function of DNA addition. The extent of hypochromicity in the charge-transfer band as a function of DNA binding, plotted reciprocally as Ao/A *versus* [M]/[DNA], is found to provide a good measure of relative binding affinity. Informatiom from Fig. 4, we can deduce easily that the Cu(II) complex intercalates deeply into the DNA base pairs than the free lignad.

Fluorescence Spectra: The ligand and its Cu(II) complex can emit weak luminescence in Tris-buffer with a max wavelength of about 373 nm and 443 nm. The results of the emission titrations for the two compounds with DNA are illustrated in the titration curves (Fig. 5). Upon addition of DNA, the emission intensities at about 443 nm of the two compounds grow to around 1.30 and 1.53 times larger, respectively, than those in the absence of DNA. The results of the emission titrations suggest that both the compounds are protected from solvent water molecules by the hydrophobic environment inside the DNA helix, and that the Cu(II) complex can be protected more efficiently than the ligand. This implies that both the compounds can insert between DNA base pairs and that the Cu(II) complex can bind to DNA more strongly than the ligand. In order to further illustrate this point clearly, changes in emission intensities for the ligand and the Cu(II) complex have been plotted against the added

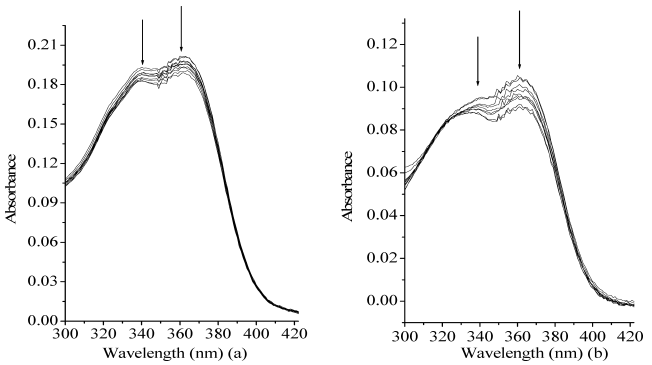

Fig. 3. (a) Electronic Spectra of the Ligand (10 μM) in the Presence of Increasing Amounts of CT-DNA

[CT-DNA]=0—22.5 μM. Arrow shows the absorbance changes upon increasing CT-DNA concentration.

(b) Electronic Spectra of the Cu(II) Complex (10 μM) in the Presence of Increasing Amounts of CT-DNA

[CT-DNA]=0—22.5 μM. Arrow shows the absorbance changes upon increasing CT-DNA concentration.

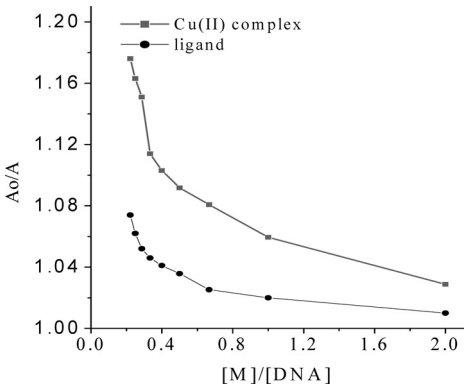

Fig. 4. Hypochromism in the Visible Charge-Transfer Band as a Function of [Compound]/[DNA]

Ao/A represents the ratio of absorbance of free compound (in the absence of DNA) to the absorbance as a function of increasing concentrations of added DNA (0—22.5 μM).

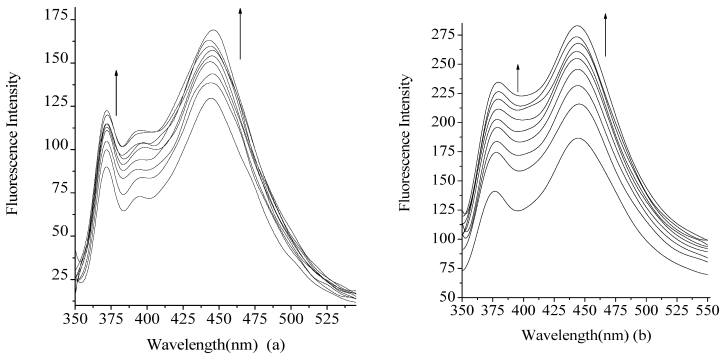

Fig. 5. (a) The Emission Enhancement Spectra of the Ligand (10 μM) in the Presence of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20 μM CT-DNA

Arrow shows the emission intensity changes upon increasing DNA concentration.

(b) The Emission Enhancement Spectra of the Cu(II) Complex (10 μM) in the Presence of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20 μM CT-DNA

Arrow shows the emission intensities upon increasing DNA concentration.

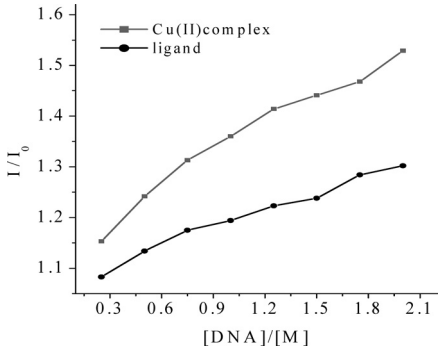

Fig. 6. Changes in Emission Intensities (at about 443 nm) for the Ligand (10 μM) and Its Cu(II) Complex (10 μM) in the Presence of Calf Thymus DNA (0—20 μM) in Buffer Solutions

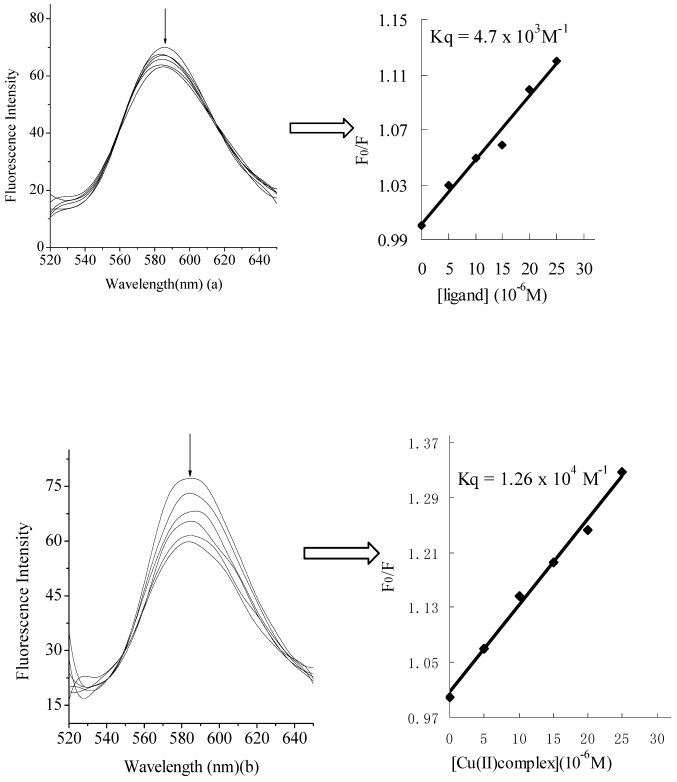

Fig. 7. (a) The Emission Spectra of DNA–EB System (10 μM DNA and 0.32 μM EB), λ_{ex}=500 nm, λ_{em}=520.0—650.0 nm, in the Presence of 0, 5, 10, 15, 20, 25, and 30 μM Ligand

Arrow shows the emission intensity changes upon increasing ligand concentration. Inset: Stern–Volmer plot of the fluorescence titration data of ligand, K_q=4.7×10^3 M^{-1}. (b) The Emission Spectra of DNA–EB System (10 μM DNA and 0.32 μM EB), λ_{ex}=500 nm, λ_{em}=520.0—650.0 nm, in the Presence of 0, 5, 10, 15, 20, 25, and 30 μM Cu(II) Complex

Arrow shows the emission intensity changes upon increasing Cu(II) complex concentration. Inset: Stern–Volmer plot of the fluorescence titration data of Cu(II) complex, K_q=1.26×10^4 M^{-1}.

DNA concentration per mole compounds at about 443 nm in Fig. 6.

Steady-state emission quenching experiments are also used to observe the binding mode of the compounds to DNA. It is well known that EB can intercalate nonspecifically into DNA, which causes it to fluoresce strongly. Competitive binding of other drugs to DNA and EB will result in displacement of bound EB and a decrease in the fluorescence intensity. This fluorescence-based competition technique can

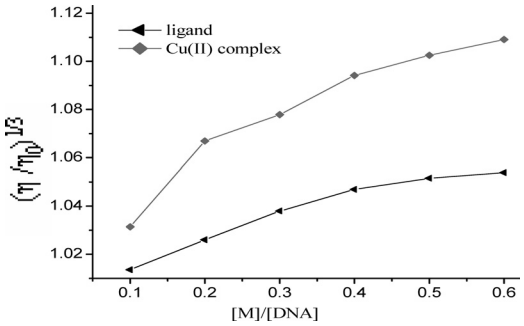

Fig. 8. Effect of Increasing Amounts of the Ligand and the Cu(II) Complex on the Relative Viscosity of CT-DNA at 25.0 °C

provide indirect evidence for the DNA-binding mode. Figure 7 shows the emission spectra of the DNA–EB system with increasing amounts of the ligand and the Cu(II) complex. The emission intensity of the DNA–EB system decreases as the concentration of the two compounds increased, which indicated that two compounds could displace EB from the DNA–EB system. The resulting decrease in fluorescence was caused by EB changing from a hydrophobic environment to an aqueous environment.[29] The quenching plots illustrate that the quenching of EB bound to DNA by the compounds are in good agreement with the linear Stern–Volmer equation. The plots of F_0/F *versus* [Q], K_q is given by the ratio of the slope to the intercept. The K_q values for the ligand and its Cu(II) complex are 4.7×10^3 and 1.26×10^4 M^{-1}, respectively. The data show that the interaction of the Cu(II) complex with DNA is stronger than that of the ligand, which is consistent with the above absorption.

Viscosity Measurements: As optical photophysical probes generally provide necessary, but not sufficient, clues to further clarify the interactions between the study on complex and DNA, viscosity measurements were carried out. Hydrodynamic measurements that are sensitive to length change (*i.e.* viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data. A classical intercalation model demands that the DNA helix lengthen as base pairs are separated to accommodate the binding ligand, leading to an increase in DNA viscosity. In contrast, a partial, non-classical intercalation of compound could bend (or kink) the DNA helix, reducing its effective length and, concomitantly, its viscosity.[19,30] Viscosity experimental results clearly show that both the compounds can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the viscosity of DNA. The effects of both compounds on the viscosity of DNA are shown in Fig. 8.

Conclusions

Taken together, we have synthesized and characterized a new coumarin derivative (8-methylcoumaro-4a,10a-pyrone-3-carbaldehyde benzoyl hydrazone) ligand and its novel Cu(II) complex. DNA-binding studies indicate that the Cu(II) complex and its free ligand can interact with calf thymus DNA by intercalation mechanism (Fig. 9). Furthermore, various comparative experiments show that the Cu(II) complex can bind to DNA more strongly than the free ligand, which attributes to chelating effect of the copper(II) ion to the free ligand. Chelating effect (metal ion to free ligand) can

Fig. 9. Simple Simulation of Interactions of the Ligand and Its Cu(II) Complex with DNA (in an Intercalative Mode)

enhance the planar functionality of metal complex, so the complex can insert and stack between the base pairs of double helical DNA more easily than the free ligand.[31] It is believed that the information obtained from the present work would ultimately be helpful to the understanding of the mechanism of metal complexes with nucleic acids, and useful in the development of potential probes of DNA structure and conformation.

Acknowledgement This work is supported by the National Natural Science Foundation of China (20475023) and Gansu NSF (3ZS041-A25-016).

References

1) Erkkila K. E., Odom D. T., Barton J. K., *Chem. Rev.*, **99**, 2777—2795 (1999).
2) Mrksich M., Dervan P. B., *J. Am. Chem. Soc.*, **115**, 9892—9899 (1993).
3) Trawick B. N., Danihe A. T., Bashkin J. K., *Chem. Rev.*, **98**, 939—960 (1998).
4) Xiong Y., Ji L. N., *Coord. Chem. Rev.*, **185**, 711—733 (1999).
5) Sigman D. S., Mazumder A., Perrin D. M., *Chem. Rev.*, **93**, 2295—2316 (1993).
6) Metcalfe C., Thomas J. A., *Chem. Soc. Rev.*, **32**, 215—224 (2003).
7) Barton J. K., *Science*, **223**, 727—734 (1986).
8) Hudson B. P., Barton J. K., *J. Am. Chem. Soc.*, **120**, 6877—6888 (1998).
9) Chan H. L., Liu H. Q., Tzeng B. C., You Y. S., Peng S. M., Yang M. S., Che C. M., *Inorg. Chem.*, **41**, 3161—3171 (2002).
10) Kumar C. V., Barton J. K., Turro N. J., *J. Am. Chem. Soc.*, **107**, 5518—5523 (1985).
11) Xu H., Zheng K. C., Chen Y., Li Y. Z., Lin L. J., Zhang P. X., Ji L. N., *Dalton Trans.*, **11**, 2260—2268 (2003).
12) Friedman A. E., Chambron J. C., Sauvage J. P., Turro N. J., Barton J. K., *J. Am. Chem. Soc.*, **112**, 4960—4962 (1990).
13) Barton J. K., Kumar C. V., Turro N. J., *J. Am. Chem. Soc.*, **108**, 6391—6393 (1986).
14) Hiort C., Lincoln P., Norden B., *J. Am. Chem. Soc.*, **115**, 3448—3454 (1993).
15) Naing K., Takahashi M., Taniguchi M., Yamagishi A., *J. Chem. Soc., Chem. Commun.*, **4**, 402—403 (1993).
16) Satyanarayana S., Dabrowiak J. C., Chaires J. B., *Biochemistry*, **31**, 9319—9324 (1992).
17) Kumar C. V., Asuncion E. H., *J. Am. Chem. Soc.*, **115**, 8547—8553 (1993).
18) Eriksson M., Leijon M., Hiort C., Norden B., Gradsland A., *Biochemistry*, **33**, 5031—5040 (1994).
19) Xiong Y., He X. F., Zou X. H., Wu J. Z., Chen X. M., Ji L. N., Li R. H., Zhou J. Y., Yu K. B., *Dalton Trans.*, **1**, 19—24 (1999).
20) Barton J. K., Danishefsky A., Golderg J., *J. Am. Chem. Soc.*, **106**, 2172—2176 (1984).
21) Efink M. R., Ghiron C. A., *Anal. Biochem.*, **114**, 199—227 (1981).
22) Sharan P., Giri S., Nizamuddin, *J. Indian Chem. Soc.*, **66**, 393—394 (1989).
23) Lacova M., Loos D., Furdik M., Matulova M., El-Shaaer H. M., *Molecules*, **3**, 149—158 (1998).
24) Geary W. J., *Coord. Chem. Rev.*, **7**, 81—122 (1971).
25) Narang K. K., Singh V. P., *Transition Met. Chem.*, **18**, 287—290 (1993).
26) Kelly J. M., Tossi A. B., McConnell D. J., OhUigin C., *Nucleic Acids Res.*, **13**, 6017—6034 (1985).
27) Tysoe S. A., Morgan R. J., Baker A. D., Strekas T. C., *J. Phys. Chem.*, **97**, 1707—1711 (1993).
28) Pyle A. M., Rehmann J. P., Meshoyrer R., Kumar C. V., Turro N. J., Barton J. K., *J. Am. Chem. Soc.*, **111**, 3051—3058 (1989).
29) Zeng Y. B., Yang N., Liu W. S., *J. Inorg. Biochem.*, **97**, 258—264 (2003).
30) Satyanarayana S., Dabrowiak J. C., Chaires J. B., *Biochemistry*, **32**, 2573—2584 (1993).
31) Xu H., Zheng K. C., Lin L. J., Li H., Gao Y., Ji L. N., *J. Inorg. Biochem.*, **98**, 87—97 (2004).