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The interaction of native DNA with Zn(II) and Cu(II) complexes of 5-triethyl ammonium methyl salicylidene orto-phenylendiimine

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Abstract

The interaction of native calf thymus DNA with the Zn(II) and Cu(II) complexes of 5-triethyl ammonium methyl salicylidene orto-phenylendiimine (ZnL^{2+} and CuL^{2+}), in 1 mM Tris–HCl aqueous solutions at neutral pH, has been monitored as a function of the metal complex-DNA molar ratio by UV absorption spectrophotometry, circular dichroism (CD) and fluorescence spectroscopy. The results support for an intercalative interaction of both ZnL^{2+} and CuL^{2+} with DNA, showing CuL^{2+} an affinity of approximately 10 times higher than ZnL^{2+}. In particular, the values of the binding constant, determined by UV spectrophotometric titration, equal to 7.3×10^4 and 1.3×10^6 M^{-1}, for ZnL^{2+} and CuL^{2+}, respectively, indicate the occurrence of a marked interaction with a binding size of about 0.7 in base pairs. The temperature dependence of the absorbance at 258 nm suggests that both complexes strongly increase the DNA melting temperature (Tm) already at metal complex-DNA molar ratios equal to 0.1. As evidenced by the quenching of the fluorescence of ethidium bromide-DNA solutions in the presence of increasing amounts of metal complex, ZnL^{2+} and CuL^{2+} are able to displace the ethidium cation intercalated into DNA. A tight ZnL^{2+}–DNA and CuL^{2+}–DNA binding has been also proven by the appearance, in both metal complex-DNA solutions, of a broad induced CD band in the range 350–450 nm. In the case of the CuL^{2+}–DNA system, the shape of the CD spectrum, at high CuL^{2+} content, is similar to that observed for ψ-DNA solutions. Such result allowed us to hypothesize that CuL^{2+} induces the formation of supramolecular aggregates of DNA in aqueous solutions.

Keywords: Copper; DNA; Intercalations; Schiff bases; Zinc

1. Introduction

Over the past decades there has been substantial interest in the deoxyribonucleic acid (DNA) binding properties toward a number of metal complexes, with the aim to develop novel reagents which can control genetic information and or prevent the growth and replication of cancerous cells through the inhibition of transcription [1–5]. The necessary requisites that such complexes should obviously possess, are to be stable and inert in biological environment and water-soluble. Among the metals with established biological properties, great attention has been devoted to copper and zinc complexes, for several reasons. Firstly, together with iron, they are the most abundant trace elements present in biological systems and several metalloproteins contain one or both elements. Moreover, their derivatives are usually toxic and therefore have often been used as biocides. In addition, it is worth to note that copper and zinc differ for one atomic number unit and, at a given oxidation state of the metal, the different number of d electrons can be used to explain differences of their

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analogous compounds in, for example, magnetic, spectroscopic, redox or structural properties [6].

Copper and zinc complexes have been found to interact with DNA through different binding modes [7–9]. In particular, copper(II) complexes in the presence of oxidizing or reducing agents are able to cleave DNA. Research on DNA cleavage by synthetic copper complexes is of considerable interest because of their efficacy as anti-tumour agents [10–13].

The interaction among DNA and metal complexes containing multidentate aromatic ligands, with square planar N_4 or N_2O_2 coordination, has been throughly considered [14–16]. Indeed, such compounds have some favourable features [15–19]: the ligand can be attached to the metal in a controlled manner; binding to DNA is usually accompanied by marked absorbance changes in the UV–vis frequency range and, sometimes, fluorescence emission too, due to excitation of charge transfer transitions. These properties, together with the analysis of the optical and thermal behaviour of DNA in the presence of these metal complexes, provide a convenient handle for monitoring the binding process.

Salen, the anion of the Schiff base [*N*,*N*′-ethylene-bis(salicylideneimine)], H_2Salen, and ligands obtained by its chemical modifications fall in this class of compounds. Indeed, they are characterized by a planar area with extended π system, coordinate metal atoms via a square planar N_2O_2 system and their complexes experience electronic transitions in the UV–vis range.

We have previously reported on the interaction of native calf thymus DNA with Fe^{III}(Salen)Cl [20]. The results obtained have been interpreted in terms of an electrostatic binding of $[Fe(Salen)]^+$ cation and the phosphate groups of DNA.

ESR measurements on DNA-fibres [21] have revealed that the orientation of water-soluble cationic Salen-type Schiff base complexes of copper(II), relative to the DNA axis, changes by modification of the chain length bridging the two imino nitrogens in the complex. Such measurements allowed to conclude that the 1,2-phenylenediamine bridge enables the metal complex to intercalate DNA. Recently, it has also been reported that a new copper(II) complex, Cu(*o*-VANAHE)$_2$, (VANAHE=2(*o*-vanillinamino)1-hydroxyethane) [22] binds to DNA and presents interesting nuclease activity.

The synthesis of copper complexes with several ligands has been extensively reported, and those factors that determine their application in biological processes deeply investigated. On the contrary, the study of the interaction of zinc(II) complexes of Schiff bases with DNA has received less attention. Since H_2Salen and most of its derivatives are usually sparingly soluble in water [23], the synthesis of water-soluble derivatives is an important first step for possible applications in biological systems. To this aim, an increasing number of papers have been reported to introduce charged groups to the salicylidene or diamine moieties [24–27]. In particular, it has been reported that cationic Schiff base complexes of copper(II) strongly interact with DNA by groove or intercalating binding mode, depending on the size and the nature of the substituents [27].

To further investigate the role of the metal ion in the interaction with DNA, in the present paper, we have synthesized the Cu(II) and Zn(II) complexes of 5-triethyl ammonium methyl salicylidene orto-phenylendiimine [21] and studied their interaction with native calf thymus DNA in aqueous solution.

2. Materials and methods

The complexes (Fig. 1) were synthesized [21] by the reaction of 5-(triethylammoniummethyl)salicylaldehyde chloride, 1,2 phenylendiamine and copper(II) or zinc(II) acetate in a 2:1.1:1 molar ratio, in doubly distilled water, and isolated as perchlorate salts. The 5-(triethylammoniummethyl) salicylaldeide chloride ligand was prepared from 5-chloromethyl salicylaldeide [28] and triethylamine in THF. The products were characterized by elemental analysis, UV spectroscopy, and/or ^{1}H NMR and ^{13}C NMR.

5-Chloromethyl salicylaldeide: ^{1}H NMR (300.13 MHz, $CDCl_3$, s, singlet; d, doublet; m, multiplet) δ (ppm): 4.59 (s, 2H, CH_2); 6.99–7.02 (d, 1H, $J = 8.4$ Hz, Ar); 7.54–7.59 (m, 2H, Ar); 9.90 (s, 1H, OH); 11.07(s, 1H, CHO); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ (ppm): 45.19; 118.30; 120.34; 129.19; 133.58; 137.27; 161.61; 196.12.

5-(Triethylammoniummethyl) salicylaldeide chloride: ^{1}H NMR (300.13 MHz, CD_3OD, t, triplet; q, quartet; dd, double doublet) δ (ppm): 1.33–1.38 (t, 9H, $J = 6.8$ Hz, CH_3); 3.11–3.23 (q, 6H, $J = 7.0$ Hz, CH_2); 4.41 (s, 2H, CH_2); 7.01–7.04 (d, 1H, $J = 9.5$ Hz, Ar); 7.59–7.64 (dd, 1H, $J = 2.5$ Hz, $J = 10.0$ Hz, Ar); 7.81–7.82 (d, 1H, $J = 2.5$ Hz, Ar); 10.08 (s, 1H, CHO); ^{13}C NMR (75.47 MHz, CD_3OD) δ (ppm): 7.06; 52.48; 59.51; 118.45; 118.88; 122.24; 136.32; 140.21; 162.97; 194.56.

$CuC_{34}H_{46}N_4O_{10}Cl_2$ ($CuL(ClO_4)_2$): (a) Anal. Calcd: C, 50.72; H, 5.76; N, 6.96; Cl, 8.81; found: C, 50.22; H, 5.69; N, 6.86; Cl, 8.59; (b) electronic absorption in Tris–HCl, pH 7.5: [nm (ε, $M^{-1}\,cm^{-1}$)]: 252 (5.35×10^4), 304 (2.49×10^4), 334 (1.95×10^4), 397 (1.79×10^4).

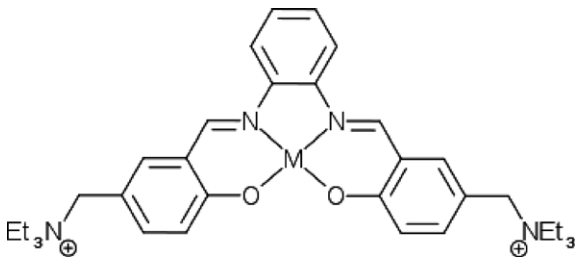

Fig. 1. Structure of the ML^{2+} complex (M = Zn, Cu, H_2L^{2+} = 5-triethyl ammonium methyl salicylidene orto-phenylendiimine).

$ZnC_{34}H_{46}N_4O_{10}Cl_2$ ($ZnL(ClO_4)_2$): (a) Anal. Calcd: C, 50.60; H, 5.74; N, 6.94; Cl, 8.79; found: C, 50.18; H, 5.68; N, 6.97; Cl, 8.78; (b) electronic absorption in Tris–HCl, pH 7.5: [nm (ε, $M^{-1}\,cm^{-1}$)]: 244 (3.97×10^4), 293 (1.69×10^4), 373 (1.51×10^4); (c) ^1H NMR (300.13 MHz, DMSO-D_6) δ (ppm): 1.23–1.27 (t, 18H, $J = 6.6$ Hz, CH_3); 3.04–3.09 (q, 6H, $J = 6.3$ Hz, CH_2); 4.25 (s, 2H, CH_2); 6.68–6.71 (d, 2H, $J = 8.7$ Hz, Ar); 7.22–7.26 (dd, 2H, $J = 2.1$ Hz, $J = 9.0$ Hz, Ar); 7.36–7.39 (m, 2H, Ar); 7.46–7.47 (d, 2H, $J = 2.1$ Hz, Ar); 7.83–7.86 (m, 2H, Ar); 8.97 (s, 2H, CH).

The NMR spectra were recorded on a Bruker Avance 300 spectrometer.

Lyophilized calf thymus DNA (Fluka, BioChemika) was resuspended in 1.0×10^{-3} M tris-hydroxymethyl-aminomethane (Tris–HCl) pH 7.5 and dialyzed as described in the literature [29]. DNA concentration, expressed in monomers units ([$DNA_{phosphate}$]), was determined by UV spectrophotometry using the molar absorption coefficient 7000 $M^{-1}\,cm^{-1}$ at 258 nm [30]. Ethidium bromide (EB) (Aldrich) was dissolved in Tris–HCl, pH 7.5.

All experiments were carried in Tris–HCl aqueous buffer at pH 7.5. Absorption measurements were performed on a Varian UV–vis Cary 1E double beam spectrophotometer, equipped with a Peltier temperature controller, using 1 cm path-length cuvettes. Circular dichroism (CD) spectra were recorded at 25 °C on a Jasco J-715 spectropolarimeter, using 1 cm path-length quartz cells. Fluorescence spectral changes were registered, at room temperature, in the range 200–1100 nm on samples excited by a Deuterium lamp (Avalight-DHS) in the frequency range 200–400 nm using an Avaspec-2048 spectrometer.

3. Results and discussion

3.1. UV–vis Absorption spectroscopy

CuL^{2+} and ZnL^{2+} (Fig. 1) show an intense absorption band at about 252 and 244 nm, respectively, and a characteristic absorption band at about 397 nm and 373 nm, respectively. The latter can be attributed to a metal perturbed infra-ligand electronic transition. In fact, an absorption band at about 370 nm has been also observed in 1 mM Tris–HCl aqueous solutions of the isolated ligand. Moreover, the similarity with the UV absorption spectrum of the Fe(III)SalenCl complex [31,32], recently investigated [20], allows us to infer that both the Zn(II) and Cu(II) cationic complexes analogously adopt a square planar O_2N_2 coordination. The UV–vis absorption spectrum of both metal complexes is significantly perturbed by the addition of increasing amounts of DNA. In details, the absorption band of ZnL^{2+} at 373 nm (black line in Fig. 2a) is red shifted by about 10 nm and shows hypochromism of about 40%. The absorption band of CuL^{2+} at 397 nm (black line in Fig. 2b) is red shifted by about 20 nm and shows hypochromism of about 30%. Moreover, the intensity of the absorption band at 260 nm (black line) is lowered by the addition of DNA up to stoichiometric ratio [$DNA_{phosphate}$]/[ML^{2+}] = 1.0 for both ML^{2+} complexes (lines red, cyan and magenta[1] for ZnL^{2+}, Fig. 2a, lines red and cyan for CuL^{2+}, Fig. 2b), while it increases at higher molar ratios. All these findings support the hypothesis of DNA intercalating interactions of both metal complexes [33] through the stacking interaction of the aromatic rings of the ligand and the base pairs of DNA, with a higher affinity of CuL^{2+} toward DNA, compared to ZnL^{2+}.

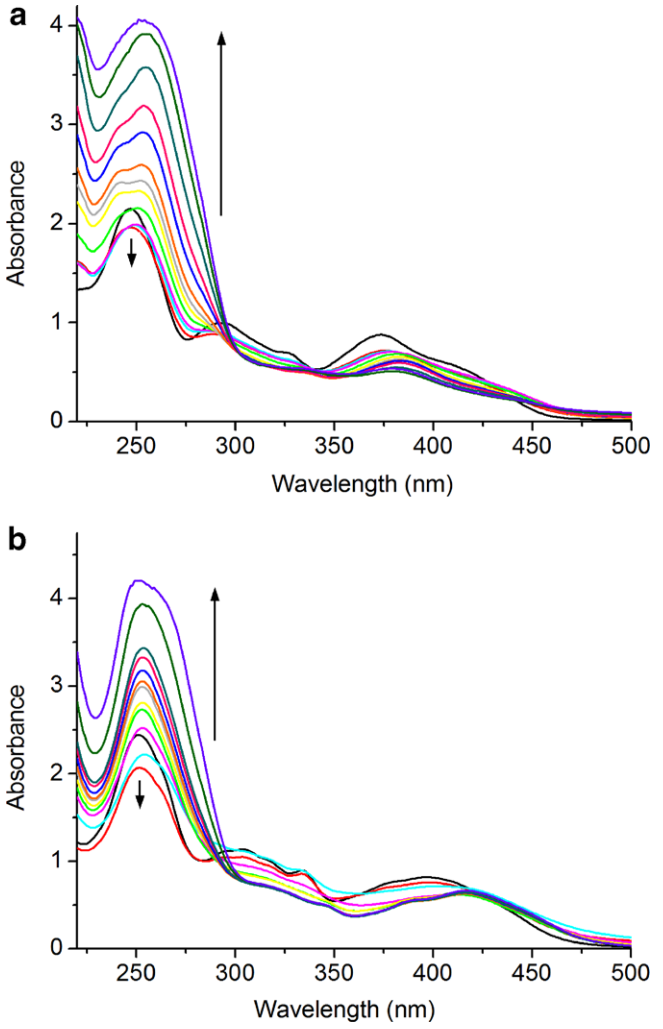

Fig. 2. Absorption spectra of ZnL^{2+} (a) and CuL^{2+} (b) in the presence of increasing amounts of CT-DNA. (a) [ZnL^{2+}] = 54.0 μM, [DNA] = 0.0 (–), 27.0 (–), 40.4 (–), 54.0 (–), 80.9 (–), 107.8 (–), 136.1 (–), 161.8 (–), 215.7 (–), 269.6 (–), 350.5 (–), 457.4 (–), 540.0 (–) μM. (b) [CuL^{2+}] = 52.7 μM, [DNA] = 0.0 (–), 26.4 (–), 52.7 (–), 79.1 (–), 105.5 (–), 132.0 (–), 158.0 (–), 184.8 (–), 211.6 (–), 238.4 (–), 263.7 (–), 394.8 (–), 527.5 (–) μM.

The quantity $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ at 373 nm for ZnL^{2+} and at 397 nm for CuL^{2+} has been plotted in Fig. 3, at increasing amounts of DNA. ε_a, ε_f and ε_b are the molar extinction coefficients of the solution containing both complex and DNA, of the free complex, and of the complex bound to

[1] For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

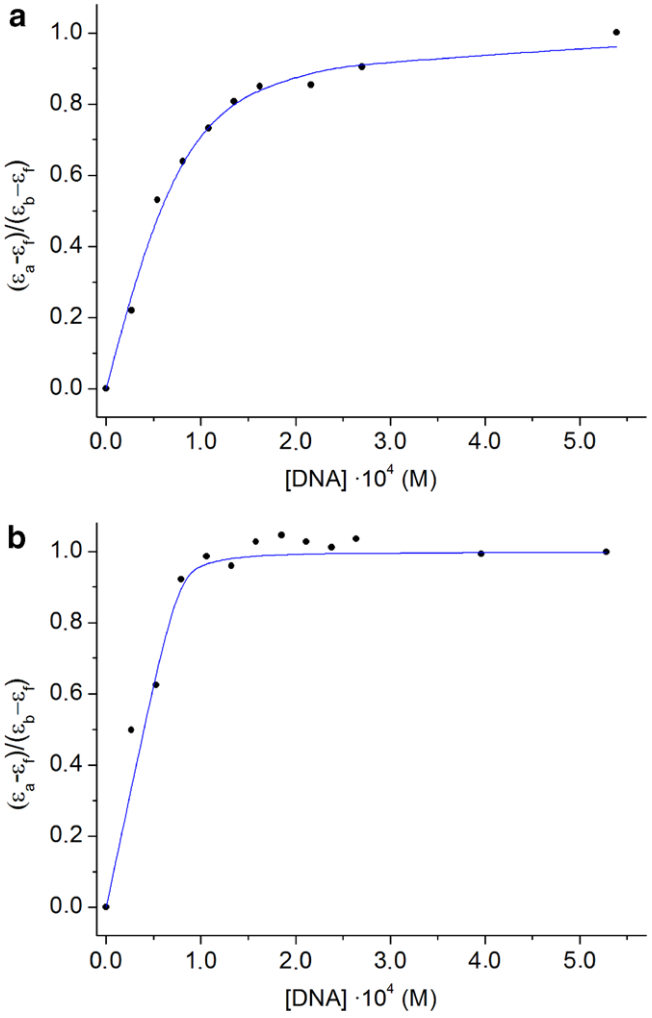

Fig. 3. Spectrophotometric titration of (a) ZnL^{2+}, at 373 nm, and (b) CuL^{2+}, at 397 nm, with CT-DNA in aqueous solution. $[ZnL^{2+}] = 54.0\ \mu M$, $[DNA] = 0.0–540\ \mu M$. $[CuL^{2+}] = 52.7\ \mu M$, $[DNA] = 0.0–527.5\ \mu M$. The solid line is the fit of the experimental data by Eq. (1).

DNA, respectively. In particular, ε_f was determined by a calibration curve of the isolated metal complexes in aqueous solution, following the Beer–Lambert law. ε_b was determined from the plateau of the DNA titration (Fig. 3), where addition of DNA did not result in further changes in the absorption spectrum. ε_a, was determined as the ratio between the measured absorbance and the ML^{2+} concentration.

Following Eq. (1) [34], such spectrophotometric titration allowed us to obtain the values of the intrinsic binding constant (K_b) and of the binding size in base pairs (s) of the ZnL–DNA and of the CuL–DNA complexes:

$$\frac{(\varepsilon_a-\varepsilon_f)}{(\varepsilon_b-\varepsilon_f)} = \frac{b-(b^2-2K_b^2C_t[DNA_{phosphate}])^{1/2}}{\frac{s}{2K_bC_t}} \quad (1a)$$

$$b = 1 + K_bC_t + \frac{K_b[DNA_{phosphate}]}{2s} \quad (1b)$$

where C_t is the total concentration of the metal complex, and $[DNA_{phosphate}]$ is the DNA concentration in monomer units. The K_b and s values obtained by nonlinear fits of the experimental data by Eq. (1) were $K_b = (1.28 \pm 0.05) \times 10^6\ M^{-1}$, $s = 0.73 \pm 0.05$ for CuL^{2+}, $K_b = (7.35 \pm 0.01) \times 10^4\ M^{-1}$, $s = 0.69 \pm 0.01$ for ZnL^{2+}. These results confirm that both metal complexes strongly interact with DNA and that the binding of CuL^{2+} to DNA is one order of magnitude tighter than that of ZnL^{2+}. These K_b values are of the same order of magnitude of that determined in analogous conditions for EB, $K_b = 1.9 \times 10^5\ M^{-1}$ [5]. Moreover, the K_b value obtained for the CuL^{2+}–DNA system is analogous to that previously determined by equilibrium dialysis and spectrophotometric methods [27], where it is reported that CuL^{2+} is a strong DNA intercalator. Finally, both complexes show an interaction stoichiometry of approximately 2 mol of DNA base pairs per 3 mol of metal complex. Similar values have been found for metal complexes–DNA systems with ascertained DNA intercalating interaction (see e.g. [4,17]). It has been in particular reported [4] that a value of 0.7 mol of base pair per mol of ligand could result from stacking of ligand molecules on the DNA surface.

3.2. DNA thermal denaturation analysis

Thermal denaturation profiles of calf thymus DNA solutions, in the presence of increasing amounts of ZnL^{2+} and CuL^{2+}, were obtained by plotting the absorbance at 258 nm as a function of temperature (see Fig. 4).

It is known that when the temperature in the solution increases, the double-stranded DNA gradually dissociates into single strands; T_m is therefore defined as the temperature where half of the total base pairs is unpaired [35]. The DNA melting temperature (T_m) is strictly related to the stability of the double helix, and the interaction of chemicals with DNA may alter T_m, by stabilizing or destabilizing the final complex. Moreover, it is also usually possible to obtain information on the strength of the interaction.

According to the literature [36–38] the intercalation of natural or synthesized compounds results in the stabilization of the DNA double helix, due to the stabilizing stacking interactions, followed by a considerable increase in the melting temperature of DNA. The presence of a positive charge on the intercalator should further increase the attractive interaction with the negatively charged phosphate groups and help the intercalative mechanism.

The melting temperature of DNA 100 μM in Tris–HCl 1 mM (53 ± 2 °C, Fig. 4) increases of about 14, 17 and 26 °C, at $[ZnL^{2+}]/[DNA_{phosphate}]$ molar ratios 0.1, 0.2 and 0.45 (Fig. 4a) and of about 21 and 37 °C, at $[CuL^{2+}]/[DNA_{phosphate}]$ molar ratios 0.1 and 0.2 (Fig. 4b). These results are indicative of a strong metal complex–DNA interaction, that stabilizes the native DNA conformation. Moreover it can be argued that the stabilization, hence the complex–DNA binding, is stronger for CuL^{2+} compared to ZnL^{2+}.

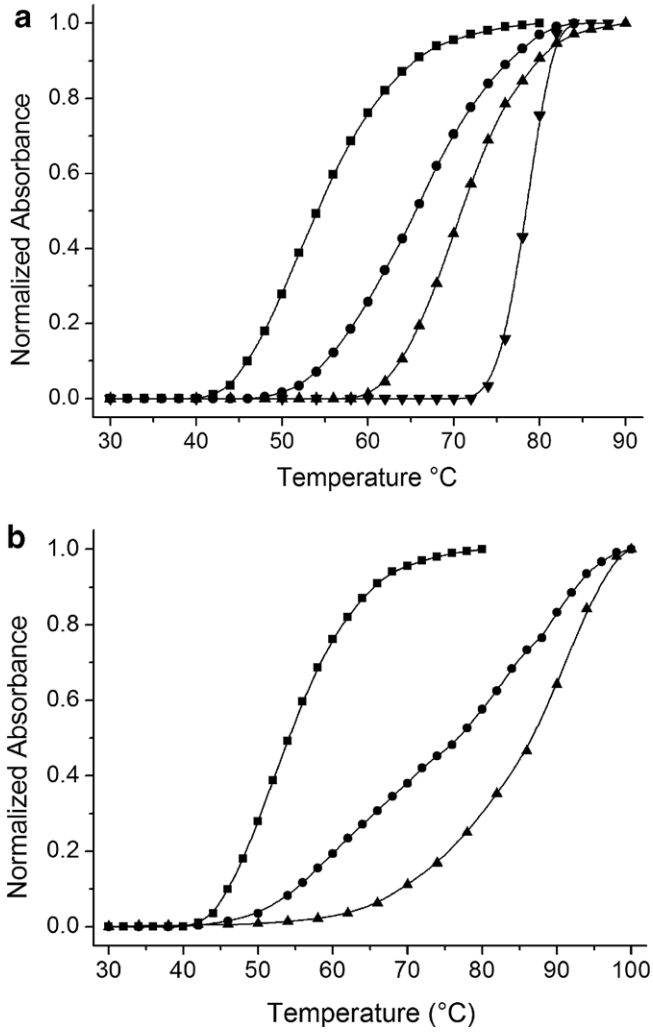

Fig. 4. Thermal denaturation profiles of CT-DNA in the presence of increasing amounts of (a) ZnL^{2+} and (b) CuL^{2+}, in 1.0 mM Tris–HCl (pH 7.5). $[DNA_{phosphate}] = 100.0$ μM; (a) $[ZnL^{2+}] = 0.0$ (■, $T_m = 53$ °C), 10.0 μM (●, $T_m = 67$ °C), 20.0 μM (▲, $T_m = 70$ °C), 45.0 μM (▼, $T_m = 79$ °C); (b) $[CuL^{2+}] = 0.0$ μM (■, $T_m = 53$ °C), 10.0 μM. (●, $T_m = 74$ °C), 20.0 μM (▲, $T_m = 90$ °C).

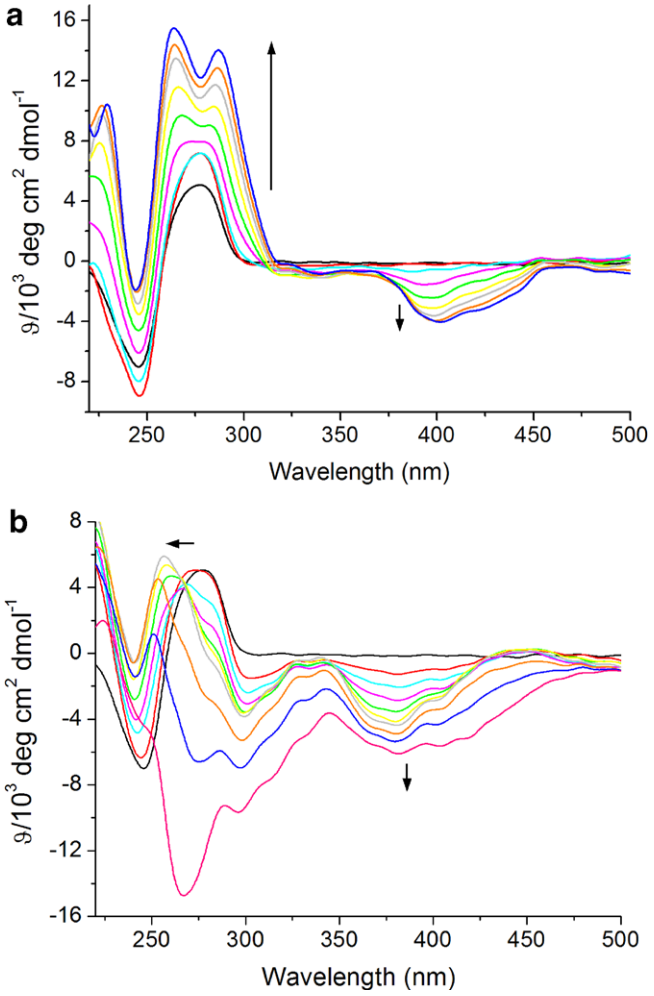

Fig. 5. Circular dichroism spectra of CT-DNA in the presence of increasing amounts of (a) ZnL^{2+} and (b) CuL^{2+}, in 1.0 mM Tris–HCl (pH 7.5). $[DNA_{phosphate}] = 100.0$ μM, (a) $[ZnL^{2+}] = 0.0$ (–), 11.5 (–), 17.2 (–), 23.0 (–), 28.7 (–), 34.5 (–), 40.2 (–), 46.0 (–), 51.7 (–) μM; (b) $[CuL^{2+}] = 0.0$ (–), 10.0 (–), 15.0 (–), 20.0 (–), 25.0 (–), 30.0 (–), 35.0 (–), 40.0 (–), 45.0 (–), 50.0 (–) μM.

The peculiar trend of the DNA melting profile observed in the presence of CuL^{2+} at $[CuL^{2+}]/[DNA_{phosphate}]$ molar ratios 0.1 (Fig. 4b), induces us to hypothesize that there are at least two different melting transitions involved occurring at about 65 and 85 °C. The transition at the lower temperature is no more observed in the presence of higher amounts of CuL^{2+} in the DNA solutions.

3.3. Circular dichroism

CD spectra of CT-DNA 100.0 μM, in 1.0 mM Tris–HCl (Fig. 5), were recorded in the presence of increasing amounts of ZnL^{2+} and CuL^{2+}, up to $[ML^{2+}]/[DNA_{phosphate}]$ molar ratios approximately 0.5.

The DNA dichroic band at ca. 275 nm (black line in Fig. 5a) is monotonously increased, and splitted in two bands at 264 and 287 nm, by the addition of increasing amounts of ZnL^{2+}. Moreover, an induced CD band appears in the range 375–450 nm. The CD of native DNA (black line in Fig. 5b) is drastically modified by the addition of increasing amounts of CuL^{2+}. In particular, a decrease and a blue shift of the positive CD band of DNA is observed and, also in this case, an induced CD band appears in the range 350–450 nm.

These findings are indicative of deep conformational changes of the DNA double helix following the interaction of DNA macromolecule with the metal complexes. Moreover, it can be argued that a tight binding exists between the metal complexes and DNA. In fact, the presence of induced CD shows that both ZnL^{2+} and CuL^{2+} moieties supply a further chromofore appended to the chiral backbone of the DNA double helix [39].

Finally, the CD spectrum recorded in the presence of $[CuL^{2+}] = 50$ μM is similar to that observed for the condensed ψ-DNA forms [40]. This would indicate that the copper complex at higher concentrations induces the

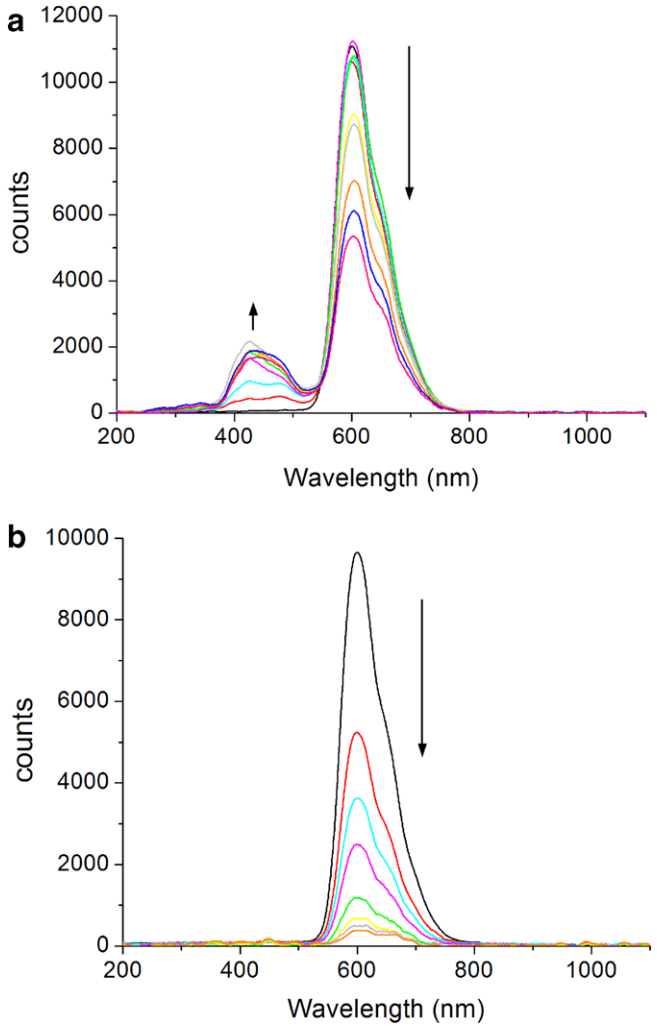

Fig. 6. Fluorescence spectra of the EB-DNA complex in the presence of increasing amounts of (a) ZnL^{2+} and (b) CuL^{2+}. [EB] = 4.0 μM, $[DNA_{phosphate}]$ = 25.0 μM; (a) $[ZnL^{2+}]$ = 0.0 (–), 2.5 (–), 7.5 (–), 10.0 (–), 12.5 (–), 17.5 (–), 20.0 (–), 30.0 (–), 40.0 (–), 60.0 (–) μM; (b) $[CuL^{2+}]$ = 0.0 (–), 1.3 (–), 1.9 (–), 2.5 (–), 3.8 (–), 5.0 (–), 6.3 (–), 7.5 (–) μM.

formation of supramolecular DNA aggregates. Such results could be put in relation with the DNA melting profile recorded at $[CuL^{2+}]/[DNA_{phosphate}]$ molar ratio 0.1 (Fig. 4b). In fact, this would mean that in the presence of low amounts of CuL^{2+}, two forms of DNA could be present, i.e. the non aggregate and the aggregate forms. By increasing the CuL^{2+} concentration, an increase of the amount of supramolecular DNA structure should occur.

3.4. Fluorescence spectroscopic studies

No fluorescence was observed for the CuL^{2+} solution, while an emission band at 440 nm is present in the spectrum of the ZnL^{2+} complex. On the other hand, it is know that CT-DNA does not give fluorescence, while its emission intensity is greatly enhanced in the presence of Ethidium bromide (EB). EB is also weakly fluorescent, but the EB-DNA complex is remarkably more fluorescent at about 600 nm, as a consequence of the intercalation of EB between adjacent DNA base pairs [41].

The emission spectra of the EB-DNA complex in Tris–HCl 1 mM, in the presence of increasing amounts of ZnL^{2+} and CuL^{2+}, are shown in Fig. 6. It can be noted that the intensity of the fluorescence spectrum of the EB-DNA complex is lowered by the addition of increasing amounts of both ZnL^{2+} (Fig. 6a) and of CuL^{2+} (Fig. 6b). Both complexes then seem to displace EB from the DNA cavities. This result confirms that both complexes interact with DNA by an intercalating mechanism, competing with EB for the same binding sites.

The relative intensity values (F_0/F) at 600 nm, of the fluorescence spectra of the EB-DNA complex in the presence of increasing amounts of ZnL^{2+} and of CuL^{2+} are reported in Fig. 7.

In Fig. 7a it is possible to distinguish two linear trends of the emission band at ca. 600 nm with the amount of ZnL^{2+},

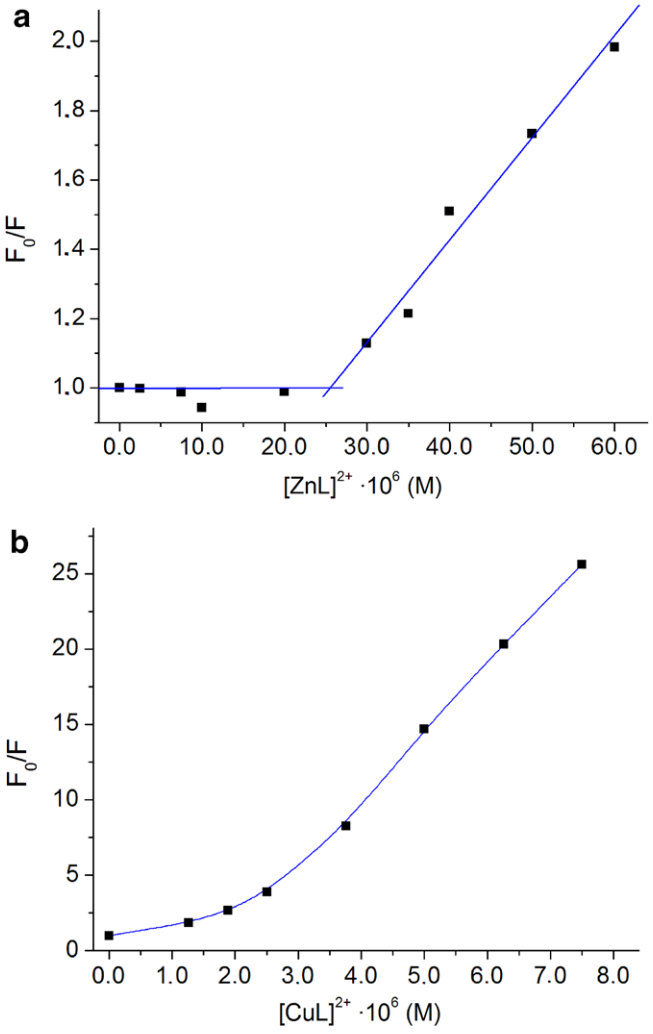

Fig. 7. Relative intensity (F_0/F) at 600 nm, of the fluorescence spectra of the EB-DNA complex in the presence of increasing amounts of (a) ZnL^{2+} and (b) CuL^{2+}. [EB] = 4.0 μM, $[DNA_{phosphate}]$ = 25.0 μM; (a) $[ZnL^{2+}]$ = 0–60 μM; (b) $[CuL^{2+}]$ = 0–7.5 μM.

showing that: at $[ZnL^{2+}]/[DNA_{phosphate}]$ molar ratios roughly less than 1, there is no evidence of EB quenching; at $[ZnL^{2+}]/[DNA_{phosphate}]$ molar ratios greater than 1, the emission intensity monotonously decreases by increasing the ZnL^{2+} concentration.

On the other hand, Fig. 7b shows that the emission band at ca. 600 nm monotonously decreases by increasing the amount of CuL^{2+} at concentrations 10 times lower than ZnL^{2+}.

This result shows that CuL^{2+} is more able than ZnL^{2+} in replacing the strong DNA intercalator EB, in agreement with the higher value of K_b spectrophotometrically determined (see above).

This result is consistent with a DNA intercalation mechanism for both metal complexes, being CuL^{2+} characterized by a higher affinity toward DNA, compared to ZnL^{2+}.

4. Conclusions

The results obtained collectively show that ZnL^{2+} and CuL^{2+} strongly interact with native DNA, presumably by an intercalative mechanism. CuL^{2+}, seems to be a stronger DNA intercalator than ZnL^{2+}.

The interaction occurrence is supported by the following three findings:

(i) the high values of K_b, $(1.28 \pm 0.05) \times 10^6$ M^{-1}, and $(7.35 \pm 0.01) \times 10^4$ M^{-1}, obtained for CuL^{2+} and ZnL^{2+}, respectively;
(ii) the noteworthy increase of the DNA melting temperature, T_m, of about 14, 17 and 26 °C, at $[ZnL^{2+}]/[DNA_{phosphate}]$ molar ratios 0.1, 0.2 and 0.45, and of about 21 and 37 °C, at $[CuL^{2+}]/[DNA_{phosphate}]$ molar ratios 0.1 and 0.2;
(iii) the appearance of an induced CD band in the range 350–450 nm at increasing $[ML^{2+}]/[DNA_{phosphate}]$ molar ratios.

On the other hand, the intercalation mechanism is supported by the following three evidences:

(i) the UV hypochromism and bathochromic shift of the absorption bands at 373 nm and 397 nm, for ZnL^{2+} and CuL^{2+}, respectively, in the presence of increasing amounts of DNA;
(ii) the decrease of the fluorescence band at 600 nm of EB-DNA solutions by the addition of increasing amounts of ML^{2+} complexes;
(iii) the pronounced modifications of the CD of DNA in the presence of increasing amounts of both ML^{2+} complexes.

Finally, it is worth mentioning that the shape of the CD of the CuL^{2+}–DNA solutions, at the highest $[CuL^{2+}]/[DNA_{phosphate}]$ molar ratios investigated, is indicative of the formation of DNA aggregates.

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