Metal-assisted red light-induced efficient DNA cleavage by dipyridoquinoxaline-copper(ii) complex†

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Complete cleavage of double stranded pUC19 DNA by the complex [Cu(dpq)$_2$(H$_2$O)](ClO$_4$)$_2$ (dpq, dipyridoquinoxaline) has been observed on irradiation at 694 nm from a pulsed ruby laser, assisted by the metal d-band transition as well as the quinoxaline triplet states in the absence of any external additives.

Designing molecules that cleave DNA at ~ 700 nm is of great importance in photodynamic therapy (PDT) because of significant skin penetration of light in the near IR region.[1–3] The currently used PDT drug Photophrin II® is a porphyrin-based compound that generates cytotoxic singlet oxygen ($^1O_2$, $^1\Delta_g$) for cell necrosis upon irradiation at 630 nm.[3] The present work stems from our interest in probing the photo-nuclease activity of transition metal complexes that can satisfy the basic requirements of PDT. Toshima and coworkers have recently shown that DNA intercalator quinoxalines, similar to those present in the antitumor antibiotics like echinomycin or triostin, are capable of cleaving double stranded DNA in the ultraviolet at 365 nm without any external additives.[4] They expected the conjugated C=N bond in quinoxaline to generate the photo-excited ^{3}(n-π*) and/or ^{3}(π-π*) state(s) which could cause DNA cleavage by H-abstraction and/or electron transfer pathway(s).

In this communication, we report that the nuclease activity of the quinoxaline ligand enhances drastically when bound to a metal like copper(ii) ion and the complex exhibits complete cleavage of supercoiled (SC) DNA on exposure to red light of ~ 700 nm. We have used a copper(ii) complex [Cu(dpq)$_2$(H$_2$O)](ClO$_4$)$_2$ (**1**)[5] containing dipyrido-[3,2-*d*:2′,3′-*f*]-quinoxaline (dpq) as a model quinoxaline having metal binding sites with an aim to probe the role of metal centered transitions on its photo-induced DNA cleavage activity. Like other reported[4] quinoxalines, the dpq ligand shows significant photo-nuclease activity on exposure to UV light. However, it is inactive on red light irradiation. Interestingly, complex **1** shows enhanced DNA cleavage activity on photo-irradiation at UV as well as visible red light in the absence of any additives. The results are of significance as the number of non-porphyrinic copper-based photosensitizers showing visible light-induced DNA cleavage is rare.[6,7] Also for PDT applications, known copper-porphyrin complexes are less efficient than their free bases since the latter exhibit higher cytotoxicity derived from the production of singlet oxygen than the former.[8]

The nuclease activity of **1** has been studied using SC pUC19 DNA in a medium of Tris-HCl/NaCl buffer (pH 7.2) under dark conditions on photolysis at 312 nm or at 694 nm.‡ For mechanistic studies, cleavage reactions in the presence of several reagents are carried out using a CW He–Ne laser of 632.8 nm. Selected photo-cleavage data are given in Table 1 and relevant gel electrophoresis diagrams are shown in Fig. 1 and 2. A 50 μM solution of the dpq ligand and a 25 μM solution of **1** show significant cleavage of SC DNA (0.5 μg) on exposure to UV light at 312 nm for 10 min. Observation of complete inhibition of cleavage by sodium azide indicates the involvement of singlet oxygen ($^1O_2$) as the reactive species. As

suggested by Toshima *et al.*, the triplet states resulting from the n-π* and π-π* photo-excitation in the UV radiation could activate oxygen to form $^1O_2$ that cleaves DNA.[4] The enhancement of cleavage activity of the quinoxaline ligand on binding to copper is a significant result.

A 50 μM solution of **1** on irradiation at 694 nm from a ruby laser (40 mJ P^{-1}) for 60 min shows complete cleavage of SC DNA. It is also possible to achieve complete DNA cleavage by **1** at lower complex concentration (25 μM) but with longer exposure time (2 h). Interestingly, the bis-phen analogue[9] of **1** is photo-cleavage inactive even on UV light irradiation at 312 nm. This establishes that the quinoxaline moiety in dpq is essential

Table 1 Selected SC DNA Cleavage Data^a of **1** and the dpq ligand

Sl. No	Reaction condition	λ (nm)	Conc. (μM)	t (min)	Form-I (%)	Form-II (%)
1	DNA + **1**	312	25	10	39	61
3	DNA + dpq	312	50	10	57	43
4	DNA + NaN$_3$ (150 μM) + **1**^{b}	312	25	10	93	7
5	DNA + DMSO (2 μL) + **1**	312	25	10	41	59
5	DNA + **1**^{c}	694	25	120	15	85
6	DNA + **1**	694	50	120	2	98
7	DNA + **1**	632.8	80	30	50	50
8	DNA + NaN$_3$ (150 μM) + **1**	632.8	80	30	49	51

^{a} Form-I, SC-DNA; Form-II, NC-DNA; *t*, exposure time. ^{b} With dpq (50 μM) alone, 9% cleavage. ^{c} With dpq (200 μM) alone, 3% cleavage.
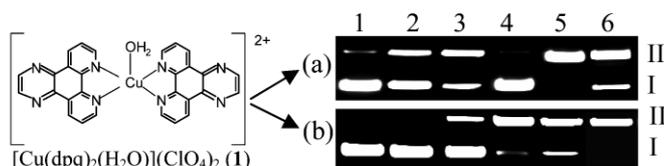

Fig. 1 SC DNA (0.5 μg) cleavage activity of **1** and dpq using (a) UV light (312 nm), 10 min exposure: lane 1, DNA control; lane 2, DNA + dpq (50 μM); lane 3, DNA + **1** (25 μM); lane 4, DNA + NaN$_3$ (150 μM) + **1** (25 μM); lane 5, DNA + D$_2$O (14 μL) + **1** (25 μM); lane 6, DNA + DMSO (2 μL) + **1** (25 μM) and (b) pulsed ruby laser (694 nm, 40 mJ P^{-1} peak power): lane 1, DNA control (2 h); lane 2, DNA + dpq (200 μM, 2 h); lane 3, DNA + **1** (10 μM, 2 h); lane 4, DNA + **1** (25 μM, 2 h); lane 5, DNA + **1** (50 μM, 30 min); lane 6, DNA + **1** (100 μM, 30 min).
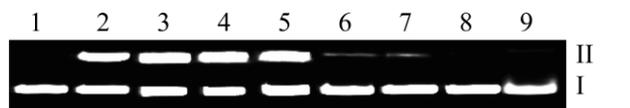

Fig. 2 Cleavage of SC DNA (0.5 μg) by **1** (80 μM) in presence of different reagents using CW laser of 632.8 nm (3 mW) for 30 min exposure time in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing DMF (10%). Lanes 1–7, 9 in air; lane 8 under argon. Lane 1, DNA control; lane 2, DNA + **1**; lane 3, DNA + NaN$_3$ (150 μM) + **1**; lane 4, DNA + L-Histidine (100 μM) + **1**; lane 5, DNA + D$_2$O (14 μL) + **1**; lane 6, DNA + DMSO (2 μL) + **1**; lane 7, DNA + Mannitol (100 μL) + **1**; lane 8, DNA + **1** (under argon); lane 9, DNA + KI (0.8 mM) + **1**.

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† Electronic supplementary information (ESI) available: Figs. S1–S5. SC DNA cleavage. See http://www.rsc.org/suppdata/cc/b3/b308344f/

for photo-nuclease activity. The ruby laser wavelength is close to the λ_{max} value (673 nm) of the d–d band of the copper(II) ion in **1** (Fig. 3). Observation of excellent nuclease activity at 694 nm implies the involvement of the d–d band in the cleavage.

The mechanistic aspects of the photo-nuclease activity of **1** have been further investigated at 632.8 nm using a CW He–Ne laser. The experiments carried out under aerobic conditions show significant photo-cleavage with a concentration of 80–100 μM. This complex is, however, cleavage inactive under an argon atmosphere indicating the necessity of oxygen in the reaction process. Use of singlet oxygen inhibitors like sodium azide or L-histidine as additives has no apparent effect on the cleavage activity. Similarly, use of D_2O as a solvent, in which singlet oxygen has longer lifetime, has no significant effect on the cleavage efficiency. All these results provide sufficient evidence that while singlet oxygen is involved in the photo-cleavage under UV light, the red light-induced DNA cleavage follows a different mechanism. This observation assumes significance as the ternary copper(II) complexes having sulfur containing ligands or the copper-porphyrin complexes are known to cleave DNA involving singlet oxygen on visible light irradiation.[6,8]

To understand the mechanism of DNA cleavage by **1** under red light, we have studied the effect of hydroxyl radical scavengers on the cleavage efficiency. Bis(phen)copper complexes are known to involve hydroxyl radicals in their "chemical nuclease" activity.[9] Both mannitol and DMSO show complete inhibition of the photo-cleavage of **1**. The preliminary results suggest the involvement of hydroxyl radical in the photo-cleavage reaction of **1** under red light irradiation. Generation of hydroxyl radical on photo-sensitization is not uncommon. Collet *et al*. have shown the formation of hydroxyl radical on photo-excitation of sulfur and selenium analogs of psoralen at 365 nm.[10] The pathway proposed by them involves the formation of superoxide anion ($O_2{}^{\cdot-}$) that subsequently converts to OH$\cdot$. The other possibility is the formation of hydroxyl radical from hydrogen peroxide in a mechanistic pathway similar to the one proposed for the "chemical nuclease" activity of bis(phen)copper complex involving the Cu(II)/Cu(I) redox couple.[9] A pertinent question is why complex **1** would follow such a mechanistic pathway involving one-electron or two-electron reduction of the oxygen molecule rather than forming the singlet oxygen which is generally observed in the PDT cycle. One possibility is that the relatively high redox potential of the Cu(II)/Cu(I) couple in **1** (0.09 V *vs.* SCE in DMF-Tris-HCl/0.1 M KCl)[5] enable the metal to undergo reduction from +2 (d^9) to +1 (d^{10}) state by an electron transfer in the excited state. The resulting Cu(I) centre then activates oxygen to form the hydroxyl radical. Observation of complete inhibition of DNA cleavage by **1** in presence of KI as

an additive provides evidence for the formation of hydrogen peroxide, a source of OH$\cdot$ radical, under photolytic conditions.[11]

In summary, this is the first report on a metal-promoted efficient DNA cleavage activity using a quinoxaline-copper(II) complex at ~700 nm. This wavelength is better suited for PDT applications considering greater transparency of human tissue at this wavelength compared to 630 nm used for Photofrin II®. This work opens up further scope for design and development of new copper(II) complexes containing hybrid quinoxaline ligands for cellular use. The present data are also of importance for understanding the function of currently used quinoxaline-based antitumor antibiotics in the presence of bio-essential copper ions in a cellular medium. The dual mechanistic pathways involving hydroxyl radical under red light exposure and singlet oxygen for UV irradiation by complex **1** are new propositions in the chemistry of light-induced DNA cleavage.

Notes and references

‡ For DNA cleavage experiments, SC pUC19 DNA in 50 mM tris(hydroxymethyl)methane-HCl (Tris-HCl) buffer of 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 18 μL. Photo-exposures were done in a dark room using 312 nm (96 W) UV-transilluminator, pulsed ruby laser of 694 nm (Lumonics, 1/6 Hz, 20 ns, 40 mJ P^{-1} in single shot mode) and CW He–Ne laser of 632.8 nm (Scientifica-Cook make, UK, 3 mW). The inhibition reactions were carried out by addition of 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. The extent of cleavage was measured from the intensities of the bands using UVITEC Gel Documentation System. Electronic spectral data in DMF-Tris buffer (1:4 v/v), [λ, nm (ε, dm^3 mol^{-1} cm^{-1})]: 262 (14,800), 282 (9,800), 324 (3,300), 341 (2,900) for dpq and 262 (15,600), 291 (9,500), 337 (3,600), 673 (70) for **1** that was prepared by a known method.[5]

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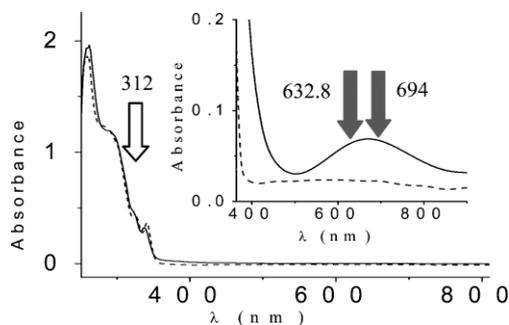11 O. Baudoin, M.-P. Teulade-Fichou, J.-P. Vigneron and J.-M. Lehn, *Chem. Commun.*, 1998, 2349.

Fig. 3 Electronic spectra of dpq (---) and **1** (—) in DMF-Tris-buffer (1 : 4 v/v) (compound concentration, 0.5 mM). The wavelengths used for the photo-cleavage reactions are shown.