Journal of Inorganic Biochemistry 104 (2010) 877–884

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Metal complexes of modified cyclen as catalysts for hydrolytic restriction of plasmid DNA

Joel A. Krauser, Aarti L. Joshi, Ismail O. Kady *

Department of Chemistry, East Tennessee State University, Box 70,695, Johnson City, TN 37614, USA

ARTICLE INFO

Article history:
Received 3 December 2009
Received in revised form 15 April 2010
Accepted 16 April 2010
Available online 24 April 2010

Keywords:
DNA
Acridine
Nuclease models
Metal complexes
Cyclen
Intercalation

ABSTRACT

Simple and novel nuclease models have been synthesized. These involve metal-binding ligand 1,4,7,10-tetraazlcyclododecane (cyclen) tethered to an acridine ring (a DNA-binding group) by amide linkers of various lengths. Binding of these probes to DNA was studied by monitoring changes in their UV–visible spectra affected by the presence of DNA. Titration of these compounds with increasing amounts of pBR322 DNA caused hypochromic effects and shifted the acridine absorption at 360 nm to a longer wavelength. Under biologically relevant conditions (37 °C and pH 7.4), specific transition metal complexes of these compounds are found to be highly effective catalysts toward the hydrolysis of plasmid DNA. This is demonstrated by their ability to convert the super-coiled DNA (form I) to open-circular DNA (form II). Structure-activity correlation studies show that hydrolytic activity depends on both the structure of ligand (L_1>L_2>L_3) and the nature of metal ion cofactor (Co^{3+}>Zn^{2+}>Cr^{2+}>Ni^{2+}>Cu^{2+}>Fe^{3+}).

1. Introduction

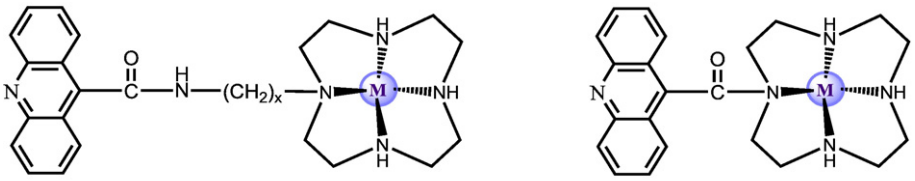
Molecular recognition and sequence-specific cleavage of DNA by synthetic probes have been the subject of intensive studies for a number of years [1–5]. Such restriction enzyme analogs, if proven sufficiently efficient and specific, could have numerous applications, such as DNA structure and sequence determination, recombinant DNA manipulation, gene isolation and analysis, and development of therapeutic agents [6–8]. Such applications have been confined to a limited number of natural restriction endonucleases. Under physiological conditions, phosphodiester bonds of DNA are found to be remarkably stable and resistant to hydrolysis. Deoxyribonucleases, in the presence of metal ion cofactors, are known to accelerate the hydrolysis of DNA phosphodiester bonds by a factor of up to 10^{16}. However, new tools for site-selective DNA hydrolysis are still needed for further advancement in biotechnology and molecular biology, such as manipulation of large DNA molecules of higher plants and animals that cannot be manipulated by the natuarally occuring restriction endonucleases [9–12].

Many DNA-intercalating agents can bind reversibly to DNA by intercalation of their planar, mostly aromatic, chromophores between the nitrogen base pairs. One effect of clinically used DNA-intercalating agents, such as the antitumor drug Amsacrine AMSA, is their ability to selectively inhibit DNA synthesis, which is attributed to inhibition of DNA polymerase I [13]. Yet another major effect of DNA-intercalating drugs is their ability to induce DNA strand breaks which result in disruption of the topoisomerase enzyme function [14]. DNA topoisomerase enzymes, such as mammalian *topo* I and *topo* II, can untwist the densely packed DNA by generating temporary breaks within the DNA strands which result in topological changes in the tertiary structure of the macromolecule. It is believed that molecules that can stimulate permanent strand breaks in DNA would be of significantly important in terms of developing new chemotherapeutic agents and in elucidating the specific mechanisms involved in DNA-cleaving processes [15]. However, mere intercalation of such drugs with DNA is not sufficient for anti-neoplastic activity. Evaluation of a series of substituted acridine derivatives showed that DNA intercalation, coupled with the action of appropriately placed active side-groups, is needed for antitumor and anti-neoplastic activities of such agents [16,17]. Therefore, the central premise, underlying the design of chemical agents that can mediate chemical reactions on nucleic acids (and proteins), is that the juxtaposition of a target recognizing domain with a catalytic moiety will result in molecules with properties that are superior to the sum of the individual component parts; this strategy provides a novel platform for drug design and development.

Inspired by our previous work [18–21] and that of others [22–27] which showed that certain metal complexes exhibit remarkable activity toward hydrolysis of the phosphodiester bonds, and in an effort to design relatively small synthetic molecules that can mimic deoxyribonucleases, we synthesized a series of compounds L_1–L_3. These molecules involve a DNA-binding group (acridine) tethered to a metal-binding 1,4,7,10-tetraazlcyclododecane cyclen ligand (Scheme 1). This work demonstrates the effectiveness of some metal complexes of these

* Corresponding author. Tel.: +1 423 439 6910; fax: +1 423 439 5835.
E-mail address: kadyi@etsu.edu (I.O. Kady).

0162-0134/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2010.04.007



ML_1, x = 1
ML_2, x = 2

ML_3

Scheme 1. Compounds synthesized and tested in this study.

probes in catalyzing the hydrolysis of DNA under biologically relevant conditions.

2. Experimental

2.1. General information

All chemical reagents of puriss quality were purchase either from Aldrich or Sigma company and were used without further purification. Solvents were purchased from Fisher Scientific Company and used untreated unless mentioned otherwise. Plasmid DNA pBR322 was obtained from Sigma company, and concentrations of all DNA solutions (base pairs per liter) were determined spectrophotometrically. Ultra pure sterilized water was used in all experiment. The free base ligands were synthesized as described below and purified by crystallization of their HBr salts. Their purity was verified by ^{1}H NMR and mass spectroscopy to be >99%. UV–visible spectra were recorded on Shimadzu UV-1700 spectrophotometer equipped with a temperature control unit. NMR spectra were obtained on 400 MHz JEOL JNM-ECS spectrometer, sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 in D_2O and tetramethylsilane (TMS) in $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR measurements. The abbreviations used to describe the peaks splitting patterns in the reported ^{1}H NMR spectra are as follows: d = doublet, t = triplet, m = multiplet, and brd = broad. Thin-layer chromatography (TLC) was performed using silica gel plates with fluorescent indicator UV-254 from Aldrich; column chromatography were performed using silica gel (60–200) from TSI Chemicals. Electrophoresis was performed using 1% agarose gels submersed in TAE buffer (20 mM TRIS base, 20 mM acetic acid, 1 mM EDTA) containing 1% SDS.

2.2. Synthesis

2.2.1. (2-hydroxyoethyl)-acridine-9-carboxamide (1)

9-acridinecarbonyl chloride was prepared from 9-acridinecarboxylic acid using modified literature procedures [28]. Specifically, 9-acridinecarboxylic acid hydrate (0.48 g, 2 mmol) was refluxed in freshly distilled thionyl chloride (5 mL) for 2 h under anhydrous nitrogen atmosphere. The excess thionyl chloride was distilled under reduced pressure and the remaining yellow solid product was further dried under vacuum (0.1 mmHg) overnight and used without further purification. The product (0.483 g, 2 mmol) was dissolved in anhydrous acetonitrile (10 mL) then added gradually, over a period of 2 h, to a solution of 2-ethanolamine (0.3 g, 5 mmol) in acetonitrile (20 mL) containing 0.5 mL of triethylamine. The reaction mixture was stirred at room temperature (under nitrogen) for 12 h. The white precipitate was separated by filtration and solvent was evaporated under reduced pressure, the excess triethylamine and 2-ethanolamine were evaporated in vacuum (0.1 mmHg) to leave a yellow solid. The crude product was purified by silica gel column chromatography (methylene chloride containing 10% methanol and 0.5% conc. NH_4OH). Yield (0.23, 48%); mp: 173 °C (dec). IR (KBr): 3210, 3035, 2953, 2941, 2857, 2835, 1732, 1605, 1523, 1458, 1445, 1305, 1163, 1012 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 8.26 (d, 2H, J = 8.2 Hz), 8.15 (d, 2H, J = 8.3 Hz), 7.89 (t, 2H), 7.77 (t, 2H), 3.83 (t, 2H), 3.42 (t, 2H). ^{13}C NMR (DMSO-d6): δ 173.45 (C O), 142.52, 133.48, 129.23, 127.42, 126.49, 125.28, 120.19, 63.27, 27.64. MS (m/z, %): 266 (M^+, 9), 248 (78), 238 (27), 236 (32), 220 (31), 206 (36), 178 (100), 88 (15). Anal. calc. for $C_{16}H_{14}N_2O$: C, 72.05; H, 5.31; N, 10.52. Found: C, 72.14; H, 5.21; N, 10.41.

2.2.2. (3-hydroxyopropyl)-acridine-9-carboxamide (2)

A procedure similar to that of (2-hydroxyoethyl)-acridine-9-carboxamide (1) was used, except using 3-aminopropanol in place of 2-ethanolamine. Yield (54%); mp: 141 °C (dec). IR (KBr): 3220, 3028, 2949, 2931, 2863, 2826, 1725, 1603, 1518, 1442, 1435, 1300, 1038 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 8.31 (d, 2H, J = 7.9 Hz), 8.17 (d, 2H, J = 8.1 Hz), 7.85 (t, 2H), 7.73 (t, 2H), 3.92 (t, 2H), 3.38 (t, 2H), 2.05 (m, 2H). ^{13}C NMR (DMSO-d6): δ 174.03 (C O), 141.25, 132.52, 128.95, 126.98, 125.23, 123.18, 120.11, 61.25, 50.95, 27.58. MS, m/z (%): 281 (M^+ + 1, 12), 262 (80), 250 (23), 234 (17), 206 (35), 178 (100), 59 (8). Anal. calc. for $C_{17}H_{16}N_2O_2$: C, 72.83; H, 5.75; N, 9.99. Found: C, 72.75; H, 5.64; N, 9.89.

2.2.3. Acridine-tethered cyclen ligands (L_1)

(2-Hydroxyoethyl)-acridine-9-carboxamide **1** (0.266 g, 1 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 10 mL) containing anhydrous pyridine (0.316 g, 4 mmol). This solution was added gradually to a solution of p-toluenesulfonyl chloride (Ts–SO_2Cl, 0.285 g, 1.5 mmol) in THF (10 mL), cooled in ice bath under nitrogen atmosphere. The solution was stirred at room temperature for 4 h, and the white precipitate was removed by filtration. The filtrate was added gradually to a solution of 1,4,7,10-tetraazlcyclododecane (cyclen 0.344 g, 2 mmol) in 10 mL of anhydrous THF. The reaction mixture was stirred at room temperature for 8 h under nitrogen. The solvent was removed under reduced pressure and the resulting oil was taken up in 5 mL of 5% HCl, and the solution was washed three times with methylene chloride (3 × 10 mL). The aqueous layer was treated with 2 mL of 20% NaOH and the product was extracted from the alkaline solution with methylene chloride (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and solvent was evaporated under diminished pressure. The resulting oil was dissolved in methanol (3 mL) and treated with HBr solution (48%, 0.5 mL) to precipitate the hydrobromide salt. This prodedure was repeated once more to obtain pure $L_1 \cdot 4HBr \cdot 4H_2O$, yield (0.35 g, 43%), m.p (102 °C, dec.). IR (KBr): 3410, 2915, 2750, 2466, 2374, 1735, 1618, 1575, 1457, 1433, 1401, 1359 cm^{-1}. ^{1}H NMR (D_2O, pD 2): δ 8.73 (2H, brd), 8.28 (2H, brd), 8.18 (2H), 8.12(2H), 3.96 (2H, t, –CONH–CH_2), 2.80–3.52 (18H, brd multiplet, –CH_2N). ^{13}C NMR (D_2O, pD 2): δ 176.24 (C O), 149.57, 141.63, 133.89, 130.23, 126.27, 122.13, 119.91, 59.15, 53.45, 51.25, 49.34, 46.96, 26.89. Anal. calc. for $C_{24}H_{44}N_6O_5Br_4$: C, 35.31; H, 5.44; N, 10.30. Found: C, 35.29; H, 5.32; N, 10.38.

2.2.4. Acridine-tethered cyclen ligands (L_2)

The ligand $L_2 \cdot 4HBr \cdot 4H_2O$ was prepared from (3-hydroxyopropyl)-acridine-9-carboxamide (**2**) following a procedure similar to the one used to synthesize L_1.4HBr.4H_2O. Yield (3.98 g, 48%); m.p 95 °C, dec.).

IR (KBr): 3408, 2935, 2745, 2474, 2354, 1739, 1628, 1567, 1437, 1428, 1405, 1336 cm^{-1}. ^1H NMR (D_2O, pD 2): δ 8.77 (2H, brd), 8.35 (2H, brd), 8.28 (2H), 8.10 (2H), 4.26 (2H, t,–CONH–CH_2), 2.80–3.52 (18H, brd multiplet,–CH_2N), 1.81 (2H). ^{13}C NMR (D_2O, pD 2): δ 174.34 (C O), 150.12, 142.33, 134.02, 129.95, 125.17, 121.21, 118.95, 61.31, 54.35, 50.98, 48.31, 44.86, 39.96, 26.95. Anal. calc. for $C_{25}H_{46}N_6O_5Br_4$: C, 36.16; H, 5.60; N, 10.12. Found: C, 36.25; H, 5.49; N, 10.18.

2.2.5. Acridine-tethered cyclen ligands (L_3)

Asolution of 9-acridinecarbonyl chloride (0.241 g, 1 mmol) in anhydrous THF (5 mL) was added gradually, over a period of 1 h, to a solution of 1,4,7,10-tetraazlcyclododecane (cyclen, 0.344 g, 2 mmol) in 5 mL of anhydrous THF containing pyridine (0.158 g, 2 mmol). The solution was stirred at room temperature for 4 h and the white precipitate was filtered. The solvent was removed under reduced pressure, the residual oil was dissolved in 5 mL of 5% HCl, and the solution was washed three times with methylene chloride (3 × 10 mL). The aqueous layer was treated with 2 mL of 20% NaOH and the product was extracted from the alkaline solution with methylene chloride (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and solvent was evaporated under diminished pressure. The resulting oil was dissolved in methanol (3 mL) and treated with HBr solution (48%, 1 mL) to precipitate the hydrobromide salt of the product. This prodedure was repeated once more to obtain pure $L_3 \cdot 3HBr \cdot 3H_2O$. Yield (0.487 g, 63%); m.p (125 °C, dec.). IR (KBr): 3428, 2935, 2735, 2432, 1725, 1609, 1573, 1455, 1429, 1413 cm^{-1}. ^1H NMR (D_2O, pD 2): δ 8.71 (2H), 8.19 (2H), 8.08 (2H), 7.95 (2H), 2.80–3.52 (16H, brd multiplet, –CH_2N). ^{13}C NMR (D_2O, pD 2): δ 175.18 (C O), 148.25, 142.58, 133.19, 129.29, 125.97, 123.12, 118.89, 56.19, 52.25, 50.06, 48.394. Anal. calc. for $C_{22}H_{36}N_5O_4Br_3$: C, 39.18; H, 5.39; N, 10.38. Found: C, 39.15; H, 5.37; N, 10.40.

2.2.6. Acridine-tethered cyclen Zn^{II} complex $[Zn^{II}L_1]Cl_2.H_2O$

A solution of $L_1 \cdot 4HBr \cdot 4H_2O$ (0.408 g, 0.5 mmol) was dissolved in 5 mL of 10% NaOH. The alkaline solution was extracted with methylene chloride (3 × 10 mL), the combined organic layers were washed with distilled water (5 mL), dried over anhydrous Na_2CO_3, and evaporated under reduced pressure. The oil residue was dissolved in 5 mL methanol and was added to a solution of zinc(II) chloride (0.273 g, 2 mmol) in 10 mL of hot methanol. The solution was refluxed for 2 h under nitrogen, the yellow solid were collected by filtration and recrystallized from aqueous methanol. Yield of $[Zn^{II}L_1]Cl_2 \cdot H_2O$ (0.235 g, 82%). IR (KBr): 3418, 3285, 2925, 2795, 2486, 2335, 1739, 1609, 1579, 1477, 1453, 1425, 1402, 1369, 1278, 1163, 1109, 998, 923 cm^{-1}. ^1H NMR ($CDCl_3$ + 10% CD_3OD): δ 8.53 (2H, brd, J = 7.9), 8.27 (2H, J = 8.9), 8.03 (2H), 7.85(2H), 4.12 (2H, t, –CONH–CH_2), 3.65 (2H, t, CH_2–N), 2.98–3.48 (16H, brd multiplet, cyclen CH_2N). ^{13}C NMR ($CDCl_3$ + 10% CD_3OD): δ 172.59 (C O, amide), 170.9, 148.55, 142.65, 132.38, 129.51, 125.17, 121.19, 119.35, 55.19, 52.36, 50.26, 48.94, 45.89, 30.57. Anal. calc. for $C_{24}H_{34}Cl_2N_6O_2Zn$: C, 50.14; H, 5.97; N, 14.62. Found: C, 50.02; H, 5.95; N, 14.54.

2.2.7. Acridine-tethered cyclen Zn^{II} complex $[Zn^{II}L_2]Cl_2.H_2O$

This compound was prepared following a procedure similar to that used in the synthesis of $[Zn^{II}L_1]Cl_2.H_2O$, yield (0.247 g, 84%). IR (KBr): 3435, 3275, 2930, 2767, 2448, 2374, 1719, 1601, 1538, 1484, 1444, 1421, 1411, 1336, 1259, 1146, 1120, 987, 942 cm^{-1}. ^1H NMR ($CDCl_3$ + 10% CD_3OD): δ 8.48 (2H, brd, J = 8.1), 8.23 (2H, J = 9.0), 8.02 (2H), 7.77(2H), 4.32 (2H, t, –CONH–CH_2), 3.64 (2H, t, CH_2–N), 2.90–3.52 (16H, brd multiplet, cyclen CH_2N), 1.63 (2H, m). ^{13}C NMR ($CDCl_3$ + 10% CD_3OD): δ 173.57 (C O, amide), 147.98, 141.59, 131.88, 128.91, 126.15, 120.18, 118.95, 56.12, 51.33, 49.86, 47.17, 44.88, 37.93, 29.97. Anal. calc. for $C_{25}H_{36}Cl_2N_6O_2Zn$: C, 50.98; H, 6.17; N, 14.27. Found: C, 50.86; H, 6.16; N, 14.18.

2.2.8. Acridine-tethered cyclen Zn^{II} complex $[Zn^{II}L_3]Cl_2.H_2O$

This compound was prepared following a procedure similar to that used to synthesize $[Zn^{II}L_1]Cl_2 \cdot H_2O$, yield (0.200 g, 75%). IR (KBr): 3465, 3271, 2928, 2758, 2432, 2360, 1702, 1612, 1571, 1448, 1428, 1418, 1402, 1329, 1242, 1136, 1125, 958 cm^{-1}. ^1H NMR ($CDCl_3$ + 10% CD_3OD): δ 8.36 (2H, brd, J = 8.1), 8.20 (2H, J = 9.0), 8.06 (2H), 7.86 (2H), 2.80–3.48 (16H, brd multiplet, cyclen CH_2N). ^{13}C NMR ($CDCl_3$ + 10% CD_3OD): δ 172.52 (C O, amide), 148.25, 140.19, 132.84, 129.10, 125.98, 121.12, 118.62, 56.72, 52.10, 51.66, 49.24. Anal. calc. for $C_{22}H_{29}Cl_2N_5O_2Zn$: C, 49.68; H, 5.51; N, 13.17. Found: C, 49.59; H, 5.49; N, 13.03.

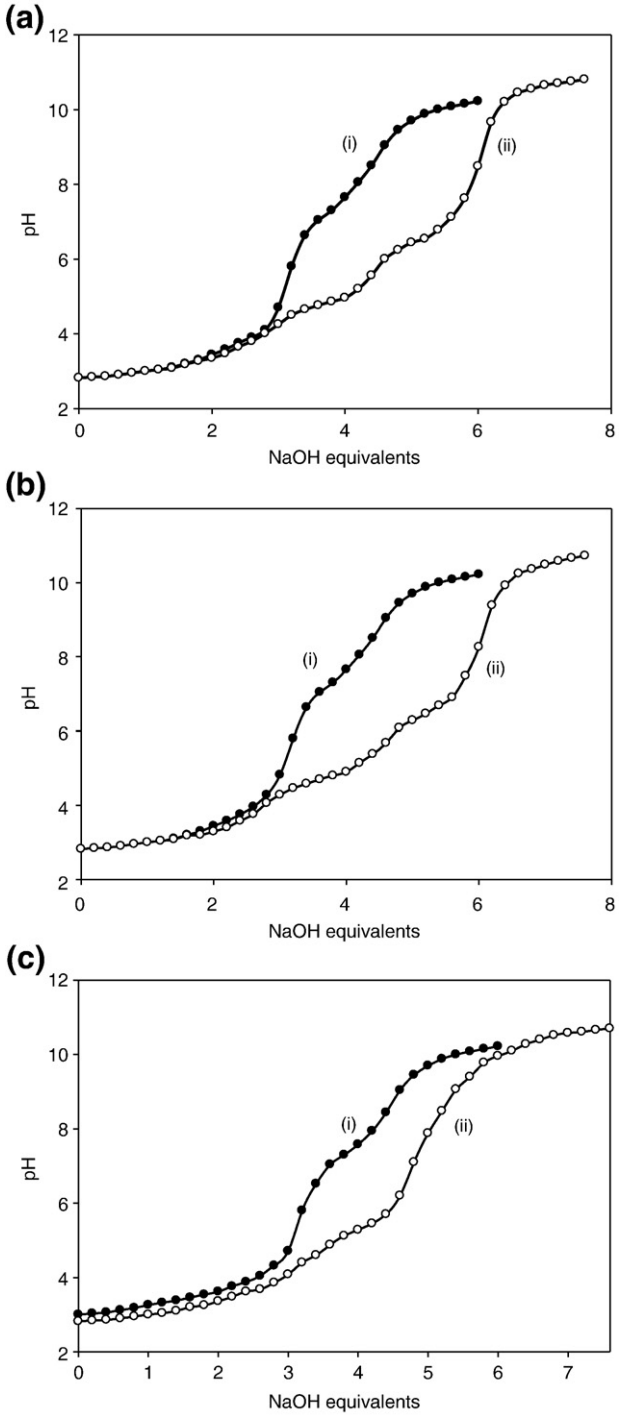


Fig. 1. pH-Potentiometric titration curves of the ligands (L_1–L_3) at 25 °C, ionic strength I = 0.10 M $NaNO_3$: (a) i. 1.0 mM $L_1.5H^+$; ii. 1.0 mM $L_1.5H^+$ + 1.0 mM Zn^{2+}; (b) i. 1.0 mM $L_2 \cdot 5H^+$; *ii.* 1.0 mM L_2 $5H^+$ + 1.0 mM Zn^{2+}; (c) i. 1.0 mM L_3 $4H^+$; ii. 1.0 mM L_3 $4H^+$ + 1.0 mM Zn^{2+}.

2.3. pH measurments and potentiometric titration

2.3.1. Calibration of pH meter

All pH measurements were carried out using a Cole Parmer (model 5996-50) pre-calibrated electrode and pH meter. The pH meter was regularly calibrated in the 2–12 pH range before measurements. The calibration was done at 25 ± 0.5 °C, using 100 ml of 4.00 mM HCl solution ($I = 0.10$ M $NaNO_3$) to obtain the lower pH limit (pH_1); then 8 mL of 0.10 M carbonate-free NaOH solution was added and the upper pH limit (pH_2) was measured. The corresponding theoretical values of pH_1 and pH_2 were calculated to be 2.48 and 11.45, respectively [29]. This method provided an experimental confidence limit of ± 0.01 in the 2–12 pH range.

2.3.2. pH-potentiometric titrations

The equilibrium constants for protonation of the free ligands and deprotonation constants of the metal-bound water were determined by potentiometric pH-titration. HYPERQUAD-2008 program was used to calculate the protonation constants of the free ligands and the stability constants of the metal complexes based the e.m.f. data [30–32]. Titrations were carried out using carbonate-free NaOH solution, at 25 °C and at a constant ionic strength ($I = 0.10$ M $NaNO_3$), in pH range 2–10. The average of three independent titrations was considered in the calculations. The value of $K_w = [H^+][OH^-]$ used under these conditions was $10^{-13.4}$. Fig. 1 shows pH-titration curves for the protonated ligands ($L \cdot 5H^+$) in the absence and in the presence of equimolar amount of Zn^{2+}.

Table 1
Log of protonation constant ($K = [LH_n]/[LH_{n-1}][H^+]$), log stability constant of Zn^{II} complex ($K_{ZnL} = [Zn^{II}L]/[Zn^{II}][L]$), and pK_a of Zn^{II}-bound water, at 25 °C and $I = 0.1$ M $NaNO_3$.

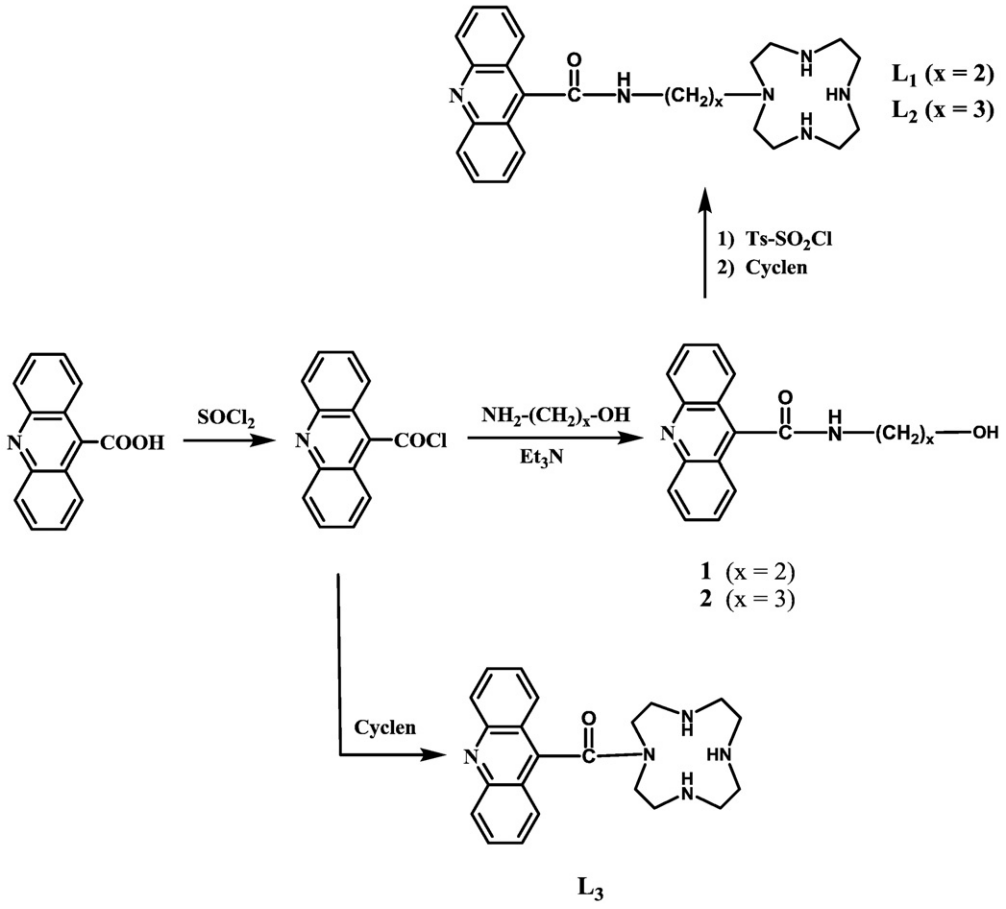
Equilibrium Reaction	log*K* (cyclen)[a]	log*K* (L_1)	log*K* (L_2)	log*K* (L_3)
$L + H^+ \rightleftharpoons LH^+$	10.7	9.76	9.68	11.32
$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	9.7	7.57	7.38	10.83
$LH_2^{2+} + H^+ \rightleftharpoons LH_3^{3+}$	<2	4.54	4.62	6.42
$LH_3^{3+} + H^+ \rightleftharpoons LH_4^{4+}$	<2	<2[b]	<2[b]	–
$LH_4^{4+} + H^+ \rightleftharpoons LH_5^{5+}$	–	<2[c]	<2[c]	–
$L + Zn^{II} \rightleftharpoons Zn^{II}L(H_2O)$	16.2	11.42	11.36	8.84
$Zn^{II}L(H_2O) \rightleftharpoons Zn^{II}L(HO^-) + H^+$	$pK_a = 7.88$	7.39	7.46	7.25

[a] Based on reference [31].
[b] Protonation constant of the tertiary amine nitrogen.
[c] Protonation constant of the acridine nitrogen.

2.4. DNA experiments

2.4.1. DNA-binding studies

Stock solutions (100 mM) of the ligands (free base) in tris–HCl buffer (20 mM, pH 7.4, $I = 0.10$ M NaCl), containing 10% by volume dimethylsulfoxide DMSO were prepared. Spectroscopic titrations were performed by adding DNA to the ligands' stock solutions; the resulting solutions were then diluted with buffer to get the desired concentrations of both the ligand and DNA. Concentration of ligands in the final solutions were 50 μM and the molar ratio between ligand and DNA (base pairs) ranged from 1:0 to 1:1. All UV–visible spectroscopic measurements were performed at 25 °C using 1 cm quartz cuvettes.



Scheme 2. Synthetic scheme for preparation of ligands L_1, L_2, and L_3.

2.4.2. DNA-cleaving studies

All solvents and buffer solutions used in DNA-cleaving experiments were sterilzed and free of oxygen; argon gas was bubbled through the solutions for 30 min before reactions. In case when metal complexes were prepared *in situ*, deoxygenated equimolar aqueous solutions of both the ligand and the metal salt were mixed under argon atmosphere for 2 h before they were added to the pre-deoxygenated buffer solution of DNA. DNA cleavage experiments were typically performed by incubating the DNA sample (100 μM, base pairs) at 37 °C in 20 μL of a buffered solution (10 mM tris–HCl, pH 7.4, $I = 0.10$ M NaCl) containing equimolar concentrations of either the metal complex ($Zn^{II}L$), or the pre-mixed ligand/metal solution. Reactions were allowed to proceed for 6 h under argon atmosphere. DNA fragments were separated on a 1% agarose gel electrophoresed in TAE buffer (20 mM TRIS base, 20 mM acetic acid, 1 mM EDTA) containing 1% SDS. DNA bands were visualized by staining with ethidium bromide solution and photographed. The relative intensities of the DNA bands were quantified by densitometric analysis using a Biorad 620 interfaced with a PC workstation. After adjusting for the background and multiplying by a factor of 1.44 to account for the reduced affinity of DNA for ethidium bromide, the results were normalized and compared to the controls.

3. Results and discussion

3.1. Synthesis

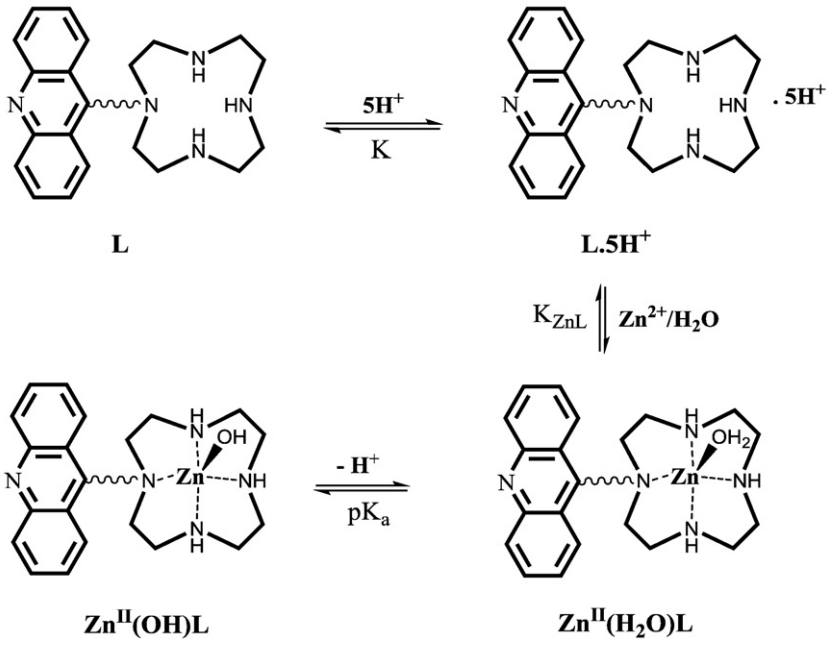
Compounds L_1 and L_2 were synthesized from 9-acridinecarbonyl chloride [28], upon treatment first with at least two equivalents of $H_2N–(CH_2)_xOH$, followed by tosylation. The tosylate derivatives were treated with excess cyclen (1,4,7,10-tetraazacyclododecane) to give the desired products (Scheme 2). Compound L_3 was prepared directly from 9-acridinecarbonyl chloride upon treatment with two equivalents of cyclen in THF. After the reactions were complete, solvent was evaporated and excess unreacted cyclen was recovered by sublimation under reduced pressure. Products were obtained as yellow oil. Purification of crude products was achieved by converting the free base ligands into their hydrobromide salts, followed by recrystallization from methanol. All analytical and spectral data of the products were consistent with the assigned structures.

Only zinc complexes were synthesized and characterized, since these complexes (and that of cobalt) showed the highest DNA hydrolytic activities, and because no signifcant difference in activity was observed between pre-synthesized metal complexes and metal complexes that were synthesized *in situ*. Thus we did not attempt to isolate and characterize complexes of other metals, such complexes were prepared *in situ* prior to reaction with DNA. The zinc complexes of L_1–L_3 were synthesized by refluxing a methanolic solution containing the free-base ligands and excess amount of zinc chloride.

3.2. Potentiometric titration

Solution properties of the ligands and their Zn^{II} complexes were studied by pH-titration of the ligands in the absence and presence of equimolar amounts of Zn^{II}, at 25 °C and constant ionic strength $I = 0.10$ M $NaNO_3$. The logarithm of protonation equilibrium constants ($\log K$) of the free ligands and the formation constants ($\log K_{ZnL}$) of zinc complexes were determined from the plot of pH versus the number of sodium hydroxide equivalents added (Fig. 1). The results, summarized in Table 1, show that the cyclen component of both ligands L_1 and L_2 has one set of three relatively more basic amine groups (the primary amine groups with $\log K = 4.54, 7.57, 9.76$ for L_1, and $\log K = 4.62, 7.38, 9.68$ for L_2), and a second set of less basic tertiary amine group (with $\log K < 2$). The protonation constant of the acridine nitrogen and that of the tertiary nitrogen of the macrocycle were too low to measure by this method. The pH-titration curves showed a buffer region (3 < NaOH equivalents < 5) corresponding to neutralization of the protonated nitrogen atoms of the macrocycle, accompanied by the formation of the metal complex. The titration curves of L_1 and L_2 show breaks when 5 equivalents of NaOH (4 equivalents in the case of L_3) were added, after which deprotonation of the metal-bound water becomes evident (Scheme 3). Interestingly, the acidity of the Zn^{II}-bound water (expressed as pK_a values) of such tethered cyclen complexes is enhanced ($pK_a = 7.39, 7.46$, and 7.25 for L_1, L_2, L_3, respectively) relative to that of un-tethered cyclen ($pK_a = 7.88$) [31]. This acidity enhancement could be attributed to weakening of the bond between Zn and the tertiary nitrogen of the macrocycle in L_1, L_2, and more so between the metal and the amide nitrogen in L_3 [33].

The equilibrium constants for the formation of Zn^{II} complexes were determined from the pH-titration data. The calculated $\log K_{ZnL}$ for L_1, L_2, and L_3 are 11.42, 11.36, 8.84 $mol^{-1}\ L^{-1}$, respectively. The



Scheme 3. Protonation of ligands and formation of Zn(II) complexes.

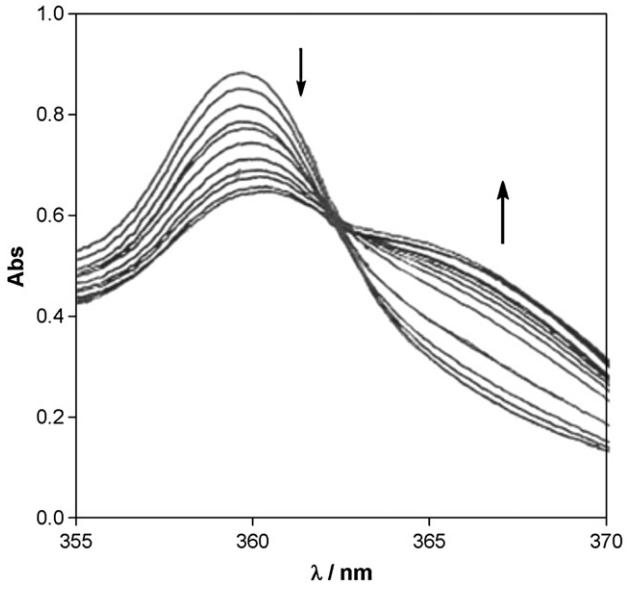


Fig. 2. Changes in the UV–visible absorption spectrum of L_1 (50.0 μM) upon titration with plasmid pBR322 DNA in tris–HCl buffer, at pH 7 and 25 °C. The bathochromic shift and hypochromocity of the 360 nm band is dependent on the concentration of DNA.

relatively small stability constant for L_3, compared to that of L_1 and L_2, may be attributed to the weaker bond between Zn and the amide nitrogen compared to the Zn-tertiary amine bond.

3.3. DNA experiments

3.3.1. DNA-binding studies

The ability of these compounds to bind to plasmid DNA was demonstrated by monitoring the changes in their absorption spectra when DNA is added. UV–visible absorption spectrum of a buffered solution of L_1 (in 10 mM tris–HCl, pH 7.4, at 25 °C and $I = 0.10$ M NaCl), in the absence of DNA, shows four absorption bands: 340 nm ($\varepsilon = 1.5\times10^4$ M^{-1} cm^{-1}), 360 nm ($\varepsilon = 1.8\times10^4$ M^{-1} cm^{-1}), 405 nm ($\varepsilon = 5.5\times10^3$ M^{-1} cm^{-1}), and 429 nm ($\varepsilon = 3.2\times10^3$ M^{-1} cm^{-1}). The 360 nm band is believed to be due to the extended π-bonds conjugation of the acridine ring [34]. Only the binding of L_1 to DNA was studied since complexes of this ligand exhibited the highest hydrolytic activity. The extent of binding was determined by monitoring the change in the 360 nm absorption band upon increasing the concentration of DNA (Fig. 2). Comparative studies of the features of the absorption spectrum of L_1, in the absence and presence of DNA showed that the extent of the red-shift of the 360 nm band depends on DNA concentration. Such a shift can be attributed to the strong DNA-ligand interaction [24,35]. In addition, DNA-ligand interaction resulted in a significant hypochromic effect on the absorption spectrum of the ligand. These results indicate clearly that the acridine group in these compounds interacts strongly with the double-stranded DNA, mostly through intercalation. Interactions of this nature between acridine derivatives and DNA have been previously observed in other related studies [23,36,37].

3.3.2. DNA hydrolysis

Hydrolytic reactions between selected metal complexes of these ligands and DNA were studied by monitoring the conversion of closed-

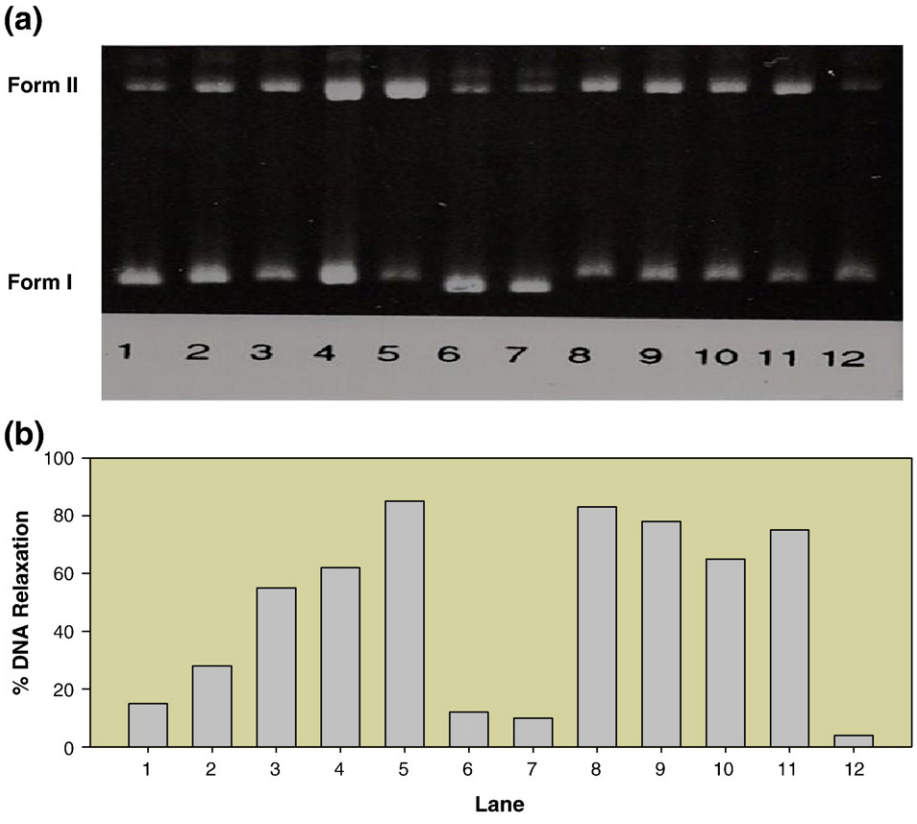


Fig. 3. (a) 1% agarose gel showing cleavage of plasmid pBR322 DNA by metal complexes of compounds L_1, L_2, and L_3, at 37 °C for 6 h. All reactions were conducted under argon atmosphere in 20 μL of degassed buffer (20 mM Tris–HCl, pH 7.4, $I = 0.10$ M NaCl) containing 100 μM DNA (base-pair). Lane 1: 100 μM of each of cyclen and acridine; lane 2: 100 μM of each of cyclen, acridine, and Co^{3+}; lane 3: 100 μM of each L_3 and Co^{3+}; lane 4: 100 μM of each L_2 and Co^{3+}; lane 5: 100 μM of each L_1 and Co^{3+}; lane 6: 100 μM of each L_1 and Fe^{3+}; lane 7: 100 μM of each L_1 and Co^{3+}, in presence of 200 μM ethidium bromide; lane 8: 100 μM of each L_1 and Zn^{2+}; lane 9: 100 μM of each L_1 and Cr^{2+}; lane 10: 100 μM of each L_1 and Cu^{2+}; lane 11: 100 μM of each L_1 and Ni^{2+}; lane 12: DNA only (control). (b) Densitometeric quantitative analysis of the relaxation of plasmid DNA by metal complexes of L_1–L_3, based on lanes analysis in part (a). % Relative relaxation = [(form II)/(form I + form II)] × 100%.

circular super-coiled cc-DNA (Form I) to the nicked (Form II) and the linear (Form III). The extent of strand scission of DNA was assessed by agarose gel electrophoresis. Typically, pBR322 plasmid DNA was incubated in a buffer solution containing either the pre-synthesized metal complex (in case of ZnL), or the ligand and equimolar amount of the metal salt. No significant differences in hydrolytic activities were observed between using pre-synthesized metal complexes versus using metal complexes that were synthesized *in situ*; therefore all the comparative DNA-cleaving studies that were performed in this study involved *in situ* syntheses of the metal complexes. DNA hydrolysis reactions were carefully carried out under oxygen-free sterilized conditions to minimize oxidative or enzymic damage to DNA. Fragments of the cleaved DNA were separated by electrophoresis (Fig. 3a). After staining the gel with ethidium bromide solution, the photograph of the gel was scanned and the bands were quantified. After adjusting for the background, the results were normalized relative to controls (Fig. 3b).

Generally, metal complexes of L_1 are more active toward hydrolysis of the phosphodiester bonds of DNA than those of L_2 and L_3, however the activity trend based on the metal ion cofactors was similar in all three ligands. Results show that the hydrolytic activities of these compounds depend on both the structure of the ligand (activity order: $L_1 > L_2 > L_3$) and the nature of metal ion cofactor (activity order: $Co^{3+} > Zn^{2+} > Cr^{2+} > Ni^{2+} > Cu^{2+} > Fe^{3}$). This was demonstarted by the conversion of cc-DNA (form I) to open circular oc-DNA (form II), a process that normally requires one nicking in cc-DNA. The high activity of L_1 could be justified on the bases of having the optimum length of linker between the metal-binding and DNA-binding groups. This structural advantage allows proper positioning of the metal complex relative to the phosphodiester bonds. A longer or shorter linking arm decreased the restriction activity. Although the highest hydrolytic activities were observed in the case of cobalt and zinc complexes, complexes of other metals such as chromium, nickel, and copper showed good activities; iron complexes on the other hand had the least restriction activity. Interestingly, control tests involving incubation of DNA in a solution containing the metal ion, acridine, or cyclen failed to elicit any activity beyond the background. This substantiates the necessity of close cooperation between all these three components.

The extent of DNA hydrolysis by these metal complexes is not significantly affected by the presence of catalase enzyme, dithiothreitol (DTT), or when excess amounts of radical scavengers such as potassium iodide or potassium formate were used. This indicates that the mode of the observed DNA cleavage is not of an oxidative nature. However, the presence of excess amounts of ethidium bromide, a known powerful intercalator, profoundly suppresses DNA cleavage, indicating that intercalation of the acridine moiety in the double-stranded DNA is required to achieve a good hydrolytic activity. Formation of linear DNA (form III), a process that normally requires at least two nearby nickings in the double-helix, was not observed except with $Co^{III}L_1$ and only under prolonged reaction time, more than 24 h (data is not shown).

The optimum pH for DNA hydrolysis is found to be around the pKa values of the metal complexes, which suggests possible involvement of metal-hydroxo intermediates in the reaction. Such intermediates are usually formed by deprotonation of at least one water molecule that is coordinated to the metal center [38–40]. To further understand the mechanism of DNA cleavage, we compared the hydrolytic activities of metal complexes at different pH values. The results show that the activity is largely dependent on the pH (Fig. 4). In the case of Zn(II)L_1 complex, the highest activity was observed at pH 7.4. Reactions at higher pH values resulted in a decrease in hydrolytic activity, possibly due to destabilization of the metal complex under alkaline conditions.

4. Conclusions

In this study, a series of compounds (L_1–L_3) were synthesized and characterized. These compounds involve attaching cyclen (metal-binding group) to an acridine ring (DNA-binding group) with linking arms of various lengths. DNA-binding experiments showed that the acridine group of these compounds can bind to double-stranded DNA by intercalating between its nitrogen base pairs. This was evident from both the strong hypochromic effect and the bathochromic shift in the absorption spectra of the ligands upon titration with increasing amounts of DNA.

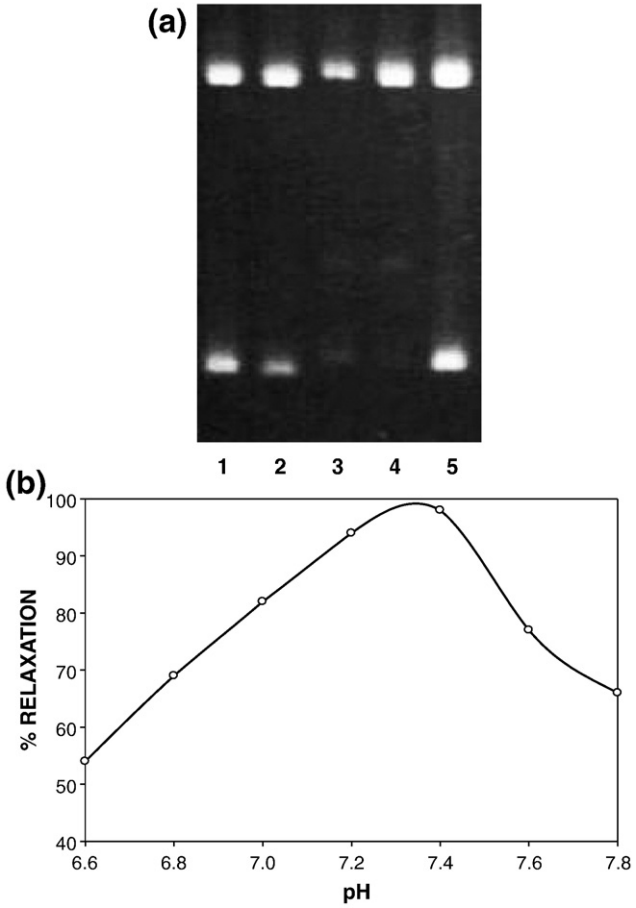


Fig. 4. (a) Agarose gel showing the effect of pH on DNA hydrolysis by $Zn^{II}L_1$ at 37 °C, $I = 0.10$ M (NaCl), reaction time 6 h. (b) The pH-profile of DNA hydrolysis in part a.

Metal complexes of these compounds are able to hydrolyze plasmid DNA effectively and under biologically relevant conditions (pH 7.4, and at 37 °C). Comparative studies based on the ligand showed that hydrolytic activity generally follows the order: $L_1 > L_2 > L_3$. Results show that activity also depends on the nature of the metal ($Co^{3+} > Zn^{2+} > Cr^{2+} > Ni^{2+} > Cu^{2+} > Fe^{3+}$). This study also clearly shows that the mode of DNA cleavage by these metal complexes is hydrolytic and not oxidative.

The higher hydrolytic efficiency of L_1, relative to that of L_2 and L_3, indicates that it has the optimum length of spacing arm between the metal-binding and the DNA-binding subgroups. This allows positioning of the metal complex in the proper location relative to the phosphodiester bonds. A longer linking arm (in L_2) as well as a shorter one (in L_3) resulted in a decrease in restriction activities.

Acknowledgment

This work was supported in part by institutional support from East Tennessee State University, and by the Schering-Plough Industrial Award (No. 3403).

References

[1] X.Q. Chen, J.Y. Wang, T.Y. Zhang, L.Z. Zhang, X.J. Peng, Chin. Chem. Lett. 19 (2008) 342–344.

[2] C. Liu, M. Wang, T. Zhang, H. Sun, Coord. Chem. Rev. 248 (2004) 147–168.
[3] A. Sreedhara, J.A. Cowan, J. Biol. Inorg. Chem. 6 (2001) 337–347.
[4] F.N. Aka, M.S. Akkaya, E.U. Akkaya, J. Mol. Catal. A: Chem. 165 (2001) 291–294.
[5] E. Kimura, E. Kikuta, J. Biol. Inorg. Chem. 6 (2000) 139–155.
[6] S. Franklin, Curr. Opin. Chem. Biol. 5 (2001) 201–208.
[7] R. Ott, R. Krämer, Appl. Microbiol. Biotechnol. 52 (1998) 761–767.
[8] J.A. Cowan, Curr. Opin. Chem. Biol. 5 (2001) 634–642.
[9] Y. Yamamoto, W. Tsuboi, M. Komiyama, Nucleic Acids Res. 31 (2003) 4497–4502.
[10] M. Komiyama, J. Sumaoka, Curr. Opin. Chem. Biol. 2 (1998) 751–757.
[11] E.L. Hegg, J.N. Burstyn, Coord. Chem. Rev. 173 (1998) 133–165.
[12] K.D. Copeland, M.P. Fitzsimons, R.P. Houser, J.K. Barton, Biochemistry 41 (2002) 343–356.
[13] P.E. Gormley, V.S. Sethi, R.L. Cysyk, Cancer Res. 38 (1978) 1300–1306.
[14] L.A. Zwelling, S. Michaels, L.C. Erickson, T.S. Ungerleider, Biochemistry 20 (1981) 6553–6563.
[15] E.M. Nelson, K.M. Tewey, L.F. Liu, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 1361–1365.
[16] O. Phanstiel, H.L. Price, L. Wang, J. Juusola, M. Kline, S.M. Shah, J. Org. Chem. 65 (2000) 5590–5599.
[17] G.J. Atwell, B.F. Cain, B.C. Baguley, G.J. Finlay, W.A. Denny, J. Med. Chem. 20 (1977) 1128–1134.
[18] D.P. Sheng, I.O. Kady, J. Appl. Catal. A: General 365 (2009) 149–152.
[19] I.O. Kady, Chem. Lett. 37 (2008) 744–745.
[20] I.O. Kady, B. Tan, Z. Ho, T. Scarborough, J. Chem. Soc., Chem. Commun. 137 (1995) 1137–1138.
[21] I.O. Kady, B. Tan, Tetrahedron Lett. 36 (1995) 4031–4034.
[22] Y.-G. Fang, J. Zhang, S.-Y. Chen, N. Jiang, H.-H. Lin, Y. Zhang, X.-Q. Yu, Bioorg. Med. Chem. 15 (2007) 696–701.
[23] K. Shinozuka, Y. Nakashima, K. Shimizu, H. Sawai, Nucleoside Nucleotides & Nucleic Acids 20 (2001) 117–130.
[24] Y. Yamamoto, W. Tsuboi, M. Komiyama, Nucleic Acids Res. 31 (2003) 4497–4502.
[25] M. Shionoya, T. Ikeda, E. Kimura, M. Shiro, J. Am. Chem. Soc. 116 (1994) 3848–3859.
[26] Q.-X. Xianga, et al., J. Mol. Cat. A: Chem. 187 (2002) 195–200.
[27] T. Gajda, Y. Dupre, I. Torok, J. Harmer, A. Schweiger, J. Sander, D. Kuppert, K. Hegetschweiler, Inorg. Chem. 40 (2001) 4918–4927.
[28] G. Kada, K. Kaiser, H. Falk, H.J. Gruber, Biochim. Biophys. Acta 1427 (1999) 44–48.
[29] T. Koike, T. Gotoh, S. Aoki, E. Kimura, M. Shoro, Inorg. Chim. Acta 270 (1998) 424–432.
[30] P. Gans, A. Sabatini, A. Vacca, J. Chem. Soc., Dalton Trans. (1985) 1195–1200.
[31] M. Kodama, E. Kimura, J. Chem. Soc., Dalton Trans. (1977) 2269–2276.
[32] A.E. Martell, R.J. Motakaitis, Determination and Use of Stability Constants, 2nd edn. VCH, New York, 1992.
[33] M. Shionoya, T. Ikeda, E. Kimura, M. Shire, J. Am. Chem. Soc. 116 (1994) 3848–3859.
[34] M. Nastasi, J.M. Morris, D.M. Rayner, V.L. Seligy, A.G. Szabo, D.F. Williams, R.E. Williams, R.W. Yip, J. Am. Chem. Soc. 98 (1976) 3979–3986.
[35] K. Fukui, K. Tanaka, Nucleic Acids Res. 24 (1996) 3962–3967.
[36] H. Tomosaka, K. Anzai, S. Omata, Biosci. Biotech. Biochem. 59 (1995) 960–961.
[37] C. Bazzicalupi, A. Bencini, C. Bonaccini, C. Giorgi, P. Gratteri, S. Moro, M. Palumbo, A. Simionato, J. Sgrignani, C. Sissi, B. Valtancoli, Inorg. Chem. 47 (2008) 5473–5484.
[38] E. Kimura, Cur. Opin. Chem. Biol. 4 (2000) 207–213.
[39] N. Williams, B. Takasaki, M. Wall, J. Chin, Acc. Chem. Res. 32 (1999) 485–493.
[40] C.S. Rossiter, R.A. Mathews, I.M.A. del Mundo, J.R. Morrow, J. Inorg. Biochem. 103 (2009) 64–71.