Hydrolytic Cleavage of DNA by Ternary Amino Acid Schiff Base Copper(II) Complexes Having Planar Heterocyclic Ligands

Pattubala A. N. Reddy,[a] Munirathinam Nethaji,[a] and Akhil R. Chakravarty*[a]

Keywords: Copper / Synthetic hydrolase / X-ray diffraction / Schiff bases / Phenanthroline bases

The ternary copper(II) complexes [Cu(salgly)L] (L = phen, **1**; dpq, **2**), [Cu(salala)L] (L = phen, **3**; dpq, **4**) and [Cu(salphe)L] (L = phen, **5**; dpq, **6**), where salgly, salala and salphe are tridentate Schiff-base ligands derived from the condensation of salicylaldehyde with glycine, L-alanine and L-phenylalanine, respectively, are prepared and their nuclease activity studied (phen, 1,10-phenanthroline; dpq, dipyridoquinoxaline). The crystal structure of **3** displays a distorted square-pyramidal (4+1) coordination geometry in which the ONO-donor Schiff base is bonded to the metal atom in the basal plane. The chelating phen ligand displays an axial–equatorial mode of bonding. The complexes exhibit a d–d band near 670 nm and a charge transfer band near 370 nm in methanol. The one-electron paramagnetic complexes display axial EPR spectra in DMF glass at 77 K, indicating a $\{d_{x^2-y^2}\}^1$ ground state. The complexes are redox-active and exhibit a quasi-reversible Cu^{II}/Cu^{I} couple in DMF at approximately –0.6 V vs. SCE. They show catalytic activity in the oxidation of ascorbic acid by molecular oxygen. The ability of the complexes to bind calf thymus (CT) DNA follows the order: **2** ≈ **4** ≈ **6** > **1** ≈ **3** ≈ **5**. Complexes **1**–**6** show oxidative DNA cleavage activity in the presence of mercaptopropionic acid as a reducing agent. All the complexes show hydrolytic cleavage activity in the absence of light or any reducing agent. The oxidative and hydrolytic DNA cleavage efficiencies follow the order: **2** ≈ **4** ≈ **6** > **1** ≈ **3** ≈ **5**. The dpq complexes, which have a greater DNA binding ability, display enhanced nuclease activity than their phen analogues. The hydrolytic DNA cleavage rate of 1.8 h^{-1}, observed for **2**, is significantly high relative to most of the known copper-based synthetic hydrolases. Mechanistic pathways involved in the nuclease activity of the complexes are discussed.

Introduction

Designing metal complexes suitable for cleaving DNA under hydrolytic conditions is of considerable importance considering the advantages of a process that produces fragments similar to those formed by the restriction enzymes, relative to other nucleolytic processes, viz. oxidative cleavage of DNA in the presence of additives or photo-induced DNA cleavage.[1–10] While photo-cleavers require the presence of a photo-sensitizer that can be activated on irradiation with UV or visible light, the redox-active "chemical nucleases" are effective cleavers of DNA in the presence of a reducing agent or H_2O_2 as an additive.[11–21] The mechanistic pathways in the oxidative process generally involve abstraction of sugar hydrogen, electron transfer, or guanine base oxidation, depending on the reaction conditions. The oxidative processes form cleaved products that are not readily amenable to further enzymatic manipulations, and thus have limited use in molecular biology. The synthetic metallohydrolases, on the other hand, follow a mechanistic pathway targeted at the phosphodiester bonds linking the nucleosides, and involves metal-promoted acid/base catalysis which leads to the formation of fragments that can be used for further enzymatic religation. Among several types of transition metal complexes used as synthetic hydrolases, copper(II) and zinc(II) complexes are better suited for the hydrolysis of DNA due to the strong Lewis acid properties of these metal ions.

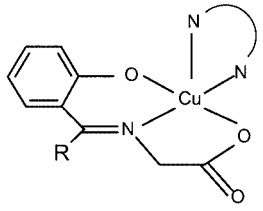
The present work stems from our interest to design ternary copper(II) complexes with amino acid based Schiff-base ligands and planar NN-donor heterocyclic ligands. While 1,10-phenanthroline (phen) and dipyridoquinoxaline (dpq) are used for binding to DNA, the Schiff bases containing different α-amino acids are designed to participate in the acid/base activity through their potentially labile carboxylate and/or phenolate oxygen atom binding site(s). We have synthesized a series of new ternary complexes, viz. [Cu(salgly)(L)] (L = phen, **1**; dpq, **2**), [Cu(salala)(L)] (L = phen, **3**; dpq, **4**) and [Cu(salphe)L] (L = phen, **5**; dpq, **6**), where salgly, salala and salphe are tridentate Schiff-base ligands derived from the condensation of salicylaldehyde with glycine, L-alanine and L-phenylalanine, respectively (Scheme 1). Complex **3** has been structurally characterized by X-ray crystallography. An important observation from

[a] Department of Inorganic and Physical Chemistry, Indian Institute of Science,
Bangalore 560012, India
Fax: (internat.) + 91-80-23600683
E-mail: arc@ipc.iisc.ernet.in

Supporting information for this article is available on the WWW under http://www.eurjic.org or from the author.

  DOI: 10.1002/ejic.200300514

this study is the efficient hydrolytic DNA cleavage activities of the dpq complexes, with complex **2** showing a hydrolysis rate that is significantly higher than those generally observed for copper(II)-based synthetic hydrolases.[4,7]



[Cu(salgly)(phen)] (R=H, **1**)
[Cu(salgly)(dpq)] (R=H, **2**)
[Cu(salala)(phen)] (R=Me, **3**)
[Cu(salala)(dpq)] (R=Me, **4**)
[Cu(salphe)(phen)] (R=CH_2Ph, **5**)
[Cu(salphe)(dpq)] (R=CH_2Ph, **6**)

Scheme 1

Results and Discussion

Synthesis and General Aspects

Complexes **1** and **2** have been prepared in high yields by the reactions of [Cu(salgly)(H_2O)]·0.5H_2O with 1,10-phenanthroline (phen) and dipyridoquinoxaline (dpq), respectively. Complexes **3**−**6** have been synthesized by the reactions of copper(II) acetate hydrate with the Schiff base H_2salala or H_2salphe in the presence of phen or dpq. The ternary complexes are stable in the solid state and moderately stable in solution. They have been characterized by elemental analysis, and from spectral and magnetic data (Table 1).

The non-electrolytic and one-electron paramagnetic ($\mu_{eff} \approx 1.8\ \mu_B$ at 25 °C) complexes show infrared spectral features that are characteristic of the Schiff base and the heterocyclic ligand. The carboxyl C=O stretch of the Schiff base is observed in the range 1620−1650 cm^{-1}. The electronic spectra of the complexes in methanol display a d-d band near 670 nm. In addition, the complexes exhibit a charge transfer (CT) transition at ca. 370 nm. They present an axial EPR spectrum in DMF-glass at 77 K, with a $g_{\|}$ value of ca. 2.22 ($A_{\|} \approx 170 \times 10^{-4}\ cm^{-1}$) and a $g_{\perp}$ value of 1.98, suggesting a $\{d_{x^2-y^2}\}^1$ ground state and a tetragonally distorted geometry for the complexes.[22] The Schiff bases H_2salala and H_2salphe are optically active. Complexes **3** and **4**, containing the dianionic salala ligand, are found to be optically active. In contrast, complexes **5** and **6** with the salphe ligand do not show any optical rotation; racemization could possibly have taken place as a result of complexation with the metal ion. A similar process has been reported earlier for phenylalanine-based Schiff-base complexes.[23,24]

Crystal Structure

Complex **3** has been characterized by single-crystal X-ray diffraction. The ORTEP view of the complex is shown in Figure 1. Selected bond lengths and angles are given in Table 2. The complex crystallizes in the monoclinic space group C_2 with two complexes and ten water molecules in the crystallographic asymmetric unit. The α-carbon atom of the amino acid Schiff base has an (*S*) configuration in both molecules. Based on the structural data, the complex has been formulated as [Cu{sal-(*S*)-ala}(phen)]·5H_2O. The crystal structure of the complex reveals the presence of a ternary structure consisting of a tridentate ONO-donor Schiff base, a bidentate N,N-donor heterocyclic base and a copper(II) center in the discrete monomeric species.[24] In the five-coordinate structure, the Schiff base is situated in the basal plane. The phen ligand displays axial-equatorial coordination. The distortion from the square-pyramidal geometry can be estimated from the τ values, 0.23 and 0.2, for two independent molecules in the asymmetric unit.[25] Significant hydrogen-bonding interactions involving the water molecules are observed. The complex displays intermolecular π-π stacking interactions of the phenolate phenyl moieties.

Redox and Catalytic Properties

The electron transfer behavior of complexes **1**−**6** was studied by cyclic and differential pulse voltammetric techniques using a glassy carbon working electrode in dimethylformamide (DMF); 0.1 M TBAP was used as the electrolyte. The electrochemical data are given in Table 1. The complexes exhibit a quasi-reversible cyclic voltammetric response near −0.6 V vs. SCE in DMF, with large ΔE_p values at a scan rate of 50 $mV \cdot s^{-1}$. The redox process is assigned to the Cu^{II}/Cu^{I} couple. The reduced species is unstable in the voltammetric time scale as can be seen from the i_{pa}/i_{pc} ratio which is less than 1 (i_{pa} and i_{pc} are the anodic and cathodic peak currents, respectively).

Table 1. Physico-chemical data for the ternary complexes **1**−**6**

Entry	Complex	IR[a] [cm^{-1}]		λ [nm] (ε [$dm^3\ M^{-1} \cdot cm^{-1}$])[b]		EPR[c]		Cyclic voltammetry[d]
		ν(C=O)	ν(C=N)	d-d band	CT band	g ($10^4 \times A$ [cm^{-1}])	$g_{\perp}$	$E_{1/2}$ [V] (ΔE_p [mV])
1	[Cu(salgly)(phen)] (**1**)	1642	1595	672 (103)	368 (3600)	2.21 (155)	1.99	−0.33 (440)
2	[Cu(salgly)(dpq)] (**2**)	1642	1605	660 (190)	369 (4300)	2.23 (175)	1.89	−0.26 (290)
3	[Cu(salala)(phen)] (**3**)	1635	1596	671 (98)	369 (4100)	2.19 (150)	1.98	−0.57 (600)
4	[Cu(salala)(dpq)] (**4**)	1605	1575	668 (229)	368 (4420)	2.22 (165)	2.00	−0.44 (255)
5	[Cu(salphe)(phen)] (**5**)	1625	1595	665 (195)	369 (3400)	2.25 (169)	2.05	−0.76 (160)
6	[Cu(salphe)(dpq)] (**6**)	1636	1599	663 (210)	369 (4150)	2.25 (175)	1.99	−0.65 (210)

[a] KBr disc. [b] In MeOH. [c] In DMF glass at 77 K. [d] CV data for the Cu^{II}/Cu^{I} couple in DMF/0.1 M TBAP. $E_{1/2} = 0.5(E_{pa} + E_{pc})$, $\Delta E_p = E_{pa} - E_{pc}$, where E_{pa} and E_{pc} are anodic and cathodic peak potentials, respectively. Scan rate, 50 mV s^{-1}.

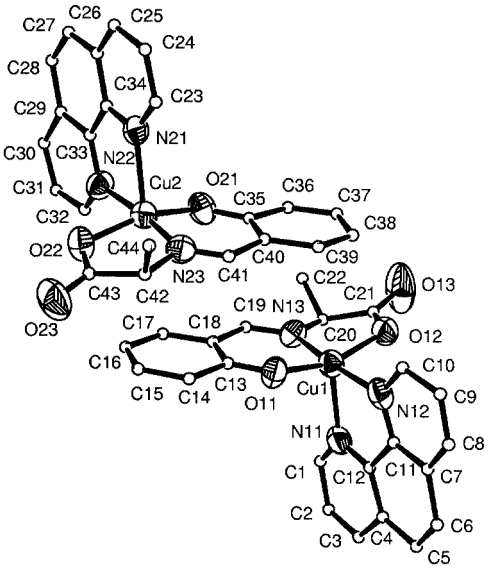


Figure 1. ORTEP diagram for two independent complexes of $[Cu(salala)(phen)]\cdot 5H_2O$ (**3**·$5H_2O$) with atom labeling scheme and thermal ellipsoids at the 50% probability level

Table 2. Selected bond lengths [Å] and bond angles [°] data for two independent molecules of $[Cu(salala)(phen)]\cdot 5H_2O$ (**3**·$5H_2O$) with e.s.d.s in the parenthesis

Molecule 1		Molecule 2	
Cu(1)−O(11)	1.918(7)	Cu(2)−O(21)	1.947(7)
Cu(1)−O(12)	1.982(7)	Cu(2)−O(22)	1.973(7)
Cu(1)−N(11)	2.280(9)	Cu(2)−N(21)	2.268(9)
Cu(1)−N(12)	1.994(9)	Cu(2)−N(22)	2.028(11)
Cu(1)−N(13)	1.899(10)	Cu(2)−N(23)	1.944(9)
O(11)−Cu(1)−O(12)	162.0(4)	O(21)−Cu(2)−O(22)	159.3(4)
O(11)−Cu(1)−N(11)	98.9(3)	O(21)−Cu(2)−N(21)	101.7(3)
O(11)−Cu(1)−N(12)	91.8(4)	O(21)−Cu(2)−N(22)	92.3(4)
O(11)−Cu(1)−N(13)	94.4(4)	O(21)−Cu(2)−N(23)	94.5(3)
O(12)−Cu(1)−N(11)	99.0(3)	O(22)−Cu(2)−N(21)	98.9(3)
O(12)−Cu(1)−N(12)	90.0(4)	O(22)−Cu(2)−N(22)	91.1(4)
O(12)−Cu(1)−N(13)	82.7(4)	O(22)−Cu(2)−N(23)	82.4(4)
N(11)−Cu(1)−N(12)	78.4(4)	N(21)−Cu(2)−N(22)	78.0(4)
N(11)−Cu(1)−N(13)	105.0(3)	N(21)−Cu(2)−N(23)	101.1(3)
N(12)−Cu(1)−N(13)	172.3(4)	N(22)−Cu(2)−N(23)	173.2(4)

Complexes **1**−**6** react readily with ascorbic acid (H_2A) in aqueous methanol to form an unstable brown copper(I) species, which readily converts to the parent copper(II) complex. The complexes retain the structure during the catalytic reaction with a H_2A/complex molar ratio of ca 30:1. Excess addition of H_2A leads to the formation of an inactive oxalato species, analyzed as [CuL(ox)] (ox = oxalate dianion). Oxalic acid is formed from the oxidation of the dehydroascorbate species (A).[26] The reduced copper(I) complex converts to the moderately stable bis(phen)- or bis(dpq)copper(I) species under an inert gas.

Oxidative Cleavage of DNA

The ability of the complexes **1**−**6** in inducing DNA cleavage has been studied by gel electrophoresis using supercoiled pUC19 DNA in 50 mM Tris-HCl/50 mM NaCl buffer (pH = 7.2). On reaction with SC DNA in the presence of mercaptopropionic acid (MPA), complexes **1**−**6** show nuclease activity (Figure 2). Control experiments using only complex **2** or MPA do not show any apparent DNA cleavage under similar experimental conditions. Complexes such as $[Cu(salgly)(H_2O)]$[27] and $[Cu(salgly)(bpy)]$[24] also do not cleave DNA in the presence of MPA. This indicates the necessity of planar heterocyclic bases such as phen or dpq for oxidative DNA cleavage. The DNA cleavage efficiencies of the complexes follow the order: **2** ≈ **4** ≈ **6** > **1** ≈ **3** ≈ **5**. The % DNA cleaved observed for the dpq complexes in the presence of MPA is similar to that observed for the bis(phen)copper(II) complex which is used as a standard (Table 3).[28] A 40 μM concentration of the dpq complex **2** showed complete conversion of the initial SC form to the NC form, while for the phen analogue, only 38% conversion of the SC form was seen. The similar values for the % DNA cleaved by the three dpq complexes suggests only a minor effect of the organic moieties, such as the methyl or benzyl group of the amino acid based Schiff bases, on the cleavage efficiency. Mechanistic studies involving the addition of the minor groove binder distamycin showed complete inhibition of the cleavage process, suggesting the minor groove binding preference of the complexes.[29] Fluorescence spectral studies using ethidium bromide as a probe shows a greater DNA binding ability of the dpq complexes than their phen analogues. The binding ability of the dpq complexes is similar to that observed for the bis(phen)copper(II) complex under similar experimental conditions. The hydroxyl radical scavenger DMSO completely inhibits the cleavage reaction. This indicates the possible involvement of the hydroxyl radical and/or the reactive copper−oxo species in the cleavage pathway. A similar pathway was pro-

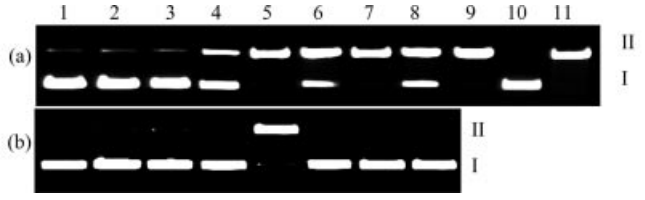


Figure 2. (a) Gel diagram showing the oxidative cleavage of supercoiled pUC19 DNA (0.5 μg) by complexes **1**−**6** and $[Cu(phen)_2(H_2O)](ClO_4)_2$ in the presence of 3-mercaptopropionic acid (MPA, 5 mM) and in the dark in 50 mM Tris-HCl/NaCl buffer containing DMF (10%): lane 1, DNA control; lane 2, DNA + MPA; lane 3, DNA + **2**; lane 4, DNA + MPA + **1**; lane 5, DNA + MPA + **2**; lane 6, DNA + MPA + **3**; lane 7, DNA + MPA + **4**; lane 8, DNA + MPA + **5**; lane 9, DNA + MPA + **6**; lane 10, DNA + MPA + $[Cu(salgly)(H_2O)]$; lane 11, DNA + MPA + $[Cu(phen)_2(H_2O)]^{2+}$; the complex concentration was 40 μM; forms I and II are supercoiled and nicked circular forms of DNA, respectively; (b) gel electrophoresis diagram for the cleavage of pUC19 DNA with complex **2** (40 μM) and MPA in the presence of different inhibiting reagents and in the dark in 50 mM Tris-HCl/NaCl buffer at 37 °C containing DMF (10%): lane 1, DNA control; lane 2, DNA + DMSO; lane 3, DNA + mannitol; lane 4, DNA + distamycin; lane 5, DNA + **2** + MPA; lane 6, DNA + DMSO + **2** + MPA; lane 7, DNA + mannitol + **2** + MPA; lane 8, DNA + distamycin + **2** + MPA; in the inhibition reactions, the reagent has been added to the DNA prior to the addition of the complex and MPA

Table 3. DNA (SC pUC19, 0.5 μg) cleavage data of **1**−**6** and bis(phen)Cu^{II} complex in the dark, and in the presence of mercaptopropionic acid (MPA, 5 mM) and in the presence of different reagents in Tris-HCl buffer

Sl no.	Reaction conditions[a]	Form I [%] (SC)	Form II [%] (NC)
1	DNA control	98	2
2	DNA + MPA	97	3
3	DNA +**2**	97	3
4	DNA + MPA + **1**	62	38
5	DNA + MPA + **2**	2	98
6	DNA + MPA + **3**	40	60
7	DNA + MPA + **4**	1	99
8	DNA + MPA + **5**	34	66
9	DNA + MPA + **6**	1	99
10	DNA + MPA + [Cu(salgly)(H_2O)]	98	2
11	DNA + MPA + [Cu(phen)$_2$(H_2O)]$^{2+}$	1	99
12	DNA + MPA + DMSO + **2**	98	2
13	DNA + MPA + mannitol + **2**	99	1
14	DNA + MPA + distamycin + **2**	98	2

[a] Complex concentration is 40 μM. Incubation period: 1 h

posed by Sigman and co-workers for the oxidative cleavage reaction of the bis(phen)copper complex.[15]

Hydrolytic Cleavage of DNA

In the absence of any reducing agent or light, the complexes are found to cleave DNA hydrolytically (Table 4). At a concentration of 1.0 mM and an incubation time of 2 h, complex **1** which contains the ligand phen cleaves 55% of SC DNA, while complete cleavage is observed for the dpq complex **2** (Figure 3, a). Complexes [Cu(salgly)(H_2O)] and [Cu(salgly)(bpy)], which do not bind DNA, do not show any hydrolytic cleavage activity under similar conditions. The cleavage efficiency follows the order: **2** ≈ **4** ≈ **6** > **1** ≈ **3** ≈ **5**. To ascertain the hydrolytic nature of the DNA cleavage process, reactions were performed in the presence of DMSO and in the absence of oxygen in dark [Figure 3 (a), lanes 9 and 10]. The cleavage efficiency does not change in the presence of DMSO. This rules out the involvement of any radical hydroxyl species in the strand cleavage. The absence of smear in the gels for an incubation time of up to 6 h also precludes any radical cleavage. Once again, the extent of DNA cleavage is found to be the same under argon, suggesting the oxygen-independent cleavage pathway. This indicates the non-oxidative nature of the DNA cleavage. The cleavage reactions have also been studied using different complex concentrations and incubation times. The % DNA cleavage for complex **2** is 52, 91 and 100% after 1.5, 6 and 10 h incubation time, respectively, at a concentration of 500 μM. The data reveal the essential role of the α-amino acid Schiff bases in promoting the Lewis acid/base activity for the enhancement of the hydrolytic cleavage, while the primary role of the heterocyclic base is to bind DNA in the minor groove.[30]

The plot showing the extent of DNA cleavage by [Cu(salgly)(dpq)] (**2**) with reaction time is displayed in Figure 4. The decrease in form I (SC) and the formation of form II (NC) of DNA with time show the expected exponential nature of the curves. The plot of log (% SC-DNA) with time gave a linear fit from which the hydrolytic pseudo-first-order rate constant ($k_{obsd.}$) is obtained (5.0×10^{-4}·s^{-1} or 1.8 h^{-1}), using a complex concentration of 1 mM (Figure 4, inset). Ito and co-workers have reported a copper(II) complex

Table 4. Hydrolytic cleavage of DNA (SC pUC19, 0.5 μg) by complexes **1**−**6** in the absence of any reducing agent or light

Sl no.	Reaction conditions	Complex concentration [mM]	Incubation time [h]	Form I [%]	Form II [%]
1	DNA control	−	10	95	5
2	DNA + **1**	1.0	2	45	55
3	DNA + **2**	1.0	2	2	98
4	DNA + **3**	1.0	2	43	57
5	DNA + **4**	1.0	2	2	98
6	DNA + **5**	1.0	2	42	58
7	DNA + **6**	1.0	2	1	99
8	DNA + [Cu(salgly)(H_2O)]	1.0	2	97	3
9	DNA + **2**	0.25	10	59	41
10	DNA + **2**	0.5	1.5	48	52
11	DNA + **2**	0.5	6	9	91
12	DNA + **2**	0.5	10	0	100
13	DNA + DMSO + **2**	1.0	2	2	98
14	DNA + **2** (under argon)	1.0	2	1	99

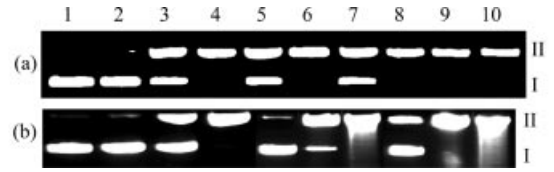


Figure 3. (a) Gel electrophoresis diagram showing the hydrolytic cleavage of supercoiled pUC19 DNA (0.5 μg) by complexes **1**−**6** (1 mM) under aerobic conditions in the dark and with an incubation time of 2 h in 50 mM Tris-HCl/NaCl buffer containing DMF (10%): lane 1, DNA control; lane 2, DNA + [Cu(salgly)(H_2O)]; lane 3, DNA + **1**; lane 4, DNA + **2**; lane 5, DNA + **3**; lane 6, DNA + **4**; lane 7, DNA + **5**; lane 8, DNA + **6**; lane 9, DNA + **2** (1 mM, under argon); lane 10, DNA + DMSO + **2** (1 mM); (b) effect of complex concentration and the incubation time on the % cleavage of supercoiled pUC19 DNA (0.5 μg) by **2** in the dark in 50 mM Tris-HCl/NaCl buffer containing DMF (10%); incubation times are 1.5 h for lanes 1−4, 6 h for lanes 5−7, and 10 h for lanes 8−10; lane 1, DNA control; lane 2, DNA + **2** (250 μM); lane 3, DNA + **2** (500 μM); lane 4, DNA + **2** (1.0 mM); lane 5, DNA + **2** (250 μM); lane 6, DNA + **2** (500 μM); lane 7, DNA + **2** (1.0 mM); lane 8, DNA + **2** (250 μM); lane 9, DNA + **2** (500 μM); lane 10, DNA + **2** (1.0 mM)

(0.095 mM) of *cis,cis*-1,3,5-triaminocyclohexane that has been shown to cleave DNA hydrolytically with a rate constant of 4.3 h^{-1}.[31] The rate of DNA hydrolysis by complex **2** is lower than this complex, but significantly higher than other copper(II) amine complexes, which have rate constants in the range 0.04−0.25 h^{-1}.[32−37] These studies were carried out under excess catalyst (pseudo-Michaelis−Menten) conditions and do not represent multiple turnover activity. The copper(II)−L-histidine complex is known to cleave DNA hydrolytically, however, at a rate of 0.76 h^{-1} with a complex concentration of 1.0 mM.[38] We have studied the saturation kinetics by varying the concentrations of both SC DNA (40−125 μM) and the complex (0.1−4.0 mM). Under Michaelis−Menten conditions, a 1.0 mM concentration of **2** is found to show saturation with a DNA concentration of 66.6 μM (Figure 5). The $k_{cat.}$ value obtained from the plot is 1.71 h^{-1} (2.85 × 10^{-2} min^{-1}).

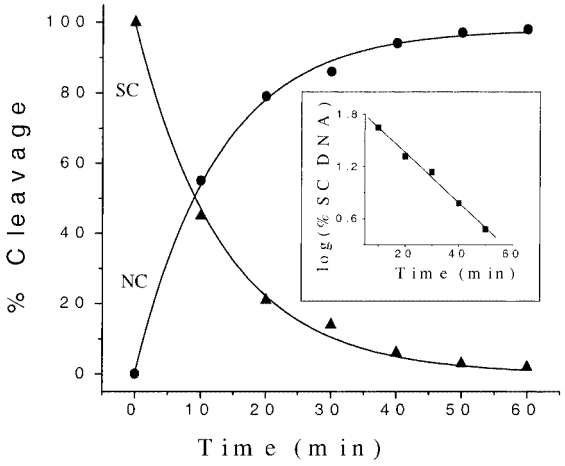


Figure 4. Hydrolytic cleavage of SC pUC19DNA (ca. 0.5 μg) showing the decrease in form I (SC DNA) and the formation of form II (NC DNA) with the incubation time (in the dark) using 1.0 mM concentration of **2**; inset shows the plot of log (% SC DNA) vs. time for complex **2** (1.0 mM) at 37 °C at pH = 7.2 for an incubation period of 10−60 min

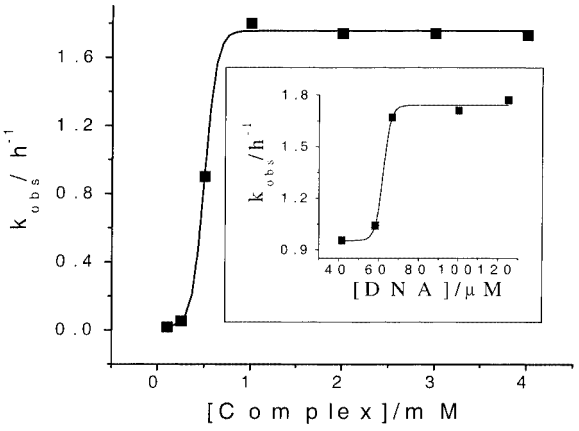


Figure 5. Saturation kinetics for the cleavage of SC pUC19 DNA (ca. 0.5 μg) by **2** at pH = 7.2 and 37 °C; inset displays the variation of the observed rate constant with SC DNA concentration (concentration of complex **2** is 1.0 mM)

Conclusion

Ternary copper(II) complexes having tridentate ONO-donor α-amino acid salicylaldimine Schiff bases and planar NN-donor heterocyclic bases are prepared and characterized. The crystal structure of **3** shows a CuN_3O_2 square-pyramidal (4+1) coordination. The complexes show efficient oxidative and hydrolytic cleavage activities. The dpq complexes, with higher DNA-binding abilities, exhibit efficient hydrolytic cleavage activities in the absence of any reducing agent and light. Mechanistic studies reveal the minor groove binding of the complexes and the involvement of the hydroxyl radical in the oxidative cleavage reactions. Hydrolytic cleavage occurs in the presence of DMSO and in the absence of oxygen. The role of heterocyclic base in complexes **1**−**6** is primarily to bind DNA at the minor groove. The amino acid Schiff bases with labile carboxylate and/or phenolato oxygen sites could be responsible for promoting the hydrolytic cleavage of DNA by utilizing the strong Lewis acid properties of copper in the +2 oxidation state. The observed DNA hydrolysis rate (1.8 h^{-1}) for **2** provides further scope for designing analogous amino acid or peptide-based copper(II) complexes or their hybrids to increase cleavage activity by enhancing the Lewis acid property of the metal ion, as well as for cellular use.

Experimental Section

Materials and Physical Measurements: All chemicals and reagents were purchased from commercial sources and were used without further purification. Glycine, L-alanine, L-phenylalanine, L-ascorbic acid and 2,2′-bipyridine were obtained from SD Fine Chemicals, Mumbai. Copper(II) acetate hydrate was bought from BDH (India). The solvents were purified by standard procedures.[39] The precursor complex [Cu(salgly)(H_2O)]·0.5H_2O, where salgly is the *N*-salicylideneglycinato dianion, was prepared by a literature method.[27] The other Schiff bases, viz. *N*-salicylidene-L-alanine

(H_2salala) and *N*-salicylidene-L-phenylalanine (H_2salphe), were prepared by the condensation of salicylaldehyde with the respective L-amino acid in the presence of LiOH according to a reported procedure.[40] The dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq) ligand, [Cu(salgly)(bpy)] and $[Cu(phen)_2(H_2O)](ClO_4)_2$ were prepared according to literature methods.[24,41,42] Calf thymus (CT) DNA and super-coiled (SC) pUC19 DNA were purchased from Bangalore Genie (India). Agarose (molecular biology grade) and ethidium bromide were from Sigma (USA). All solvents were purified before use as per standard procedures. The Tris-HCl buffer solution was prepared using deionized, sonicated triple distilled water.

Syntheses

Preparation of [Cu(salgly)(phen)] (1) and [Cu(salgly)(dpq)] (2): An aqueous methanolic solution (30 mL, 1:5 v/v) of $[Cu(salgly)(H_2O)]\cdot 0.5H_2O$ (0.39 g, 1.1 mmol) was treated with the heterocyclic base (0.22 g phen or 0.26 g dpq, 1.1 mmol) whilst stirring at 25 °C for 1 h. The product was isolated as a dark green solid in ca. 80% yield on evaporation of the solvent. The complex was crystallized by slow concentration of an aqueous methanolic solution at 5 °C. **1:** $C_{21}H_{15}CuN_3O_3$ (420.5): calcd. C 59.93, H 3.57, N 9.99; found C 59.67, H 3.88, N 9.86. IR (KBr phase): $\tilde{\nu}$ = 3417 br, 1642 s, 1595 s, 1359 m, 1199 m, 1122 m, 849 w, 719 m cm^{-1}. **2:** $C_{23}H_{15}CuN_5O_3$ (472.5): calcd. C 58.41, H 3.17, N 14.81; found C 58.18, H 3.09, N 14.75. IR (KBr disc): $\tilde{\nu}$ = 3427 br, 1642 s, 1605 s, 1528 s, 1455 m, 1342 m, 1122 m, 1086 m, 736 w, 436 w cm^{-1}.

Preparation of [Cu(salala)(phen)] (3), [Cu(salala)(dpq)](4), [Cu(salphe)(phen)] (5) and [Cu(salphe)(dpq)] (6): The complexes were prepared by a general method. An aqueous methanolic solution (20 mL, 1:4 v/v), containing copper(II) acetate hydrate (0.2 g, 1.0 mmol) and the heterocyclic base (0.22 g phen or 0.26 g dpq, 1.1 mmol), was treated with a methanolic solution (5 mL) of the Schiff base (0.19 g H_2salala or 0.27 g H_2salphe, 1.0 mmol) whilst stirring at 25 °C for 1 h. The color of the solution turned to dark green. Slow evaporation of the solvent at 5 °C gave the analytically pure product in ca. 70% yield; dark green crystalline blocks were obtained. The solid was isolated, washed with hexane and dried in vacuo over P_4O_{10}. **3:** $C_{22}H_{17}CuN_3O_3$ (434.5): calcd. C 60.76, H 3.91, N 9.67; found C 60.55, H 4.08, N 9.95. Polarimetric data: $[\alpha]_{589}$ = +26 (*c* = 10 mg/ml, MeOH). IR (KBr phase): $\tilde{\nu}$ = 3417 br, 1635 s, 1596 s, 1449 s, 1393 m, 1109 m, 1022 m, 915 m, 768 w cm^{-1}. **4:** $C_{24}H_{19}CuN_5O_3$ (488.5): calcd. C 58.96, H 3.89, N 14.33; found C 59.13, H 4.04, N 14.39. Polarimetric data: $[\alpha]_{589}$ = +29 (*c* = 10 mg/ml, MeOH). IR (KBr phase): $\tilde{\nu}$ = 3387 br, 1605 s, 1575 s, 1472 s, 1389 s, 1086 m, 822 m, 766 w, 736 w, 443 w cm^{-1}. **5:** $C_{28}H_{20}CuN_3O_3$ (509.5): calcd. C 65.95, H 3.93, N 8.24; found C 66.17, H 4.16, N 7.99. IR (KBr phase): $\tilde{\nu}$ = 3390 br, 1625 s, 1595 s, 1445 s, 1349 s, 1199 m, 1152 m, 849 m, 766 w, 499 w cm^{-1}. **6:** $C_{30}H_{20}CuN_5O_3$ (561.5): calcd. C 64.11, H 3.56, N 12.47; found C 64.30, H 3.92, N 12.27. IR (KBr phase): $\tilde{\nu}$ = 3430 br, 1636 s, 1599 s, 1450 s, 1383 s, 1129 m, 1022 m, 915 w, 768 w cm^{-1}.

DNA Binding and Cleavage Experiments: The concentration of the CT DNA, used for the binding experiments studied by spectroscopic methods, was determined by recording the absorption intensity at 260 nm with a known molar extinction coefficient of 6600 $dm^3\ mol^{-1}\ cm^{-1}$.[43] In order to compare the relative binding of complexes **1**−**6** to DNA with respect to the analogous phen complex, an ethidium bromide bound CT DNA solution in Tris-HCl / NaCl buffer (pH = 7.2) was treated with an increasing amount of the complex solution. The fluorescence intensities at 601 nm (510 nm excitation) of ethidium bromide (EB) in the bound form were plotted against the complex concentration. Competitive binding studies were done using an EB-bound DNA which show an enhanced emission intensity relative to free EB.[44,45] Binding of the complex resulted in the displacement of DNA-bound EB molecules, with a reduction of emission intensity due to fluorescence quenching of free EB by the solvent molecules.[46] The extent of complex binding to DNA was estimated from the relative decrease of the emission intensity of EB on addition of the complex. Bis(phen)copper(II) complex was used as a standard in the binding studies. The cleavage of DNA was monitored using agarose gel electrophoresis. Supercoiled pUC19 DNA (6μL, ca. 500 ng) in Tris-HCl buffer (50 mM) with 50 mM NaCl (pH = 7.2) was treated with the metal complex (40 μM in DMF) and 3-mercaptopropionic acid (2 μL, 100 μM), followed by dilution with the Tris-HCl buffer to a total volume of 20 μL. The samples were incubated at 37 °C for 1 h. A loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (3 μL) was added. Electrophoresis was performed at 60 V for 2 h in TAE buffer using 0.8% agarose gel containing 1.0 μg/mL ethidium bromide. Bands were viewed by UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled (SC) DNA to nicked circular form (NC). After electrophoresis, the proportion of DNA in each fraction was estimated quantitatively from the intensities of the bands using the UVITEC Gel Documentation System. The fraction of the original supercoiled DNA that was converted into the NC form at the end of the reaction was calculated after correcting for the low level of NC present in the original sample and the low affinity of ethidium bromide for SC relative to NC and linear forms of DNA.[47] For hydrolytic cleavage, DNA and complex were added in the dark and incubated for longer than required. Inhibition reactions were carried out by adding different reagents such as distamycin (200 μM) and DMSO (4 μL) prior to the addition of the complex.

X-ray Crystallography: Complex **3** was crystallized on slow concentration of an aqueous methanolic solution at room temperature. A crystal of approximate size 0.54 × 0.38 × 0.28 mm was mounted on a glass fiber with epoxy cement. All geometric and intensity data were collected using an automated Enraf−Nonius CAD4 diffractometer equipped with Mo-K_α radiation (λ = 0.71073 Å). The intensity data, collected using the ω-scan technique for 4550 reflections in the range $1.4° < \theta < 25°$, were corrected for Lorentz polarization effects and for absorption.[48] Structure determination was carried out using 3511 data with $I > 2\sigma(I)$ and 608 parameters using the SHELX system of programs[49] by a combination of Patterson and Fourier techniques and refined by full-matrix least squares on F^2. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed in their calculated positions and were refined using a riding model. The perspective view of the molecule was obtained using ORTEP.[50] Selected crystal data are given in Table 5. CCDC-214929 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Supporting Information: Supporting information for this article is available (see footnote on the first page of this article). Figure S1 shows the relative binding of the complexes **1** and **2** to calf thymus DNA. Figures S2 and S3 display the gel electrophoresis diagrams pertaining to the saturation kinetic studies.

Table 5. Selected crystal data for [Cu(salala)(phen)]·5H_2O (**3**·5H_2O)

Empirical formula	$C_{22}H_{27}CuN_3O_8$
Molecular mass	525.01
Temperature [K]	293
λ(Mo-K_α) [Å]	0.71073
Crystal system	monoclinic
Space group (no.)	*C*2 (5)
a [Å]	20.746(7)
b [Å]	21.592(6)
c [Å]	13.787(3)
β [°]	127.91(2)
V [Å^3]	4873(2)
Z	8
$D_{calcd.}$ [g cm^{-3}]	1.431
μ(Mo-K_α) [cm^{-1}]	9.47
F(000)	2184
Independent reflections [*R*(int)]	4403 (0.016)
Index ranges	$0 < h < 24$, $0 < k < 25$, $-16 < l < 12$
Absorption correction	ψ-scan
Goodness-of-fit on F^2	1.076
R (R_w) indices [$I > 2\sigma(I)$]	0.0694 (0.1925)
R (R_w) [all data]	0.0868 (0.2123)
Largest differential peak/hole	0.999/−0.760
Max./min. transmission	0.8275/0.7608
Weight factor: $w = [\sigma^2(F_o)^2 + (0.1598P)^2 + 1.9296P]^{-1}$; $P = (F_o^2 + 2F_c^2)/3$	

Acknowledgments

We thank the Council of Scientific and Industrial Research, New Delhi, for financial support. We also thank the Alexander von Humboldt Foundation, Germany, for donation of an electroanalytical system and the Convener, the Bioinformatics Center of IISc, for the database search. PANR thanks the CSIR for a scholarship.

[1] F. H. Westheimer, *Science* **1987**, *235*, 1173−1178.
[2] J. A. Cowan, *Chem. Rev.* **1998**, *98*, 1067−1087.
[3] D. E. Wilcox, *Chem. Rev.* **1996**, *96*, 2435−2458.
[4] A. Sreedhara, J. A. Cowan, *J. Biol. Inorg. Chem.* **2001**, *6*, 337−347.
[5] M. Komiyama, J. Sumaoka, *Curr. Opin. Chem. Biol.* **1998**, *2*, 751−757.
[6] J. K. Bashkin, *Curr. Opin. Chem. Biol.* **1999**, *3*, 752−758.
[7] E. L. Hegg, J. N. Burstyn, *Coord. Chem. Rev.* **1998**, *173*, 133−165.
[8] R. R. Breaker, *Chem. Rev.* **1997**, *97*, 371−390.
[9] R. Hettich, H.-J Schneider, *J. Am. Chem. Soc.* **1997**, *119*, 5638−5647.
[10] M. P. Fitzsimmons, J. K. Barton, *J. Am. Chem. Soc.* **1997**, *119*, 3379−3380.
[11] G. Pratviel, J. Bernadou, B. Meunier, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 746−769.
[12] B. Meunier, *Chem. Rev.* **1992**, *92*, 1411−1456.
[13] D. S. Sigman, *Biochemistry* **1990**, *29*, 9097−9105.
[14] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109−1152.
[15] D. S. Sigman, *Acc. Chem. Res.* **1986**, *19*, 180−186.
[16] D. S. Sigman, A. Mazumder, D. M. Perrin, *Chem. Rev.* **1993**, *93*, 2295−2316.
[17] W. K. Pogozelski, T. D. Tullius, *Chem. Rev.* **1998**, *98*, 1089−1107.
[18] B. Armitage, *Chem. Rev.* **1998**, *98*, 1171−1200.
[19] J. M. Veal, K. Merchant, R. L. Rill, *Nucleic Acids Res.* **1991**, *19*, 3383−3388.
[20] O. Zelenko, J. Gallagher, D. S. Sigman, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2776−2778.
[21] G. Pratviel, J. Bernadou, B. Meunier, *Adv. Inorg. Chem.* **1998**, *45*, 251−312.
[22] C. L. Foster, X. Liu, C. A. Kilner, M. T. Pett, M. A. Halcrow, *J. Chem. Soc., Dalton. Trans.* **2000**, 4563−4568.
[23] R. D. Gillard, R. Wootton, *J. Chem. Soc. B* **1970**, 364−371.
[24] P. A. N. Reddy, M. Nethaji, A. R. Chakravarty, *Inorg. Chim. Acta* **2002**, *337*, 450−458.
[25] A. W. Addison, T. N. Rao, J. Reedijk, J. V. Rijn, G. C. Verschoor, *J. Chem. Soc., Dalton Trans.* **1984**, 1349−1356.
[26] S. Lewin, *Vitamin C: Its Molecular Biology and Medical Potential*, Academic Press, London, **1976**.
[27] M. Kishita, A. Nakahara, M. Kubo, *Aust. J. Chem.* **1964**, *17*, 810−813.
[28] T. B. Thederahn, M. D. Kuwabara, T. A. Larsen, D. S. Sigman, *J. Am. Chem. Soc.* **1989**, *111*, 4941−4946.
[29] K. E. Erkkila, D. T. Odom, J. K. Barton, *Chem. Rev.* **1999**, *99*, 2777−2795.
[30] T. Gajda, Y. Düpre, I. Török, J. Harmer, A. Schweiger, J. Sander, D. Kuppert, K. Hegetschweiler, *Inorg. Chem.* **2001**, *40*, 4918−4927.
[31] U. Itoh, H. Hisada, T. Sumiya, M. Hosono, Y. Usui, Y. Fujii, *Chem. Commun.* **1997**, 677−678.
[32] J. Rammo, R. Hettich, A. Roigk, H.-J. Schneider, *Chem. Commun.* **1996**, 105−107.
[33] E. L. Hegg, J. N. Burstyn, *Inorg. Chem.* **1996**, *35*, 7474−7481.
[34] N. E. Dixon, R. J. Geue, J. N. Lambert, S. Moghaddas, D. A. Pearce, A. M. Sargesan, *Chem. Commun.* **1996**, 1287−1288.
[35] J. N. Burstyn, K. A. Deal, *Inorg. Chem.* **1993**, *32*, 3585−3586.
[36] K. A. Deal, A. C. Hengge, J. N. Burstyn, *J. Am. Chem. Soc.* **1996**, *118*, 1713−1718.
[37] K. A. Deal, J. N. Burstyn, *Inorg. Chem.* **1996**, *35*, 2792−2798.
[38] R. Ren, P. Yang, W. Zheng, Z. Hua, *Inorg. Chem.* **2000**, *39*, 5454−5463.
[39] D. D. Perrin, W. L. F. Armarego, D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, **1980**.
[40] A. S. Ceccato, A. Neves, M. A. de Brito, S. M. Drechsel, A. S. Mangrich, R. Werner, W. Haase, A. J. Bortoluzzi, *J. Chem. Soc., Dalton Trans.* **2000**, 1573−1577.
[41] J. G. Collins, A. D. Sleeman, J. R. Aldrich-Wright, I. Greguric, T. W. Hambley, *Inorg. Chem.* **1998**, *37*, 3133−3141.
[42] J. Foley, D. Kennefick, D. Phelan, S. Tyagi, B. Hathaway, *J. Chem. Soc., Dalton Trans.* **1983**, 2333−2338.
[43] M. E. Reichmann, S. A. Rice, C. A. Thomas, P. Doty, *J. Am. Chem. Soc.* **1954**, *76*, 3047−3053.
[44] M. J. Waring, *J. Mol. Biol.* **1965**, *13*, 269−282.
[45] J.-B. Le Pecq, C. Paoletti, *J. Mol. Biol.* **1967**, *27*, 87−106.
[46] L. C. Zheng, W. Jigui, W. Liufang, R. Min, J. Naiyong, G. Jie, *J. Inorg. Biochem.* **1999**, *73*, 195−202.
[47] J. Bernadou, G. Pratviel, F. Bennis, M. Girardet, B. Meunier, *Biochemistry* **1989**, *28*, 7268−7275.
[48] A. C. T. North, D. C. Phillips, F. S. Mathews, *Acta Crystallogr., Sect. A* **1968**, *24*, 351−359.
[49] G. M. Sheldrick, *SHELX-97, Programs for Crystal Structure Solution and Refinement*, University of Göttingen, Germany, **1997**.
[50] C. K. Johnson, *ORTEP, Report ORNL-5138*, Oak Ridge National Laboratory, Oak Ridge, TN, **1976**.

Received July 25, 2003
Early View Article
Published Online February 17, 2004

