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Efficient Visible Light Induced Nuclease Activity of a Ternary Mono-1,10-phenanthroline Copper(II) Complex Containing 2-(Methylthio)ethylsalicylaldimine

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Ternary Schiff base copper(II) complex [CuL(phen)](ClO_4), where HL is 2-(methylthio)ethylsalicylaldimine and phen is 1,10-phenanthroline, has been prepared and structurally characterized by X-ray crystallography. The complex shows a CuN_3OS coordination in a square-pyramidal (4 + 1) geometry with the sulfur as an equatorial ligand. The complex is an avid binder to double-stranded DNA in the minor groove and exhibits both photonuclease and chemical nuclease activity. When exposed to UV light of 312 nm (96 W) or visible light of 532 nm (125 W) under aerobic conditions, the complex causes significant cleavage of supercoiled pUC19 DNA in the absence of any externally added reducing agent or H_2O_2.

Metal-based pseudonucleases are of importance in nucleic acids chemistry for their diverse applications like footprinting, sequence-specific binding to nucleic acids, new structural probes, and therapeutic agents.[1−3] Such nucleases for which the mechanistic pathways involve damage to the sugar moiety can be broadly classified into two major types, viz., photonucleases and chemical nucleases. While photonucleases act on irradiation of the complex bound to DNA under aerobic or anaerobic conditions, chemical nucleases are in general metal complexes cleaving DNA in an oxidative manner in the presence of a reducing agent through the formation of a reactive DNA-bound metal−oxo species in the presence of dioxygen. Redox active $Cu(phen)_2^+$ in the presence of H_2O_2 is a chemical nuclease that efficiently nicks DNA.[1] The reaction involving phen, Cu^{2+}, and a reducing agent under aerobic conditions forms the cuprous species as well as H_2O_2.[4] Herein, we report a ternary copper(II) complex which besides being active as a chemical nuclease also exhibits unusual photonuclease activity in the absence of any external reducing agent. The results are of importance as the chemistry of copper-based photonucleases is virtually unexplored.[5,6] Copper being a bio-essential transition-metal ion, such copper complexes showing photonuclease activity could have better application at the cellular level in comparison to their chemical nuclease analogues requiring a reducing agent for their activity[1] or the photonucleolytic agents[2] having 4d or 5d transition metal ions.

The ternary copper(II) complex [Cu{O-2−C_6H_4CH═N(CH_2)$_2$SMe}(phen)](ClO_4) (**1**) was prepared from the reaction of copper(II) acetate hydrate with phen and 2-(methylthio)ethylsalicylaldimine (HL) in methanol followed by the addition of sodium perchlorate.[7] The crystal structure of **1** shows the presence of a Schiff base with a tridentate ONS coordination and a phen displaying a bidentate NN mode of bonding to give a square-pyramidal (4 + 1) geometry (Figure 1).[8,9] There are two independent molecules in the crystal-

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(1) (a) Sigman, D. S. *Acc. Chem. Res*. **1986**, *19*, 180. (b) Sigman, D. S.; Bruice, T. W.; Majumder, A.; Sutton, C. L *Acc. Chem. Res.* **1993**, *26*, 98. (c) Sigman, D. S.; Majumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.

(2) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem*. **1990**, *38*, 413. (c) Delaney, S.; Pascaly, M.; Bhattacharya, P. K.; Han, K.; Barton, J. K. *Inorg. Chem.* **2002**, *41*, 1966.

(3) (a) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev*. **1998**, *98*, 1089. (b) Burrows, C. J.; Muller, J. G. *Chem. Rev*. **1998**, *98*, 1109. (c) Armitage, B. *Chem. Rev*. **1998**, *98*, 1171. (d) Meunier, B. *Chem. Rev*. **1992**, *92*, 1411. (e) Pratviel, G.; Bernadou J.; Meunier, B. *Adv. Inorg. Chem*. **1998**, *45*, 251. (f) McMillin, D. R.; McNett, K. M. *Chem. Rev*. **1998**, *98*, 1201.

(4) Sigman, D. S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. *J. Biol. Chem*. **1979**, *254*, 12269.

(5) Baudoin, O.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. *Chem. Commun*. **1998**, 2349.

(6) (a) Borah, S.; Melvin, M. S.; Lindquist N.; Manderville, R. A. *J. Am. Chem. Soc*. **1998**, *120*, 4557. (b) Eppley, H. J.; Lato, S. M.; Ellington, A. D.; Zaleski, J. M. *Chem. Commun*. **1999**, 2405.

(7) The complex [CuL(phen)](ClO_4) (**1**) was prepared in 65% yield from a reaction of $Cu_2(O_2CMe)_4(H_2O)_2$ (0.5 mmol) with phen (1 mmol) and HL (1 mmol) in 10 mL of MeOH with stirring at 25 °C for 0.5 h. The complex was isolated as a perchlorate salt on addition of $NaClO_4$ to the reaction mixture. The solid was separated, washed with cold methanol, and dried in vacuo over P_4O_{10}. Anal. Calcd for $C_{22}H_{20}N_3O_5SClCu$ (**1**): C, 49.16; H, 3.72; N, 7.82. Found: C, 49.09; H, 3.96; N, 7.59. IR (KBr phase): 1091 cm^{-1} (ClO_4^-); d−d band at 661 nm (ϵ = 120 M^{-1} cm^{-1}), CT bands at 394 nm (ϵ = 1290) and 374 nm (ϵ = 1530) in the electronic spectrum in MeCN; axial X-band EPR with $g_{\|} = 2.21$ ($A_{\|} = 156 \times 10^{-4}$ cm^{-1}) and $g_{\perp} = 1.99$ in DMF glass at 77 K; $\mu_{eff} = 1.97$ μ_B. Complex **1** is soluble in MeCN, CH_2Cl_2, DMF, and MeOH; less soluble in water. **CAUTION!** Perchlorate salts of metal complexes containing organic ligands are potentially explosive. Single crystals of **1**, suitable for X-ray analysis, were obtained on slow evaporation of an aqueous methanolic solution of **1**.

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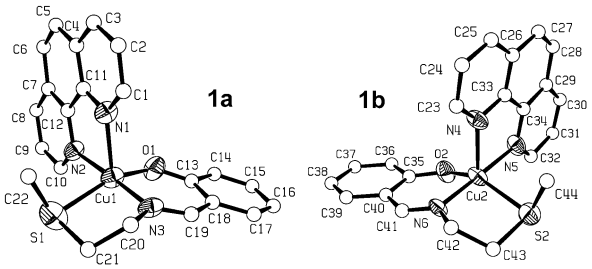

Figure 1. Perspective views of two independent molecules of **1** showing 50% probability thermal ellipsoids and atom-numbering schemes for the metal and heteroatoms [Cu(1)−S(1), 2.404(2); Cu(1)−O(1), 1.913(4); Cu(1)−N(1), 2.217(5); Cu(1)−N(2), 2.024(5); Cu(1)−N(3), 1.945(5); Cu(2)−S(2), 2.429(2); Cu(2)−O(2), 1.913(4); Cu(2)−N(4), 2.215(5); Cu(2)−N(5), 2.014(5); Cu(2)−N(6), 1.949(5) Å].

lographic asymmetric unit giving geometric parameter (τ) values of 0.34 and 0.47.[10] Molecules **1a** and **1b** display CuN_3OS coordination in which the phen ligand displays an axial−equatorial bonding mode. The cyclic voltammetry of **1** in DMF−Tris-buffer (1:1 v/v, pH 7.0) containing 0.1 M KCl as supporting electrolyte displays a quasi-reversible voltammogram at −0.12 V (ΔE_p = 204 mV) at 50 mV s^{-1}.

The binding of **1** to calf thymus DNA has been studied by the fluorescence spectral method monitoring the emission intensity of ethidium bromide (EB).[11−13] It has been observed that **1** is an avid binder to ds-DNA like $[Cu(phen)_2]^{2+}$ species.

Table 1. Selected SC pUC19 DNA Cleavage Data[a] of **1** and $Cu(phen)_2^{2+}$

no.	reaction conditions	λ (nm)	t (min)[b]	form I %	form II %
1	DNA control	312	5	91	9
2	MPA control	dark		90	10
3	DNA + **1**	FL[c]	15	52	48
4	DNA + **1**	312	1	63	37
5	DNA + **1**	312	3	39	61
6	DNA + **1**	312	5	28	72
7	DNA + $Cu(phen)_2^{2+}$	FL[c]	15	87	13
8	DNA + **1** + MPA	dark		46	54
9	DNA + $Cu(phen)_2^{2+}$ + MPA	dark		38	62
10	DNA + **1** + azide[d]	FL[c]	15	87	13
11	DNA + **1** + DMSO[e]	FL[c]	15	59	41
12	DNA + **1**[f]	312	5	69	31
13	DNA + **1**[g]	312	5	51	49
14	DNA + **1**	532	5	65	35
15	DNA + **1**	532	10	30	70

[a] Complex concentration: 80 μM (no. 3−11, 14, 15); DNA (0.5 μg); 3-mercaptopropionic acid (MPA; 5 mM). Temperature 25 °C; monochromated radiations: 312 nm (96 W) and 532 nm (125 W). Form I and form II are supercoiled (SC) and nicked circular (NC) forms, respectively. [b] t, irradiation time. [c] Under fluorescent lamp (FL) of 4 × 40 W. [d] NaN_3, 90 μM. [e] DMSO, 4 μL. [f] Complex concentration, 20 μM. [g] Complex concentration, 60 μM.

The nuclease activity of **1** has been studied using supercoiled (SC, form I) pUC19 DNA in a medium of Tris-HCl/NaCl buffer in the presence or absence of a reducing agent under illuminated or dark conditions (Table 1). The bis(phen)-copper(II) complex has been used as a standard. The extent of cleavage of SC DNA has been detected by gel electrophoresis on the basis of the conversion of the SC to nicked circular (NC, form II) DNA. Complex **1** and $Cu(phen)_2^{2+}$ are cleavage inactive when the reactions are carried out in the dark in the absence of any reducing agent (Figure 2). The same reaction, however, in the presence of visible light shows photonuclease activity of complex **1**, while $Cu(phen)_2^{2+}$ remains cleavage inactive (Figure 2a). Again, when the reactions are carried out in the presence of the reducing agent mercaptopropionic acid (MPA) in the absence of light, both complex **1** and $[Cu(phen)_2]^{2+}$ display cleavage activity (Figure 2a). The results indicate that while $[Cu(phen)_2]^{2+}$ requires a reducing agent for its DNA cleavage activity, complex **1**, besides being active as a chemical nuclease, also shows photonuclease activity in the absence of any externally added reducing agent.

To probe the reaction further, cleavage reactions are conducted with different exposure times (Figure 2b, Table 1). Using a radiation of 312 nm (96 W), the extent of cleavage has been found to increase on increasing the exposure time. The complex causes ∼80% cleavage for an exposure time of 5 min at 25 °C. We have also observed significant cleavage using visible monochromatic radiations of wavelengths 436 and 532 nm. The cleavage efficiency of complex **1** is significantly higher than those reported for the copper(II) complexes of *o*-quinacridines requiring several hours for the DNA cleavage in the absence of any added reducing agent or hydrogen peroxide.[5] Like the *o*-quinacridines, bis(9-diazo-4,5-diazafluorene)copper(II) nitrate is also known to cleave DNA in the absence of any externally added reducing agent but with an exposure time of 40 min using a radiation of ≥455 nm.[6b] These cleavage reactions

(8) Crystal data: $C_{22.5}H_{22}ClCuN_3O_{5.5}S$, M = 553.5, triclinic, space group $P\bar{1}$ (No. 2), a = 7.8487(19) Å, b = 11.573(3) Å, c = 26.276 (7) Å, α = 86.317(4)°, β = 82.347(4)°, γ = 79.416(4)°, V = 2323.2(10) Å^3, Z = 4, ρ = 1.582 g cm^{-3}, T = 293(2) K, 0.8° ≤ θ ≤ 26.1°, μ = 11.88 cm^{-1}, $F(000)$ = 1136, R1 = 0.0645, wR2 = 0.1218 for 3808 reflections with $I > 2\sigma(I)$ and 613 parameters [R1(F^2) = 0.1723 (all data)]. Weighting scheme: $w = 1/[\sigma^2(F_o^2) + (0.0539P)^2 + 0.0P]$ where $P = [F_o^2 + 2F_c^2]/3$. Intensity data for a crystal of dimensions 0.35 × 0.30 × 0.20 mm were obtained from a Bruker SMART APEX CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube Mo Kα X-ray source, with increasing ω (width of 0.3°/frame) at a scan speed of 18 s/frame. The SMART software was used for data acquisition and the SAINT software for data reduction. Absorption corrections were made on the intensity data. The structure was solved and refined with the SHELX program. There was one lattice methanol molecule in the crystallographic asymmetric unit containing two independent molecules of **1**.

(9) Sheldrick, G. M. *SHELX-97, Programs for Crystal Structure Solution and Refinement*; University of Göttingen: Göttingen, Germany, 1997.

(10) Addison, A. W.; Rao, T. N.; Reedijk, J.; Rijn, J. V.; Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349.

(11) DNA binding and cleavage experiments were done using procedures described previously. The concentration of calf thymus (CT) DNA was determined from the absorption intensity at 260 nm with a known ϵ value of 6600 M^{-1} cm^{-1}. Relative binding of **1** to CT DNA with respect to bis(phen)copper(II) complex was studied by the flourescence spectral method using ethidium bromide bound CT DNA solution in Tris-HCl/NaCl buffer (pH 7.2). The extent of SC pUC19 DNA (0.5 μg) cleavage in the reactions carried out under dark and illuminated (visible and UV light sources of 125 and 96 W, respectively, using monochromatic radiation of wavelengths 532 and 312 nm) conditions was measured from the intensities of the bands using UVITEC Gel Documentation System. The inhibition reactions were carried out by adding the reagent (distamycin, 75 μM; DMSO, 4 μL; NaN_3, 90 μM) prior to the addition of the complex. The visible light used was commercial flourescent lamps (4 × 40 W) in the laboratory for inhibition reactions, and the reaction duration was 15 min prior to incubation in the dark chamber at 37 °C followed by electrophoresis. The UV light used has a monochromatic wavelength of λ = 312 nm. Eppendorf and glass vials were used for the UV and visible light irradiations, respectively.

(12) Dhar, S.; Reddy, P. A. N.; Nethaji, M.; Mahadevan, S.; Saha, M. K.; Chakravarty, A. R. *Inorg. Chem.* **2002**, *41*, 3469.

(13) Bernadou, J.; Pratviel, G.; Bennis, F.; Girardet, M.; Meunier, B. *Biochemistry* **1989**, *28*, 7268.

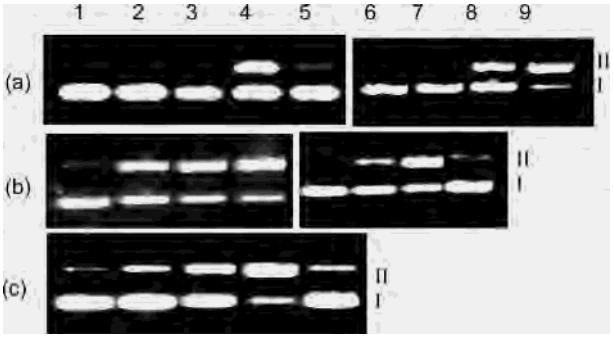

Figure 2. (a) Cleavage of SC pUC19 DNA (0.5 μg) by **1** and the bis-(phen)copper(II) species in dark and visible light conditions in the absence of MPA [lane 1, DNA control; lane 2, DNA + **1** (dark); lane 3, DNA + $Cu(phen)_2^{2+}$ (dark); lane 4, DNA + **1** (4 × 40 W fluorescent light, exposure time 15 min); lane 5, DNA + $Cu(phen)_2^{2+}$ (4 × 40 W fluorescent light, exposure time 15 min)] and in the presence of MPA [lane 6, DNA control; lane 7, MPA control; lane 8, DNA + **1** + MPA (dark); lane 9, DNA + $Cu(phen)_2^{2+}$ + MPA (dark)]. (b) Cleavage of SC pUC19 DNA using UV (312 nm, 96 W) and visible light (532 nm, 125 W) using different exposure times: lane 1, DNA control (312 nm, 5 min); lane 2, DNA + **1** (312 nm, 1 min); lane 3, DNA + **1** (312 nm, 3 min); lane 4, DNA + **1** (312 nm, 5 min); lane 5, DNA control (532 nm, 10 min); lane 6, DNA + **1** (532 nm, 5 min); lane 7, DNA + **1** (532 nm, 10 min); lane 8, DNA + Schiff base ligand, HL (532 nm, 10 min) at 25 °C. (c) Cleavage dependence at different concentrations of the metal complex using UV light (96 W) of 312 nm wavelength with 5 min exposure time at 25 °C under aerobic conditions: lane1, DNA control; lane 2, DNA + **1** (20 μM); lane 3, DNA + **1** (60 μM); lane 4, DNA + **1** (80 μM). Lane 5 shows the cleavage of SC DNA by **1** (80 μM) under argon atmosphere when exposed to light (λ = 312 nm, 96 W) for 5 min.

require high complex concentrations up to 200 μM.[5,6] We have observed that complex **1** is an effective photonucleolytic agent at a lower complex concentration (Table 1). At 80 μM concentration, **1** cleaves SC DNA to the extent of 80% with an exposure time of only 5 min with λ = 312 nm radiation (Figure 2c).[14]

The mechanistic aspects of the DNA binding and cleavage reactions are studied using distamycin as a minor groove binder and using inhibiting reagents like azide and DMSO. A complete inhibition of cleavage activity is observed when SC pUC19 DNA is incubated with distamycin prior to the addition of **1** or $[Cu(phen)_2]^{2+}$. This indicates that complex **1** is a minor groove binder like the bis-phen complex (Figure 3a). Hydroxyl radical scavenger DMSO has been found to inhibit the cleavage partially for **1** and completely for $[Cu(phen)_2]^{2+}$ in the presence of MPA under illuminated reaction condition (Figure 3b).[15] Addition of sodium azide inhibits the photonuclease activity of **1** (Figure 3b). Preliminary results are thus indicative of the involvement of singlet oxygen for the photonuclease activity of **1**. When the photonuclease reaction is carried out under an argon atmosphere, no significant photocleavage is observed (Figure 2c). The nuclease activity of **1** has also been found to be efficient on using a visible light of 532 nm (125 W). Complex **1** shows a band in its electronic spectrum at 394 nm which is assignable to the sulfur to copper(II) charge transfer transition.[16] The observed results indicate the important role of the sulfur ligand bound to copper(II) for the photonuclease activity of **1**.[17,18]

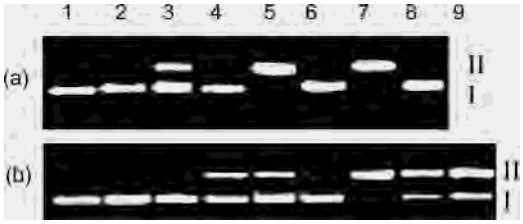

Figure 3. (a) Cleavage of SC pUC19 DNA by **1** and $Cu(phen)_2^{2+}$ using inhibition reagent distamycin under visible light (4 × 40 W fluorescent lamps): lane 1, DNA control; lane 2, distamycin control; lane 3, DNA + **1**; lane 4, DNA + distamycin + **1**; lane 5, DNA + **1** + MPA; lane 6, DNA + distamycin + **1** + MPA; lane 7, DNA + $Cu(phen)_2^{2+}$ + MPA; lane 8, DNA + distamycin + $Cu(phen)_2^{2+}$ + MPA. (b) Cleavage of SC pUC19 DNA by **1** using inhibition reagents DMSO and NaN_3 under visible light (4 × 40 W fluorescent lamps): lane 1, DNA control; lane 2, DNA + DMSO; lane 3, DNA + NaN_3; lane 4, DNA + **1**; lane 5, DNA + DMSO + **1**; lane 6, DNA + NaN_3 + **1**; lane 7, DNA + **1** + MPA; lane 8, DNA + DMSO + **1** + MPA; lane 9, DNA + NaN_3 + **1** + MPA.

In summary, the ternary copper(II) complex [CuL(phen)]-(ClO_4) with a CuN_3OS coordination and having a minor groove binder 1,10-phenanthroline shows efficient photonuclease and chemical nuclease activity. The observed efficient photonuclease activity of **1** under visible light on short exposure time and with a lesser concentration of the metal complex is a significant result in the chemistry of copper-based nucleolytic agents. Further studies are on to explore the mechanistic aspects of the reactions toward understanding the role of sulfur of the Schiff base in the photocleavage reaction.

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Supporting Information Available: Crystallographic details in CIF format. Full ORTEP diagram of the complex with atom labeling scheme. This material is available free of charge via Internet at http://pubs.acs.org.

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(14) Under similar rection conditions with a 5 min exposure time, the extent of DNA cleavage observed with monochromatic radiation of 436 and 532 nm is ~30 and ~35%, respectively. More exposure time (~10 min) is required for observing significant cleavage (~65%) using visible radiation.

(15) The true identity of the oxidizing species is presently unknown. It may be free hydroxyl radical or a copper-based oxo or hydroxo species (refs 3a,b).

(16) Kita, T.; Miura, I.; Nakayama, N.; Kawata, T.; Kano, K.; Hirota, S.; Kodera, M. *J. Am. Chem. Soc.* **2001**, *123*, 7715.

(17) Chaulk, S. G.; Pezacki, J. P.; MacMillan, A. M. *Biochemistry* **2000**, *39*, 10448.

(18) The Schiff base HL and an analogous ternary copper(II) complex [CuL′(phen)]ClO_4, where HL′ is a ONO-donor Schiff base, do not show any photonuclease activity under similar reaction conditions.