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Photosensitizer in a Molecular Bowl: Steric Protection Enhancing the Photonuclease Activity of Copper(II) Scorpionates

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Steric encumbrance caused by the tripodal ligand in the ternary tris(3-phenylpyrazolyl)borate copper(II) heterocyclic base complexes $[Cu(B)(Tp^{Ph})](ClO_4)$ (B = dipyridoquinoxaline, dipyridophenazine) leads to efficient cleavage of supercoiled DNA to its relaxed form upon exposure to red light at 632.8 and 694 nm as a result of protection of the photosensitizer in the molecular bowl of the $\{Cu(Tp^{Ph})\}$ moiety, which generates singlet oxygen as the reactive species in a type-II process.

Transition-metal complexes that are capable of cleaving DNA upon red-light irradiation in the absence of any external additives are of potential utility in the photodynamic therapy (PDT) of cancer, for which the currently used FDA-approved hematoporphyrin drug Photofrin is active at 630 nm.[1,2] Among non-porphyrinic species, tris-chelates of 3d−5d metals having planar heterocyclic bases and dirhodium complexes of dipyridophenazine (dppz) that show photoinduced DNA cleavage activity have been used as probes with varied applications in nucleic acids chemistry.[3−8] It has been observed that tris-chelate complexes of ruthenium and rhodium having the dppz ligand protected from solvent water molecules by DNA display a significant increase in the "light-switch" effect.[3,5,8]

Chart 1. Schematic Diagram Showing Steric Protection of the Photosensitizer in (A) $[Cu(dppz)(Tp^{Ph})]^+$ and (B) $[Cu(dpq)(Tp^{Ph})]^+$ Bound to DNA Grooves

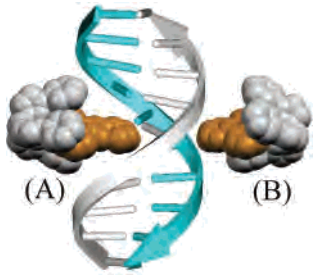
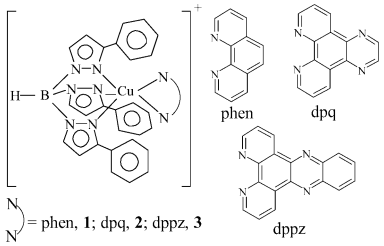


Chart 2. Copper(II) Complexes **1**−**3** and the Heterocyclic Bases



The present work stems from our interest in probing the effect of such protection of the photosensitizer from solvent molecules upon red-light-induced DNA cleavage activity of copper(II) complexes for its importance in PDT chemistry (Chart 1). We have chosen ternary copper(II) complexes of the type $[Cu(B)(Tp^{Ph})](ClO_4)$ (**1**−**3**) in which the planar heterocyclic base B, namely, 1,10-phenanthroline (phen, **1**), dipyrido[3,2-d:2′,3′-f]quinoxaline (dpq, **2**), or dipyrido[3,2-a:2′,3′-c]phenazine (dppz, **3**), is in a "molecular bowl" made of a tris(3-phenylpyrazolyl)borate copper(II) moiety $\{Cu(Tp^{Ph})\}$ that effectively encloses the photosensitizer B within its bowl (Chart 2).[9] In this communication, we report our observation of a significant enhancement of the photo-

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Table 1. Selected DNA (SC pUC19, 0.5 μg) Cleavage Data[a] for **1**−**3**

sample no.	reaction conditions	λ/nm (t/min)	conc/μM	SC (%)	NC (%)
1	DNA control	365 (40)	−	98	2
2	DNA + **1**	365 (10)	50	70	30
3	DNA + **2**	365 (10)	50	16	84
4	DNA + **3**	365 (10)	50	1	99
5	DNA + **2**	365 (40)	10	30	70
6	DNA + **3**	365 (40)	10	1	99
7	DNA control	632.8 (60)	−	99	1
8	DNA + **2**	632.8 (180)	25	2	98
9	DNA + **3**	632.8 (60)	25	1	99
10	DNA control	694 (60)	−	99	1
11	DNA + **2**	694 (60)	10	48	52
12	DNA + **3**	694 (60)	10	21	79

[a] λ is the excitation wavelength; t is the exposure time; and SC and NC are the supercoiled and nicked circular forms of DNA, respectively.

induced DNA cleavage activity of these complexes in DNA-bound form resulting from protection of the photosensitizer from the solvent molecules. The results are of interest as our previous study shows poor "chemical nuclease" activity of **1**−**3** due to steric encumbrance of the Tp^{Ph} ligand reducing the diffusion of ascorbic acid to the metal center.[9]

The nuclease activity of **1**−**3** was studied using supercoiled (SC) pUC19 DNA in a medium of Tris−HCl/NaCl buffer (pH 7.2) under dark conditions upon photolysis at 365, 632.8, and 694 nm.[10,11] Selected cleavage data are given in Table 1, and agarose gel electrophoresis diagrams are shown in Figures 1 and 2. Complexes **1**−**3** and [Cu(O_2CMe)(Tp^{Ph})] were prepared by reported procedures.[9] The acetate complex was used for control experiments. The photocleavage activity at 365 nm follows the order **3** > **2** ≫ **1** (Figure 1a). A 10 μM solution of the dppz complex, upon irradiation for 40 min at this wavelength, shows essentially complete cleavage of SC DNA, whereas its dpq analogue exhibits ∼70% cleavage. Control experiments showed [Cu(O_2CMe)(Tp^{Ph})] or the ligands alone to be cleavage-inactive under similar reaction conditions. The dpq and dppz ligands, with their photoexcited $^3(n-\pi^*)$ and/or $^3(\pi-\pi^*)$ state(s), display significantly better photosensitizing effect than the phen ligand.[12] Mechanistic aspects of the reaction were probed using external reagents. Control experiments showed that addition of singlet oxygen quencher sodium azide[13] or L-histidine[14] inhibits the cleavage whereas the hydroxyl radical scavenger DMSO shows no inhibitory effect (Figure 1b). The complexes are cleavage-inactive under argon atmosphere. The steric enclosure of the photosensitizer B by the Tp^{Ph} ligand seems to stabilize the excited triplet state for activation of dioxygen from its stable triplet state to the cytotoxic singlet state in a type-II process, oxidizing primarily the guanine base of DNA (Scheme 1).[15] The involvement of singlet oxygen is further confirmed by a significant enhancement of cleavage activity in D_2O, a solvent in which singlet oxygen has a longer lifetime than it does in water.[16]

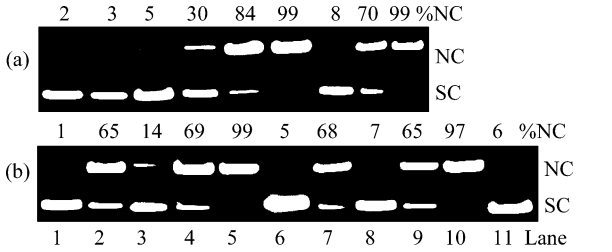


Figure 1. (a) Imaged agarose gel displaying the UV-light-induced DNA (SC pUC19, 0.5 μg) cleavage activity of complexes **1**−**3** at 365 nm (12 W): Lane 1, DNA control (40 min); lane 2, DNA + HTp^{Ph} (50 μM, 30 min); lane 3, DNA + [Cu(O_2CMe)(Tp^{Ph})] (50 μM, 30 min); lane 4, DNA + **1** (50 μM, 10 min); lane 5, DNA + **2** (50 μM, 10 min); lane 6, DNA + **3** (50 μM, 10 min); lane 7, DNA + **1** (10 μM, 40 min); lane 8, DNA + **2** (10 μM, 40 min); lane 9, DNA + **3** (10 μM, 40 min). (b) Gel electrophoresis diagram showing the cleavage of SC pUC19 DNA by **2** (25 μM, 15 min) and **3** (10 μM, 30 min) using UV light of 365-nm wavelength in the presence of different additives. The reactions were carried out under aerobic conditions except for lanes 6 and 11. Lane 1, DNA control (30 min); lane 2, DNA + **2**; lane 3, DNA + NaN_3 (25 μM) + **2**; lane 4, DNA + DMSO (2 μL) + **2**; lane 5, DNA + D_2O (14 μL) + **2**; lane 6, DNA + **2** (under argon); lane 7, DNA + **3**; lane 8, DNA + NaN_3 (25 μM) + **3**; lane 9, DNA + DMSO (2 μL) + **3**; lane 10, DNA + D_2O (14 μL) + **3**; lane 11, DNA + **3** (under argon).

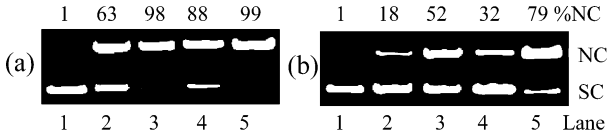
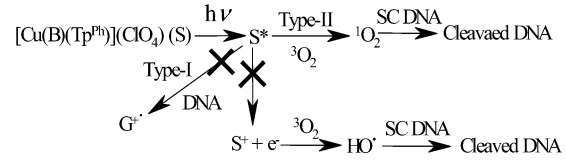


Figure 2. Agarose gel diagram showing the visible-light-induced SC DNA (0.5 μg) cleavage activity of complexes **2** and **3** at (a) 632.8 and (b) 694 nm: (a) Lane 1, DNA control (3 h); lane 2, DNA + **2** (25 μM, 1 h); lane 3, DNA + **2** (25 μM, 3 h); lane 4, DNA + **3** (25 μM, 30 min); lane 5, DNA + **3** (25 μM, 1 h). (b) Lane 1, DNA control (60 min); lane 2, DNA + **2** (10 μM, 15 min); lane 3, DNA + **2** (10 μM, 60 min); lane 4, DNA + **3** (10 μM, 15 min); lane 5, DNA + **3** (10 μM, 60 min).

Scheme 1. Mechanistic Pathway for DNA Photocleavage by **1**−**3**



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(10) Photoinduced cleavage of supercoiled (SC) pUC19 DNA by **1**−**3** was studied by agarose gel electrophoresis using procedures described elsewhere.[17] The reactions were carried out under illuminated conditions using a monochromatic UV lamp of 365-nm wavelength (12 W), a CW He−Ne laser of 632.8-nm wavelength (3 mW power, Scientifica-Cook Ltd., U.K.), and a pulsed ruby laser of 694-nm wavelength (Lumonics, 1/6 Hz, 20 ns). Eppendorf and glass vials were used for the UV- and visible-light experiments, respectively, in a dark room at 25 °C using SC DNA (0.8 μL, 0.5 μg) in 50 mM tris(hydroxymethyl)methane−HCl (Tris−HCl) buffer (pH 7.2) containing 50 mM NaCl and the complex at varied concentrations. The concentration of each complex or additive corresponded to the quantity of the sample in 2 μL of stock solution that was subsequently diluted to 18 μL with buffer prior to photolysis. For anaerobic experiments, irradiation of the solutions was done under positive pressure of argon following freeze−pump−thaw cycles.

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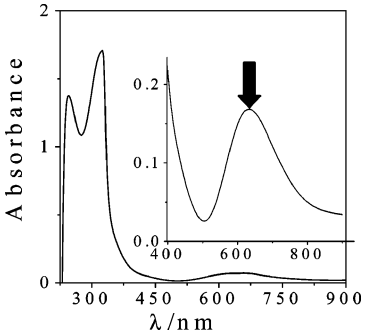

Figure 3. Electronic spectrum of **3** in DMF with the inset showing the d−d band at 633 nm.

Our earlier studies have shown that dpq and dppz complexes bind to DNA in the minor and major grooves, respectively (Chart 1).[9] Higher cleavage activity of the dppz complex than its dpq analogue could be related to the higher guanine content in the major groove or better steric protection of **3** in the major groove in comparison to **2**, in which dpq binds in the minor groove.

We probed the DNA cleavage activity of the dpq and dppz complexes at 632.8 nm using the d−d band in the photoexcitation process (Figure 3).[17,18] This wavelength falls in the PDT window of 600−800 nm and is close to the 630-nm value used for Photofrin activation. After 1 h of photoexposure, a 25 μM solution of complex **3** shows essentially complete cleavage of SC DNA to its nicked circular (NC) form (Figure 2). Complex **2** also shows a similar extent of DNA cleavage but with a longer exposure time. The efficient cleavage activity signifies effective steric protection of the photosensitizing moiety from the solvent molecules. The complexes are cleavage-inactive under argon. Use of sodium azide or L-histidine provides complete inhibition of the cleavage activity. An enhancement of cleavage is observed in D_2O. The results suggest the formation of singlet oxygen upon both UV- and red-light exposure following a type-II process in preference to the type-I and photoredox pathways (Scheme 1).[19] To probe the effect of protection of the photosensitizer by the Tp^{Ph} ligand, we performed control experiments using $[Cu^{II}(dpq)(NO_3)_2(OH_2)]$ (**I**) and $[Cu^{II}(dppz)(NO_3)_2(OH_2)]$ (**II**), in which the photosensitizers dpq and dppz are unprotected from the solvent molecules.[20] We also used complexes $[Cu^{II}(Tp)_2]$ and $[Cu^{II}(dppz)(Tp)](NO_3)$ for comparative studies [HTp = hydrotris(1-pyrazolyl)borate].[21] All of these complexes show reduced cleavage activity in comparison to their Tp^{Ph} analogues (Figure 4). The dpq and dppz scorpionates exhibit efficient photonuclease activity at 694 nm using a pulsed ruby laser (Figure 2, Table 1). A 10 μM solution of **3** displays ~80% cleavage of SC DNA after 1 h of exposure at this wavelength.

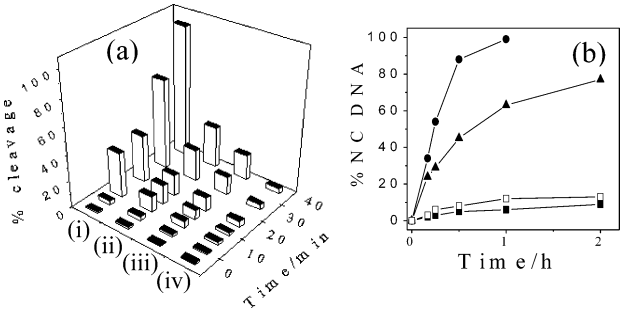

Figure 4. (a) Comparison of the SC DNA (0.5 μg) cleavage activity of complex (i) **3** with that of its analogues (ii) $[Cu(dppz)(Tp)](NO_3)$, (iii) $[Cu(dppz)(NO_3)_2(OH_2)]$ (**II**) and (iv) $[Cu(Tp)_2]$ upon photoexposure at 365 nm, where Tp is hydrotris(1-pyrazolyl)borate (complex concentration = 10 μM). (b) Extent of red-light-induced cleavage of SC DNA (0.5 μg) for complexes (▲) **2**, (●) **3**, (■) **I**, and (□) **II** at different exposure times [λ = 632.8-nm CW laser (3 mW); complex concentration = 25 mM].

In summary, we report efficient red-light-induced DNA cleavage activity of ternary copper(II) scorpionates having planar hetrocyclic bases as DNA binder cum photosensitizer. The molecular bowl of the $\{Cu(Tp^{Ph})\}$ moiety effectively encloses the photosensitizer, and its protection from the solvent molecules leads to the stabilization of the excited triplet state, which generates cytotoxic singlet oxygen species that cleave DNA in an oxidative process. The observed enhancement of cleavage activity upon steric protection is similar to the light-switch effect reported for tris-chelates of 4d metals, and the observed phenomenon offers further scope for designing and developing new photosensitizers for PDT applications.

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Supporting Information Available: Gel electrophoresis diagrams (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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