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Visible light-induced nuclease activity of a ternary mono-phenanthroline copper(II) complex containing L-methionine as a photosensitizer

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Ternary copper(II) complex, structurally characterized as [Cu(phen)(met)(MeOH)](ClO$_4$) (1) by X-ray crystallography, has 1,10–phenanthroline (phen) as an intercalator/binder to supercoiled pUC19 DNA and L-methionine (met) as a photosensitizer, and the complex displays efficient photonuclease activity on irradiation with UV (312 nm) or visible (436, 532 nm) light through a mechanistic pathway involving singlet oxygen.

Transition metal complexes that induce DNA strand scission on photolysis are of importance for their potential applications in photodynamic therapy.[1–4] The photo-activated state of the complexes with an efficient intersystem crossing to the triplet state generally leads to the formation of singlet oxygen which can cause DNA cleavage. Such synthetic photonucleases are of greater utility than their chemical nuclease analogues which require a reducing agent like ascorbic acid or a thiol for their activity.[5] The photodynamic therapeutic process necessitates the presence of a photosensitizer, a visible light source of ~600 nm wavelength, and oxygen. Among several varieties of transition metal complexes used to explore the DNA photocleavage activities, tris-polypyridyl chelates of 3d–5d metals have been extensively studied.[1] Complexes containing 4d or 5d metal ions show novel sequence-specific cleavage of DNA on photolysis. In contrast, the chemistry of copper-based photonucleases is virtually unexplored.[6] Copper being a bio-essential metal ion, its complexes could find better application at the cellular level.

Earlier studies on copper-based nucleases are primarily directed to the synthesis of complexes suitable for oxidative cleavage of DNA in the presence of a reducing agent or for hydrolysis of DNA.[3,5] The bis-phen copper complex which is an excellent chemical nuclease,[7] shows no apparent photonuclease activity. Similarly, copper-porphyrin complexes are not efficient for therapeutic applications as the cytotoxicity resulting from the singlet oxygen formed from the free base is higher than with the complex.[8] In this communication we present a copper(II) complex which is designed to have a DNA binder/intercalator and a photosensitising ligand. Using a bio-essential amino acid L-methionine (met) as a photosensitizer and 1,10-phenanthroline as DNA binder/intercalator, we were able to synthesize a ternary copper(II) complex which shows efficient photonuclease activity on irradiation with UV (312 nm) or visible (436, 532 nm) light. The complex, structurally characterized as [Cu(phen)(met)(MeOH)](ClO$_4$) (**1**), exemplifies a new class of photo-nucleolytic agent having bio-essential constituents.

Complex **1** was prepared in high yield from a reaction of the sodium salt of L-methionine with $CuSO_4 \cdot 5H_2O$ and phen in aqueous methanol and the product was isolated as a perchlorate salt.† The crystal structure of **1**‡ shows a didentate N,O-binding mode of the amino acid with an *S*-configuration and the presence of a N,N-donor 1,10-phenanthroline in a square-pyramidal (4 + 1) geometry having an axial methanol ligand (Fig. 1).[9] There are two independent molecules in the crystallographic asymmetric unit belonging to the space group $P2_1$ with the perchlorate anions showing no apparent bonding with the metal ions. The complex displays a d–d band at 609 nm in DMF and an axial EPR spectrum in DMF glass at 77 K giving $g_{\|} = 2.17$ ($A_{\|} = 140 \times 10^{-4}$ cm^{-1}) and $g_{\perp} = 1.99$. Complex **1** exhibits a quasi-reversible cyclic voltammetric response at −0.2 V (ΔE_p = 160 mV at 50 mV s^{-1} scan rate) for the Cu(II)/Cu(I) couple in DMF-Tris-HCl buffer (1 : 4 v/v; pH, 7.2) containing 0.1 M KCl as supporting electrolyte.

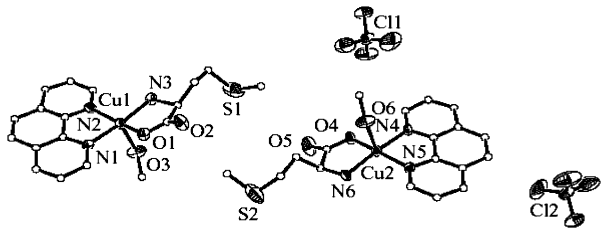

Fig. 1 Perspective view of two independent molecules of **1** showing 30% probability thermal ellipsoids and atom numbering schemes for the metal and hetero-atoms. Selected bond distances (Å) and angles (°) are: Cu(1)–O(1) 1.938(6), Cu(1)–O(3) 2.274(6), Cu(1)–N(1) 1.978(7), Cu(1)–N(2) 2.034(7), Cu(1)–N(3) 1.975(7), Cu(2)–O(4) 1.902(6), Cu(2)–O(6) 2.313(6), Cu(2)–N(4) 2.015(7), Cu(2)–N(5) 1.991(7), Cu(2)–N(6) 1.988(7); N(1)–Cu(1)–N(2) 82.4(3), O(1)–Cu(1)–N(3) 84.8(3), N(4)–Cu(2)–N(5) 82.1(3), O(4)–Cu(2)–N(6) 85.1(3).

The nuclease activity of **1** has been studied using supercoiled (SC) pUC19 DNA in a medium of Tris-HCl/NaCl buffer (pH 7.2) in the presence or absence of a reducing agent (2-mercaptopropionic acid, MPA) under illuminated or dark conditions (Table 1).[10]§ Bis(phen)copper(II) complex has been used as a standard for comparative studies. The extent of DNA cleavage has been measured by gel electrophoresis on the basis of the conversion of the SC to nicked circular (NC) forms of DNA. Complex **1** and Cu(phen)$_2^{2+}$ are cleavage inactive in the absence of light and MPA. A 40 μM solution of **1** and Cu(phen)$_2^{2+}$ show 58 and 62% cleavage of SC DNA, respectively, in presence of MPA (50 mM) under dark reaction conditions. In the absence of MPA, complex **1** shows nuclease

Table 1 Selected DNA (SC pUC19, 0.5 μg) cleavage data[a] of **1**

Sl. No.	Reaction conditions[b]	λ/nm (*t*/min)	**1**/μM	Form-I (%)	Form-II (%)
1	DNA Control	312 (20)		93	7
2	DNA + **1**	Dark	200	86	14
3	DNA + L- Methionine[c]	312 (20)		91	9
4	DNA + Bis-phen Cu^{2+}	312 (20)		86	14
5	DNA + **1**	312 (20)	200	11	89
6	DNA + **1**	436 (10)	200	60	40
7	DNA + L-Methionine[c] + **1**	436 (10)	200	75	25
8	DNA + **1**	532 (30)	300	57	43
9	DNA + Distamycin[d] + **1**	312 (5)	200	89	11
10	DNA + **1**	312 (5)	200	44	56
11	DNA + D_2O[e] + **1**	312 (5)	200	36	64
12	DNA + DMSO[f] + **1**	312 (5)	200	52	48
13	DNA + NaN_3[g] + **1**	312 (5)	200	88	12

[a] λ, excitation wavelength; *t*, exposure time. Forms -I and -II are SC and NC forms of DNA, respectively. [b] In Tris-buffer (pH 7.2). Bis-phen Cu^{2+}, 200 μM. [c] 200 μM. [d] 200 μM. [e] 14 μL. [f] 4 μL. [g] 200 μM.

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activity on irradiation with monochromatic radiation of 312 nm wavelength, while $[Cu(phen)_2]^{2+}$ remains nuclease inactive (Fig. 2a). On photolysis at 312 nm for 20 min using a 200 μM concentration of **1**, a conversion of 89% to the NC form is observed. Complex **1** is also efficient in cleaving DNA on visible light irradiation at 436 and 532 nm. It shows a 43% cleavage for an exposure time of 30 min at 532 nm using a 300 μM concentration of the complex (Fig. 2b).

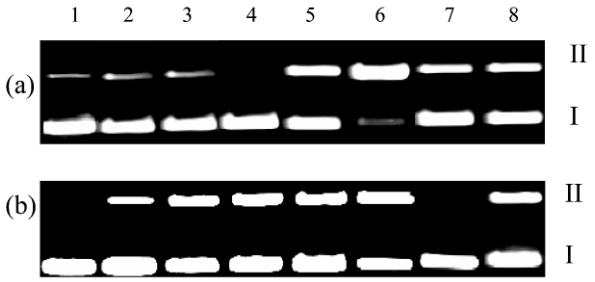

Fig. 2 (a) Cleavage of SC pUC19 DNA (0.5 μg) by **1** and the bis(phen)copper(II) species in the dark or under UV light (312 nm, 96 W) exposure followed by incubation under dark conditions and electrophoresis: Lane 1, DNA control (312 nm, 20 min); lane 2, DNA + **1** (dark, 200 μM, 20 min); lane 3, DNA + $Cu(phen)_2^{2+}$ (312 nm, 200 μM, 20 min); lane 4, DNA + L-methionine (312 nm, 200 μM, 20 min); lane 5, DNA + **1** (200 μM, 312 nm, 5 min); lane 6, DNA + **1** (200 μM, 312 nm, 20 min); lane 7, DNA + **1** (50 μM, 312 nm, 20 min); lane 8, DNA + **1** (100 μM, 312 nm, 20 min). (b) Cleavage of SC pUC19 DNA (0.5 μg) by visible light (i) at 532 nm (125 W): Lane 1, DNA control (30 min); lane 2, DNA + **1** (200 μM, 5 min); lane 3, DNA + **1** (200 μM, 10 min); lane 4, DNA + **1**(200 μM, 20 min); lane 5, DNA + **1** (100 μM, 30 min); lane 6, DNA + **1** (300 μM, 30 min) and (ii) at 436 nm (125 W): lane 7, DNA control (30 min); lane 8, DNA + **1** (200 μM, 5 min).

The mechanistic aspects of the photocleavage reaction have been studied using different reagents (Fig. 3). A complete inhibition of the cleavage activity is observed on incubating SC-DNA with distamycin prior to the addition of **1**. This suggests minor groove binding of the complex. This is in accordance with earlier observations with copper complexes having phen ligands showing minor groove binding.[5,10] Again, inhibition of cleavage is observed when the photolysis is done under an argon atmosphere suggesting the necessity of oxygen for the photocleavage reaction. Inhibition of cleavage in the presence of sodium azide and enhancement of cleavage in the presence of D_2O indicate the involvement of singlet oxygen as a reactive species in the scission reactions. The hydroxyl radical scavenger DMSO has no major effect on the photocleavage activity. Preliminary results suggest the role of phen ligand as a binder/intercalator to DNA and met as a photosensitizer.

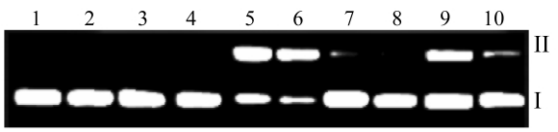

Fig. 3 Cleavage of SC pUC19 DNA (0.5 μg) by **1** (200 μM) using different reagents under UV light (312 nm, 5 min exposure): lane 1, DNA control; lane 2, DNA + NaN_3; lane 3, DNA + DMSO; lane 4, DNA + distamycin; lane 5, DNA + **1**; lane 6, DNA + **1** in D_2O; lane 7, DNA + **1** under argon; lane 8, DNA + NaN_3 + **1**; lane 9, DNA + DMSO + **1**; lane 10, DNA + distamycin + **1**.

In summary, the ternary copper(II) complex [Cu(phen)(met)(MeOH)](ClO_4) (**1**) with a CuN_3O_2 coordination binds to DNA in the minor groove and shows efficient cleavage activity on photolysis with UV or visible light by a pathway involving the formation of singlet oxygen. Complex **1**, having the bio-essential constituents copper and L-methionine, is likely to find use in photodynamic therapy.

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Notes and references

† [Cu(phen)(met)(MeOH)](ClO_4) (**1**) was prepared in 70% yield from a reaction of $CuSO_4 \cdot 5H_2O$ (1.25 g, 5.0 mmol) with L-methionine (0.75 g, 5.0 mmol) in water (25 mL) on initial treatment with an aqueous solution (10 mL) of NaOH (0.2 g, 5.0 mmol) and then followed by addition of 1,10-phenanthroline (0.94 g, 5.0 mmol) in methanol (10 mL). The solution was stirred for 2 h at 60 °C. On cooling the solution to ambient temperature, an aqueous solution (10 mL) of $NaClO_4$ (0.7 g, 5.0 mmol) was added. The solution was filtered and the filtrate on slow evaporation gave blue coloured crystals of **1** in ~5 days. The solid was isolated and washed with cold water before drying over P_4O_{10}. The complex was found to be soluble in methanol, DMF, DMSO; moderately soluble in ethanol, and sparingly soluble in water. Found: C, 41.0; H, 4.0; N, 8.1. Calc. for $C_{18}H_{22}ClCuN_3O_7S$ (**1**): C, 41.3; H, 4.2; N, 8.0%. IR (KBr phase): 3436br, 3327w, 3284w, 3058w, 1636s, 1519m, 1428m, 1387m, 1342w, 1315w, 1088vs (ClO_4^-), 853m, 722m, 614m, 564m cm^{-1} (br, broad; vs, very strong; s, strong; m, medium; w, weak). Electronic spectrum in DMF [λ_{max}/nm (ε/$dm^3\ mol^{-1}\ cm^{-1}$)]: 275 (32700), 294sh (16800), 609 (150) (sh, shoulder). **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 the complex.

‡ Crystal data for **1**: $C_{18}H_{22}ClCuN_3O_7S$, M = 523.44, monoclinic, space group $P2_1$ (no. 4), a = 9.246(4), b = 20.531(9), c = 11.864(5) Å, β = 96.985(8)°, U = 2235.4 (17) $Å^3$, Z = 4, D_c = 1.555 g cm^{-3}, T = 293 (2) K, $1.73 \leq \theta \leq 26.07°$, μ = 12.34 cm^{-1}, $F(000)$ = 1076, $R1$ = 0.0534, $wR2$ = 0.1329 for 5323 reflections with $I > 2\sigma(I)$ and 567 parameters [$R1(F^2)$ = 0.0872 (all data)]. Weighting scheme: $w = 1/[\sigma^2(F_o^2) + (0.0894P)^2 + 0.0P]$, where $P = [F_o^2 + 2F_c^2]/3$. CCDC reference number 207304. See http://www.rsc.org/suppdata/cc/b3/b303442a/ for crystallographic data in CIF or other electronic format.

§ DNA cleavage experiments were done using procedures described previously.[10] The extent of SC pUC19 DNA cleavage was monitored by agarose gel electrophoresis. The DNA in 50 mM tris(hydroxymethyl-)methane-HCl (Tris-HCl) buffer (pH 7.2) containing 50 mM NaCl was treated with the metal complex (2 μL in DMF) followed by dilution with the buffer to a total volume of 16 μL. Cleavage in the dark or under illumination (λ = 312 nm, 96 W; 436 and 532 nm, 125 W source attached to a monochromator from Applied Photophysics Limited) at 25 °C was measured from the intensities of the bands using the UVITEC Gel Documentation System. The inhibition reactions were done by adding the reagent prior to complex addition. The solutions were incubated in the dark for 1 h at 37 °C followed by gel electrophoresis for 2 h at 60 V in Tris-acetate-EDTA (TAE) buffer. Bands were visualized by UV light and photographed for analysis. Eppendorf and glass vials were used for UV and visible light experiments respectively.

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