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Copper(II) Complexes with *N*,*N*′-Dialkyl-1,10-phenanthroline–2,9-Dimethanamine: Synthesis, Characterization, DNA-Binding Thermodynamical and Kinetic Studies

Zhong-Ming Wang, Hua-Kuan Lin,* Zhi-Fen Zhou, Meng Xu, Tian-Fu Liu, Shou-Rong Zhu and Yun-Ti Chen

Department of Chemistry, Nankai University, 300071, Tianjin, PR China

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Abstract—Copper(II) complexes (Cu–L, L = *N*,*N*′-dialkyl-1,10-phenanthroline-2,9-dimethanamine) were synthesized and characterized by elemental analyses, IR spectra and conductance measurement. The interaction of the copper(II) complex with calf thymus DNA was studied by means of UV melting experiments, fluorescence spectra and circular dichroic spectra. Using ethidium bromide as a fluorescence probe, the binding mode of the complexes Cu–L with calf-thymus DNA was studied spectroscopically. The results indicate that the complexes Cu–L perhaps interact with calf-thymus DNA by both intercalative and covalent binding. Kinetics of binding of the cupric complexes to DNA was studied for the first time using ethidium bromide as a fluorescence probe with stopped-flow spectrophotometer under pseudo-first-order condition. The stronger binding of two steps in the process of the complexes Cu–L interacting with DNA was observed, and the probable interaction process was discussed in detail. The corresponding k_{obs} and E_a of binding to DNA (where k_{obs} is the observed pseudo-first-order rate constant, E_a is the observed energy of activation) were obtained.

Introduction

1,10-Phenanthroline (Phen) is one of the earliest and most extensively studied *N*-heterocyclic chelating agents. Currently, in the field of supramolecular chemistry, 1,10-phenanthroline units, as an important building block, play an important role for the development of the supramolecular chemistry,[1–3] with many novel supramolecular compounds including 1,10-phenanthroline units. In the field of inorganic medicinal chemistry, several metal complexes with 1,10-phenanthroline and natural products incorporating this heterocyclic nucleus possess interesting anticancer properties.[4–6] In the field of bioinorganic chemistry, the complex of copper(II) with two 1,10-phenanthrolines in the presence of hydrogen peroxide efficiently cleaves DNA by oxidative attack on deoxyribose units from the DNA minor groove.[7,8] Recently, Clip-Phen and its derivatives, a new type of ligand with two 1,10-phenanthroline entities linked via their C2-carbon by a short flexible arm, were synthesized by Meunier et al.,[9,10] and the corresponding copper complexes are highly efficient DNA cleaver. Additionally, the simple substitution-inert octahedral metal complexes derived from 1,10-phenanthroline are capable of selectively binding DNA through intercalation.[11–14]

Recently, we prepared the other type of conjugate of 1,10-phenanthroline, *N*,*N*′-dialkyl-1,10-phenanthroline-2,9-dimethanamine (L) (Chart 1).[15] The corresponding cupric complexes are sure to be different from the above-mentioned cupric complexes. Thus, here we are interested in reporting the synthesis, DNA-binding thermodynamic study of copper complexes with L, and kinetic process of the cupric complexes to DNA was studied for the first time using ethidium bromide as a

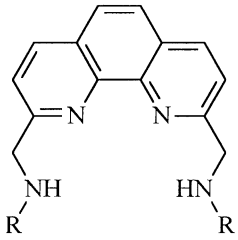

Chart 1. Structure of the ligands L. L1: R = CH_3; L2: R = CH_2CH_3; L3: R = $(CH_2)_2CH_3$; L4: R = $(CH_2)_3CH_3$; L5: R = $CH(CH_3)_2$; L6: R = $C(CH_3)_3$.

*Corresponding author. Tel.: +86-22-23501320; fax: +86-22-23502458; e-mail: linhk@public.tpt.tj.cn

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fluorescence probe with stopped-flow spectrophotometer under pseudo-first-order condition.

Results and Discussion

Syntheses of the complexes CuL(1–6)

All ligands in hydrochloride salt were transferred as free ligands to obtain the desirable products. All complexes were characterized by elemental analyses, IR spectrum and conductance measurement.

The content of copper for all complexes CuL(1–6) was determined by EDTA titration. Analyses for C, H and N were performed with a Perkin–Elmer 240C elemental analyzer. Their content was given in Table 1. These complexes are in accordance with their proposed formula.

For all complexes CuL(1–6), some characteristic IR spectral data are given in Table 2. The absorption bands 1606–1609, 1350–1354 and 879–883 cm^{-1} of Phen ring[18] are shifted to 1596–1600, 1379–1385 and 858–861 cm^{-1} after coordination, respectively, which proves the Phen ring coordinates with Cu(II) ion through N. By respective comparison with the free ligand L1 ~ 6, the appearance of ν(La–N) at 248–256 cm^{-1} (Phen ring) in the complexes CuL(1–6) further proves coordination of Phen ring. For the imido groups of the complexes CuL(1–6), the stretching vibration absorption bands 3383–3423 cm^{-1} of ν(N–H)[18] are shifted to ca. 3440 cm^{-1} after coordination, which indicates the imido groups coordinate with Cu(II) ion. Moreover, there exists an absorption peak of $Cu–N_{(NH)}$ in lower wavelength number region (414–437 cm^{-1}). It further indicates that imido groups coordinate with cupric ion. In addition, the ClO_4^- group vibration at 1094–1108 cm^{-1} and 623–626 cm^{-1}, which is typical for a non-coordinated perchlorate ion,[19] are present for the complex CuL(1–6).

The molar conductivity in methanol for all complexes CuL(1–6) is listed in Table 3. The results of the molar conductivity indicate that the complexes CuL(1–6) (159–187 S cm^2 mol^{-1}) are in the range expected for 1:2 electrolytes,[20] which also indicated that the perchlorate anions are outside the coordination sphere, in agreement with IR result. According to the structures of the ligand L(1–6), the values of the molar conductivity for the complexes CuL(1–6) suggest that imido groups coordinate with cupric ion.

On the basis of the above information, the ligands L1–6 are coordinated to the cupric ion by the nitrogen atoms of Phen ring and two imido groups. The structures of the complexes are shown in Chart 2.

Thermal denaturation of DNA

The effect of the complexes CuL(1–6) such as CuL1 on the melting temperature (T_m) of calf thymus DNA in buffer is shown in Figure 1.

Melting of DNA is a phenomenon observed when double-stranded DNA molecules are heated and separated into two single strands: it occurs due to disruption of the intermolecular force such as π–π stacking and hydrogen bonding interacting between the DNA base pairs. As shown in Figure 1, the DNA melting experiment reveal that T_m of the calf thymus DNA was 82.3 and 82.8 °C in the absence and presence of CuL1 complex, respectively. The increase in T_m indicates a slightly increased affinity of the DNA double helix due to the unwinding of the DNA helix and suggests stacking of the DNA bases upon, to some extents, interaction of complex. Some studies[7,8] indicated that $(Phen)_2Cu$ complex in the presence of hydrogen peroxide efficiently cleaves DNA by oxidative attack on deoxyribose units from the DNA minor groove. Additionally, copper complexes with Clip-Phen or its derivatives show the highly cleavage activity of DNA.[9,10] One of the consequences of DNA after cleaved is that T_m apparently decreases.

CD spectroscopy

CD spectral variations of calf thymus DNA by the addition of the complexes CuL(1–6) were recorded on a Jasco J-715 spectropolarimeter. Figure 2 shows an example of the CD spectra of calf thymus DNA treated with different concentrations of the complex CuL5. In Figure 2, DNA solutions containing the complex CuL5 show CD with opposite signs at near 260 nm, indicating that the DNA keeps the double helix B-form structure[21] in the presence of the complex CuL5. With the addition of the complex, the ellipticity value of DNA increases at the band 270–290 nm, suggesting that the change of the stacking style or orientation of base pairs of DNA occur. In addition, the slightly red shift of the CD spectra of DNA at 270–290 nm band suggests that there exists interaction between the complex and DNA. Similar phenomenon was observed in other complexes.

Binding studies

To obtain the complexes binding to calf thymus DNA, the fluorescence Scatchard plot is an important tool, which can provide the binding mode of the complexes to DNA.[8]

Saturation curves for fluorescence intensity of a series of DNA–metal complexes at increasing concentrations of the complexes CuL(1–6) (r_f, the molar ratio of the complex/DNA, is 0–0.835) were obtained by the addition of increasing concentrations of EB (2.0–20 mmol dm^{-3}). The fluorescence Scatchard plots for binding of the complexes CuL(1–6) to EB–DNA complex can be obtained, and one typical example (CuL5) are given in Figure 3. Unlike those metal complexes of platinum[12] or palladium,[21] they can show a typical competitive or noncompetitive inhibition of EB binding to calf thymus DNA. The complexes CuL(1–6) does not show a typical noncompetitive or competitive inhibition of EB binding in which both the slop that is K_{obs} (the observed association constant) and the intercept of the abscissa that is n (number of binding sites per nucleotide) decrease with

Table 1. Data of elemental analyses of the complexes CuL(1–6)

Complexes	Formula	%Found (%Calcd)			
		Cu%	%C	%H	%N
CuL1	$Cu(C_{16}H_{18}N_4)(ClO_4)_2 0.5H_2O$	11.95 (11.82)	36.10 (35.74)	3.62 (3.56)	10.22 (10.42)
CuL2	$Cu(C_{18}H_{22}N_4)(ClO_4)_2$	11.22 (11.41)	38.84 (39.32)	4.17 (3.98)	9.95 (10.07)
CuL3	$Cu(C_{20}H_{26}N_4)(ClO_4)_2$	10.45 (10.87)	41.21 (41.09)	4.50 (4.48)	9.58 (9.27)
CuL4	$Cu(C_{22}H_{30}N_4)(ClO_4)_2 0.5H_2O$	10.41 (10.22)	42.15 (42.49)	4.72 (5.02)	9.03 (9.01)
CuL5	$Cu(C_{20}H_{26}N_4)(ClO_4)_2 0.75H_2O$	10.48 (10.62)	40.18 (40.16)	4.70 (4.63)	9.37 (9.37)
CuL6	$Cu(C_{22}H_{30}N_4)(ClO_4)_2 0.5H_2O$	10.44 (10.22)	42.46 (42.49)	5.43 (5.02)	8.85 (9.01)

Table 2. Some characteristic IR spectral data (cm^{-1}) for the complexes CuL(1–6)

Complexes	ν(N–H)	ν(phen-ring)	$\nu(ClO_4^-)$	ν(Cu–N, NH)	ν(Cu–N, Phen)
CuL1	3441, 3261, 1507	1597, 1387, 858	1101, 624	414	249
CuL2	3441, 3253, 1506	1596, 1384, 861	1094, 625	421	252
CuL3	3440, 3247, 1508	1599, 1387, 858	1106, 625	422	248
CuL4	3440, 3249, 1508	1600, 1385, 858	1108, 624	422	249
CuL5	3441, 3237, 1506	1597, 1385, 860	1108, 626	421	252
CuL6	3440, 3272, 1506	1597, 1379, 868	1107, 623	437	256

Table 3. Molar conductivity for the complexes CuL(1–6) at 1.0×10^{-3}mol dm^{-3} in methanol (S cm^2 mol^{-1})

Complexes	CuL1	CuL2	CuL3	CuL4	CuL5	CuL6
Λ_m/Scm^2mol^{-1}	162.5	172.3	159.1	165.9	161.8	172.1

increase in concentrations of the complexes CuL(1–6) as given in Table 4. In this table, the reduction in K_{obs} and n, relative to that of EB, is clear. However, with the increase of the complex, the change of n is not notable, which suggests that these complexes maybe bind to DNA selectively. Additionally, the affect of the substituents to the values of K and n is not remarkable. Thus, it is obvious that Cu(II) and phenanthroline ring play an important role in this system.

Here, the data suggest that the complexes CuL(1–6) maybe bind to DNA by both covalent binding and intercalative binding mode. According to the structures of the complexes and the characters of the cupric ion and calf thymus DNA, the N or O of base pairs in DNA can bind the cupric ion of the complex at axial orientation. Additionally, the contribution to the electrostatic interaction with DNA of the R–NH–CH_2– moiety in these complexes cannot be ignored. The longer the chain of the substituent R is, the stronger the electrostatic interaction is. Of course, the π–π interaction and the hydrophobic interaction maybe occur between the aromatic rings of 1,10-phenanthroline and those of the base pairs of DNA. Some studies indicated the complex of cupric ion with two 1,10-phenanthrolines in the presence of hydrogen peroxide efficiently cleaves DNA by oxidative attack on deoxyribose units from the DNA minor groove.[7,8] So, it is possible that the complexes CuL(1–6) bind from the minor groove side of DNA.

Kinetic studies of the complexes binding to DNA

The steady state fluorescence by Scatchard plots is only capable of showing the binding mode of a compound to DNA, however, it is not able to give any information about the interaction process of the compound to DNA. With EB as a fluorescence probe, the time process of the complexes CuL(1–6) binding to DNA can be recorded using stopped-flow spectrophotometer by monitoring EB fluorescence intensity change.

One typical example, such as the interaction process between the complex CuL1 and EB-ctDNA, of the fluorescence intensity change with time is shown in Figure 4. It is discussed in detail. Similar phenomena were observed in other complexes.

Although the kinetic curve is obtained within 1500 s, it is almost unchanged even continuing for a longer time, indicating that the fluorescence change in 1500 s cover the whole process. The fluorescence intensity of EB is greatly enhanced on its intercalation between base pairs of DNA.[22] Since the fluorescence intensity of EB-DNA can be reduced on binding of metal complex to DNA–EB complex,[12,21] the quenching of fluorescence at two times

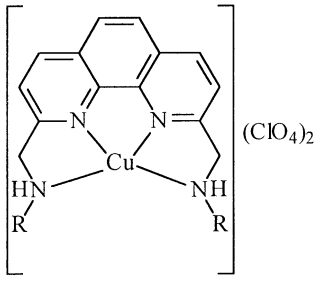

Chart 2. Proposed structure of the complexes CuL(1–6). CuL1: R = CH_3; CuL2: R = CH_2CH_3; CuL3: R = $(CH_2)_2CH_3$; CuL4: R = $(CH_2)_3CH_3$;CuL5: R = $CH(CH_3)_2$; CuL6: R = $C(CH_3)_3$.

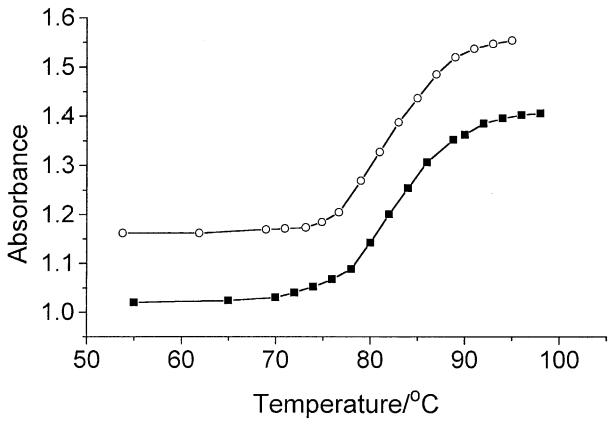

Figure 1. Thermal denaturation of calf thymus DNA (1.54×10^{-5} mol dm^{-3}) in the presence (○) and absence (■) of 1.00×10^{-5} mol dm^{-3} CuL1 complex.

on the kinetic curve represents the interaction of two steps of the complex CuL1 to DNA. However, the two-time quenching of fluorescence only represents the stronger interaction of two steps, and does not indicate that there are only two steps in the whole process of the complex CuL1 binding to DNA because it is possible that there are other steps during binding which have no effect on fluorescence, in other words, there are at least two steps in the whole process of the complex CuL1 binding to DNA.

By monitoring fluorescence intensity, the stronger interaction of two steps can be clearly observed. It is shown from Figure 4 that the fluorescence is sharply quenched at the beginning and increases after 300 s. The increase of fluorescence intensity is finished at about 700 s. The fluorescence of EB comes back again to almost the same extent as the fluorescence of the initial value. Then the fluorescence quenches slowly and the quenching is finished at about 1400 s. However, the quenching extent of fluorescence at the second step is 1/2 than that of one at the first step.

Under physiological conditions, the ionized phosphate groups in DNA are the anionic charge of the polyion surface. So, the electrostatic interaction maybe occur between the complexes and the ionized phosphate groups in DNA. It was believed that it causes DNA duplex with serious perturbations, which result in a large number of EB molecules squeezed out from DNA helix. As shown in Figure 4, the fluorescence of EB is sharply quenched in the first step for the complex CuL1. However, the base pairs remain basically present, so the squeezed-out EB molecules can intercalate between the base pairs again after the first step is finished, and fluorescence of EB comes back again to almost the same extent as the fluorescence of the initial. Considering the character of cupric ion and structures of the complexes, the interaction between the complexes and DNA is not finished. The complexes can interact continuously with DNA by coordinating with N or O of the base pairs in DNA at axial orientation, and the π–π interaction and the hydrophobic interaction maybe occur between the aromatic rings of 1,10-phenanthroline and those of the base pairs of DNA. Of course, it is also possible that the R–NH–CH_2– moiety seems to contribution to the electrostatic interaction with DNA. The interaction can cause DNA duplex with the second perturbation, and result in a large number of EB molecules squeezed out from DNA helix again. So, the complexes CuL(1–6) can make the fluorescence of EB is partially quenched again. Then the fluorescence intensity of the system is constant, indicating that the conformance of DNA becomes stable again.

The observed pseudo-first-order rate constant for the quenching reaction (the first step) at four temperatures (288.1, 298.1, 303.2 and 310.0 K), k^1_{obs}, are obtained and given in Table 5. The fitted exponential curve of the complex CuL1 binding to DNA in the first step at 288.1 K is given in Figure 5. The k^2_{obs} for the quenching reaction (the second step) at four same temperatures are also obtained and listed in Table 5. The fitted exponential curve of the complex CuL1 binding to DNA the second step at 288.1 K is given in Figure 6. At the same time, it is found that there is a linear relationship between ln k_{obs} and $1/T$ in the temperature range 288.1–310.2 K, and the linear regression coefficients of the first step and the second step are −0.999 and −0.996, respectively. The slope (E_a) is obtained according to the equation $\ln k_{obs} = \ln A - E_a/RT$.

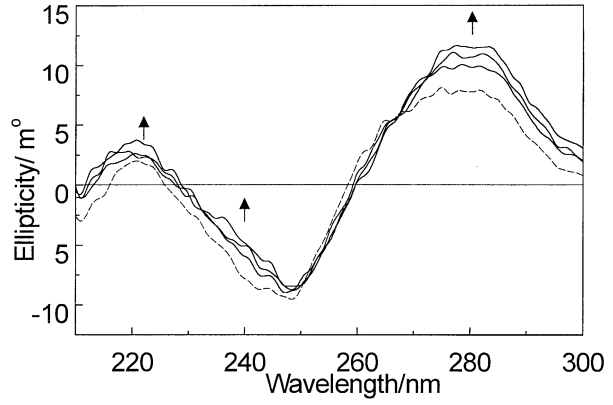

Figure 2. CD spectral variations of calf thymus DNA by the addition of the complex CuL5. [DNA] = 8.0×10^{-5} mol dm^{-3}, [CuL5] = 0, 6.67×10^{-6}, 1.33×10^{-5} and 2.00×10^{-5} mol dm^{-3}.

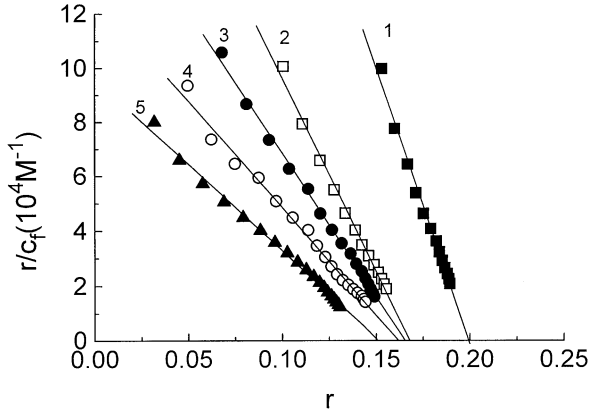

Figure 3. Fluorescence Scatchard plots for the binding of EB (2.0–20 μmol dm^{-3}) to DNA in the absence (line 1) and the presence (lines 2–5) of increasing concentrations of the complex CuL5. r_f increases in the order of 0.000, 0.045, 0.084, 0.418, and 0.835 for lines 1–5, respectively. [DNA] = 35.94 μmol dm^{-3}.

Table 4. Binding parameters for the effect of the complexes CuL(1–6) on the fluorescence of EB in the presence of calf thymus DNA

Complexes	R[a]	$K^b\times10^6$	n^c	Complexes	R[a]	$K^b\times10^6$	n^c
—	0.000	2.029	0.199	CuL4	0.045	1.235	0.163
CuL1	0.045	1.077	0.175		0.418	0.723	0.160
	0.418	0.754	0.174		0.835	0.489	0.157
	0.835	0.510	0.168	CuL5	0.045	1.416	0.168
CuL2	0.045	0.984	0.156		0.084	1.045	0.165
	0.418	0.785	0.153		0.418	0.783	0.162
	0.835	0.598	0.149		0.835	0.631	0.152
CuL3	0.045	1.239	0.169	CuL6	0.045	1.055	0.178
	0.418	0.755	0.160		0.418	0.743	0.173
	0.835	0.519	0.151		0.835	0.518	0.168

[a]R is the formal ratio of the compound concentration to nucleotide concentration.
[b]K is the association constant in $(\text{mol dm}^{-3})^{-1}$.
[c]n is the number of binding sites per nucleotide.

In Table 5, it is clear that the k_{obs} of the first step is greater than that of the second step, which may be due to the more steric hindrance in the intercalation of phenanthroline ring in the second step relative to the first step. This is also consistent with the larger E_a in the binding of the second step than that of the first step.

Experimental

Materials and methods

The ligand L was synthesized according to our previous method.[15] Commercially pure chemicals such as cupric perchlorate (Tianjin Chem. Corp., China), ethidium bromide (EB) (Fluka, Switzerland), calf thymus DNA and tris(hydroxymethyl) aminomethane (Tris) (Sigma, USA) have been purchased and used as received. Calf thymus DNA concentration was determined spectrophotometrically[16] by using the molar extinction coefficient 6600 $(\text{mol dm}^{-3})^{-1}\ \text{cm}^{-1}$ at 260 nm. Other chemicals used were of analytical reagent or high purity grade. Solvent was purified by standard techniques and was freshly distilled prior to use. The buffer used was 50 mmol dm^{-3} Tris–HCl, pH 7.0, containing 10 mmol dm^{-3} NaCl. Unless stated otherwise, all experiments in solution were carried out in this buffer.

Elemental analyses were performed on a Perkin–Elmer 240C elemental analyzer. IR spectra were obtained as KBr disks on a Nicolet 170 SX FT-IR spectrometer. Conductance measurements were carried out with a DDS-12A digital conductivity meter. A dip-type conductivity cell containing plantinized electrodes was used. Fluorescence measurements were carried out on a Shimadzu RF-540 spectrofluophotometer.

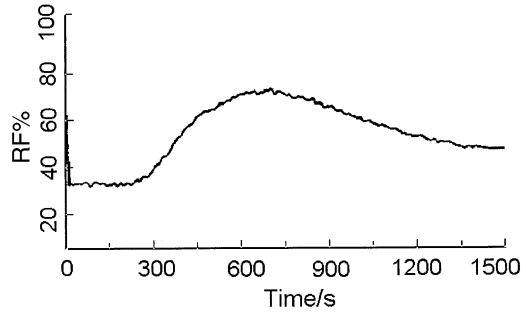

Figure 4. CuLA1 binding to DNA and conformation change monitored by the EB fluorescence intensity as a function of time ($\lambda_{ex}=540$ nm, $\lambda_{em}=590$ nm, $T=288.2\pm0.1$ K. RF denotes relative fluorescence intensity).

General procedure for preparations of cupric complexes [CuL(1-6)](ClO$_4$)$_2$

The hydrochloride salt of the ligand (2 mmol) dissolved in distilled water (100 cm^3) was mixed with slight excess NaOH (>6 mmol). After 10 min stirring, a clear light yellow solution was obtained. The clear solution was extracted with CH_2Cl_2 (40 $\text{cm}^3\times4$). The organic extract was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to yield yellow oil. The oil was dissolved in ethanol (30 cm^3). The $Cu(ClO_4)_2 6H_2O$ (2 mmol) dissolved in 10 cm^3 of ethanol was added dropwise with stirring magnetically over 10 min. The solution was stirred continuously for 1 h. The resulting blue solid was collected by filtration and washed with a small portion anhydrous ethanol and ethyl ester. The product was dried in a vacuum desiccator. Yield: 80–90%.

Thermal denatural studies

Thermal denaturation studies were conducted in a Shimadzu UV-240 spectrophotometer supplied with a thermostatted cell holder. The temperature was controlled by a TB-85 type thermostatic bath (±0.1 °C). The absorbance at 260 nm was monitored for either the DNA (1.54×10^{-5} mol dm^{-3}) or a mixture of the DNA (1.54×10^{-5} mol dm^{-3}) with the complex such as CuL1 (1.00×10^{-5} mol dm^{-3}). The UV melting temperature (T_m) is defined as the temperature at which half the helical structure is lost.

CD spectroscopy

All circular dichroic (CD) spectra were recorded on a Jasco J-715 automatic recording spectropolarimeter using matched 10 mm path length quartz cell. The concentration of calf thymus DNA was 80 μmol dm^{-3}. The respective complex stock solution (1.0×10^{-3} mol dm^{-3}) 20 μdm^3 was added to the 3.0 cm^3 DNA solution every

Table 5. The observed pseudo-first-order rate constant k_{obs1} and k_{obs2} and the corresponding observed activation energy E_{a1} and E_{a2} of the complexes CuLA1–6 binding to DNA at four different temperatures. (temperature accuracy is within 0.1 K)

Complexes	k (s^{-1})	T (K)				E_a (kJ/mol)
		288.2	298.2	303.2	310.2	
CuLA1	k_{obs1}	17.18	24.30	36.37	48.50	34.8
	$k_{obs2}/10^3$	1.13	3.94	7.53	12.82	83.6
CuLA2	k_{obs1}	16.97	27.86	37.06	48.55	36.0
	$k_{obs2}/10^3$	1.29	4.78	7.89	15.75	84.6
CuLA3	k_{obs1}	16.65	27.31	35.35	50.23	37.2
	$k_{obs2}/10^3$	1.10	3.84	7.67	12.74	84.6
CuLA4	k_{obs1}	16.34	27.47	35.96	47.65	36.5
	$k_{obs2}/10^3$	1.17	3.72	7.67	14.78	87.1
CuLA5	k_{obs1}	16.59	27.34	35.39	50.11	37.3
	$k_{obs2}/10^3$	1.15	4.91	7.83	14.30	86.8
CuLA6	k_{obs1}	16.59	26.13	34.75	48.76	36.5
	$k_{obs2}/10^3$	1.11	4.11	8.23	14.00	87.5

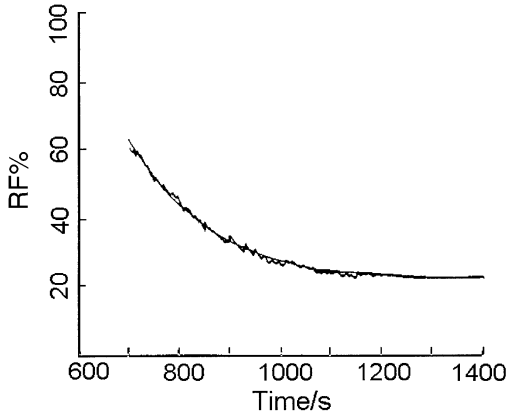

Figure 6. The fitted exponential curves of the CuLA1 complex interaction with DNA in the scond step at 288.1 K. RF denotes relative fluorescence intensity.

time. The sample solution was mixed and was allowed to warm up to room temperature (16±1 °C) prior to measurement. Each sample solution was scanned in the range 200–300 nm. A CD spectrum was generated which represented the mean of three scans from which the buffer background had been electronically subtracted.

Binding studies

The interaction of the respective complexes CuL(1–6) with calf thymus DNA in vitro was studied by fluorescence spectroscopic methods as described in the literature.[12,17] The entrance and exit slits were maintained at 5.0 and 10 nm, respectively, for all fluorescence measurements. The sample was excited at 540 nm and its emission observed at 590 nm. The samples were incubated 8 h at room temperature (20±0.2 °C) before spectral measurements. Under the condition, the fluorescence intensity of the respective complexes, calf thymus DNA and EB was very small and could be ignored.

Kinetic studies of the complexes binding to DNA

The kinetics of the complexes interacting with DNA was studied using EB as fluorescence probe with stopped-flow spectrophotometer, which was equipped with a versatile Union Giken RA-451 refrigerated circulating bath (temperature accuracy is within 0.1 K). The experiments were conducted under pseudo-first-order condition with DNA at 4.964 μmol dm^{-3} having been exposed to EB at 0.612 μmol dm^{-3} for 8 h and the complexes concentration 40-fold in excess at least. The complexes, DNA and EB were dissolved in the same buffer as the above.

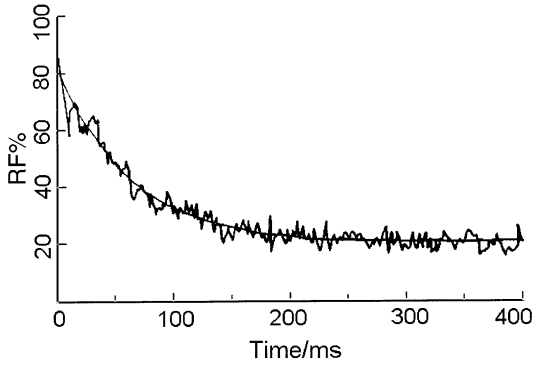

Figure 5. The fitted exponential curves of the CuLA1 complex interaction with DNA in the first step at 288.1 K. RF denotes relative fluorescence intensity.

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