Inorg. Chem. **2002**, *41*, 6196–6198

Inorganic Chemistry
Communication

Visible Light Induced Photocleavage of DNA by a Mixed-Metal Supramolecular Complex: $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$

Shawn Swavey and Karen J. Brewer*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received June 5, 2002

The mixed-metal supramolecular complex, $[\{(bpy)_2Ru(dpp)\}_2RhCl_2](PF_6)_5$ (bpy = 2,2′-bipyridine and dpp = 2,3-bis(2-pyridyl)pyrazine) coupling two ruthenium light absorbers (LAs) to a central rhodium, has been shown to photocleave DNA. This system possesses a lowest lying metal to metal charge transfer (MMCT) excited state in contrast to the metal to ligand charge transfer states (MLCT) of the bpm and Ir analogues. The systems with an MLCT excited state do not photocleavage DNA. $[\{(bpy)_2Ru(dpp)\}_2RhCl_2](PF_6)_5$ is the first supramolecular system shown to cleave DNA. It functions through an excited state previously unexplored for this reactivity, a Ru → Rh MMCT excited state. This system functions when irradiated with low energy visible light with or without molecular oxygen.

Recent emphasis has been placed on developing reagents capable of cleaving DNA, applicable as structural probes and therapeutic agents, with many transition metal complexes being reported.[1−18] Photochemical approaches are of particular interest as they offer reaction control and can be highly targeted.[10−15,19] One popular approach involves the sensitization of molecular oxygen.[5,7,8,20]

The development of photosensitizers that absorb low energy light, are tunable, and function in the absence of molecular oxygen is of interest. Oxygen independent systems function under conditions of low oxygen content and often have a different mechanism of photocleavage.[16] A photosensitizer which can be excited with low energy light can avoid the base damage induced by UV light.[21,22]

Rhodium and ruthenium complexes photocleave DNA. Photolysis at 310 nm of rhodium(III) complexes of phi (9,10-phenanthrenequinone diimine) leads to hydrogen abstraction from the 3′-carbon of deoxyribose, leading to DNA cleavage.[23] Cleavage selectivity can be modulated by ancillary[24] and active[25] ligand variation or by tethering to DNA.[26−29] $[Rh(phi)_2(phen)]^{3+}$ has recently been shown to stabilize duplex DNA inhibiting transcription.[30] $Rh_2(O_2CCH_3)_4L_2$ (L = H_2O[14] or PPh_3[31]) has exhibited the ability to photocleave DNA when irradiated in the presence of electron acceptors. Studies have shown site specific oxidative cleavage of DNA using $[Ru^{IV}(tpy)(bpy)O]^{2+}$ and $[Ru^{III}(tpy)(bpy)OH]^{2+}$ (tpy = 2,2′:6′,2″-terpyridine).[32,33] Photoexcitation of ruthenium(II)

---

* To whom correspondence should be addressed. E-mail: kbrewer@vt.edu. Phone: (540) 231-6579. Fax: (540)231-3255.

(1) Absalon, M. J.; Wu, w.; Stubbe, J. *Biochemistry* **1995**, *34*, 2076.
(2) Tullius, T. D. *Nature* **1988**, *332*, 663.
(3) Sardesai, N. Y.; Zimmermann, K.; Barton, J. K. *J. Am. Chem. Soc*. **1994**, *116*, 7502.
(4) Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc*. **1995**, *117*, 3325.
(5) Hergueta-Bravo, A.; Jimenez-Hernandez, M. E.; Montero, F.; Oliveros, E.; Orellana, G. *J. Phys. Chem. B.* **2002**, *106*, 4010.
(6) Juskowiak, B.; Dominiak, A.; Takenaka, S.; Takagi, M. *Photochem. Photobiol*. **2001**, *74*, 391.
(7) Moucheron, C.; Kirsch-DeMesmaeker, A.; Kelly, J. M. *J. Photochem. Photobiol., B* **1997**, *40*, 91.
(8) Tossi, A. B.; Kelly, J. M. *Photochem. Photobiol*. **1989**, *49*, 545.
(9) Barton, J. K.; Raphael, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6460.
(10) Barton, J. K.; Holmlin, R. E. *Inorg. Chem*. **1995**, *34*, 7.
(11) Fleisher, M. B.; Waterman, K. C.; Turro, N. J.; Barton, J. K. *Inorg. Chem*. **1986**, *25*, 3549.
(12) Bhattacharya, S.; Mandal, S. S. *Chem. Commun*. **1996**, 1515.
(13) Farinas, E.; Tan, J. D.; Baidya, N.; Mascharak, P. K. *J. Am. Chem. Soc*. **1993**, *115*, 2996.
(14) Fu, P. K.-L.; Bradley, P. M.; Turro, C. *Inorg. Chem*. **2001**, *40*, 2476.
(15) Nunez, M. E.; Rajski, S. R.; Barton, J. K. *Methods Enzymol*. **2000**, *319*, 165.
(16) Armitage, B. *Chem. Rev*. **1998**, *98*, 1171.
(17) Dunn, D. A.; Lin, V. H.; Kochevar, I. E. *Biochemistry* **1992**, *31*, 11620.
(18) Paillous, N.; Vincendo, P. *J. Photochem. Photobiol., B* **1993**, *20*, 203.
(19) Kim, J.; Sistarc, M. F.; Carter, P. J.; Thorp, H. H. *Coord. Chem. Rev*. **1998**, *171*, 341.
(20) Bonnett, R. *Chemical Aspects of Photodynamic Therapy*; Taylor and Francis, Inc.: Philadelphia, 2000; Vol. 1, Chapter 4, pp 57−70.
(21) Boutorine, A. S.; Brault, D.; Takasugi, M.; Delgado, O.; Helene, C. *J. Am. Chem. Soc*. **1996**, *118*, 9469.
(22) Murray, V.; Martin, R. *Nucleic Acids Res*. **1994**, *22*, 506.
(23) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc*. **1992**, *114*, 2303.
(24) Turro, C.; Hall, D. B.; Chen, W.; Zuilhof, H.; Barton, J. K.; Turro, N. J. *J. Phys. Chem. A* **1998**, *102*, 5708.
(25) (a) Jackson, B. A.; Henling, L. M.; Barton, J. K. *Inorg. Chem*. **1999**, *38*, 6218. (b) Jackson, B. A.; Barton, J. K. *Biochemistry* **2000**, *39*, 6176.
(26) Copeland, K. D.; Fitzsimons, M. P.; Houser, R. P.; Barton, J. K. *Biochemistry* **2002**, *41*, 343.
(27) Williams, T. T.; Barton, J. K. *J. Am. Chem. Soc*. **2002**, *124*, 1840.
(28) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.
(29) Nunez, M. E.; Barton, J. K. *Curr. Opin. Chem. Biol*. **2000**, *4*, 199.
(30) Fu, P. K.-L.; Turro, C. *Chem. Commun*. **2001**, 279.
(31) Bradley, P. M.; Fu, P. K. -.L.; Turro, C. *Comments Inorg. Chem*. **2001**, *22*, 393.
(32) Cheng, C. C.; Goll, J. G.; Neyhart, G. A.; Walch, T. W.; Singh, P.; Thorp, H. H. *J. Am. Chem. Soc*. **1995**, *117*, 2970.

10.1021/ic0257726 CCC: $22.00

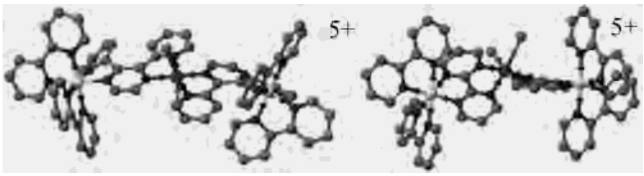


Figure 1. Mixed-metal trimetallics $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ and $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$.

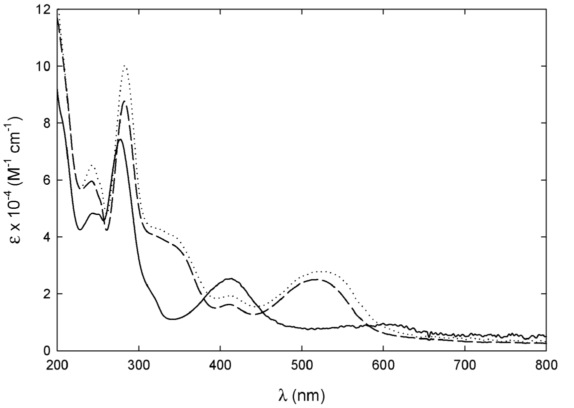


Figure 2. Electronic absorption spectra for $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ (—), $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ (- - - -), and $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$ (⋯⋯) in doubly distilled water, ddH$_2$O.

polypyridyl systems has resulted in oxidative damage to DNA in the presence of an electron acceptor[34−36] and cleavage by oxygen sensitization.[5,7,8] Rh(III) complexes intercalated into DNA serve as electron acceptors for excited Ru chromophores via long-range electron transfer.[37,38]

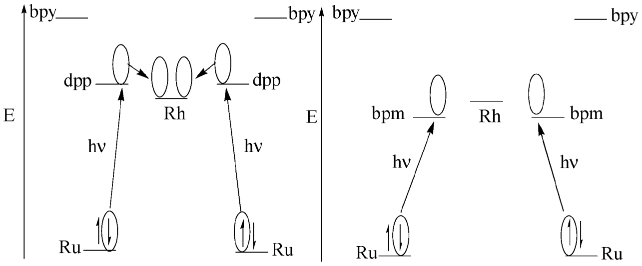
Trimetallic complexes coupling light absorbing ruthenium centers to reactive metal centers have been of interest. $[\{(bpy)_2Ru(BL)\}_2MCl_2]^{5+}$ (M = Rh or Ir and BL = 2,3-bis(2-pyridyl)pyrazine (dpp)[39] or 2,2′-bipyrimidine (bpm)[40]) complexes, shown in Figure 1, display quite varied electrochemical properties and differing lowest lying excited states. They are good chromophores with the high energy region of the electronic absorption spectra dominated by ligand based ($\pi \rightarrow \pi^*$) transitions. The visible region contains metal to ligand charge transfer (MLCT) transitions to both acceptor ligands with the BL transition being the lowest energy.

Table 1. Electrochemical Properties for a Series of Ru(II) and Ru(II)/Rh(III)/Ru(II) Trimetallic Complexes Where bpy = 2,2′-Bipyridine, dpp = 2,3-Bis(2-pyridyl)pyrazine, and bpm = 2,2′-Bipyrimidine

complex	$E_{1/2}$, V[a]	assignment
$[\{(bpy)_2Ru(dpp)\}_2RhCl_2](PF_6)_5$	1.60	$2Ru^{III/II}$
	−0.39[b]	$Rh^{III/I}$
	−0.79	dpp,dpp/dpp,dpp$^-$
	−1.02	dpp,dpp$^-$/dpp$^-$dpp$^-$
$[\{(bpy)_2Ru(bpm)\}_2RhCl_2](PF_6)_5$	1.70	$2Ru^{III/II}$
	−0.13	bpm,bpm/bpm,bpm$^-$
	−0.26	bpm,bpm$^-$/bpm$^-$,bpm$^-$
	−0.78	$Rh^{III/I}$
$[\{(bpy)_2Ru(dpp)\}_2IrCl_2](PF_6)_5$	1.56	$2Ru^{III/II}$
	−0.39	dpp,dpp/dpp,dpp$^-$
	−0.54	dpp,dpp$^-$/dpp$^-$dpp$^-$

[a] Potentials reported versus the Ag/AgCl (0.29 V vs NHE) reference electrode in 0.1 M $Bu_4NPF_6 \cdot CH_3CN$. [b] $E_p{}^c$ value.

Scheme 1. Orbital Energy Diagram for $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ and $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$



The electronic absorption spectra of these supramolecular complexes are shown in Figure 2. All three complexes possess lowest lying Ru(dπ) → BL CT bands that occur in the low energy visible region. For $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ and $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$, the Ru(d$\pi$) → dpp($\pi^*$) CT transition occurs at 525 nm. The Ru(dπ) → bpm(π^*) CT transition for $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ occurs at 594 nm. The Ir and Rh analogues, $[\{(bpy)_2Ru(dpp)\}_2MCl_2]^{5+}$, have spectroscopy that is virtually identical owing to their similar supramolecular structure and the dominance of the Ru light absorbers on the spectroscopic properties of these systems. The electrochemical properties vary with BL and M for $[\{(bpy)_2Ru(BL)\}_2MCl_2]^{5+}$, summarized in Table 1. The complexes exhibit a single reversible oxidation wave in the anodic region (1.56 and 1.70 V vs Ag/AgCl) attributed to the overlapping $Ru^{III/II}$ redox couple for the two equivalent Ru centers. $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ exhibits reversible bridging ligand reductions prior to reduction of the central Rh metal.[40] $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ undergoes an irreversible two electron reduction of the Rh(III) metal center prior to reduction of dpp BL. This orbital inversion, Scheme 1, of the dpp(π^*) and Rh(dσ^*) orbitals, allows the Rh to function as an electron acceptor giving a lowest lying, Ru → Rh metal to metal charge transfer (MMCT) excited state in this complex. It is this state we exploit for DNA photocleavage.

The lack of a Rh(dσ^*) LUMO in the $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ system allows us to use this as a very similar supramolecular architecture control system with a lowest lying MLCT state. The Ir analogue, $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$, serves as a spectroscopically matched system with a lowest lying MLCT state. pUC18 and pBluescript were used to probe photocleavage of DNA by gel electrophoresis.[14,26,41,42] Figure 3a,c shows imaged ethidium bromide stained agarose gels that reveal that the excited state of $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ photocleaves DNA.

---

(33) Farrer, B. T.; Thorp, H. H. *Inorg. Chem.* **2000**, *39*, 44.
(34) Stemp, E. D. A.; Arkin, M. R.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 2921.
(35) Delaney, S.; Pascaly, M.; Bhattacharya, P. K.; Han, K.; Barton, J. K. *Inorg. Chem.* **2002**.
(36) Ambroise, A.; Maiya, B. G. *Inorg. Chem.* **2000**, *39*, 4264.
(37) Purugganan, M. D.; Kumar, C. V.; Turro, N. J. *Science* **1988**, *241*, 1645.
(38) Arkin, M. R.; Stemp, E. D. A.; Holmlin, R. E.; Barton, J. K.; Hormann, A.; Olsen, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 475.
(39) Molnar, S. M.; Jensen, G. E.; Vogler, L. M.; Jones, S. W.; Laverman, L.; Bridgewater, J. S.; Richter, M. M.; Brewer, K. J. *J. Photochem. Photobiol., A* **1994**, *80*, 315.
(40) Nallas, G. N.; Jones, S. W.; Brewer, K. J. *Inorg. Chem.* **1996**, *35*, 6974.
(41) Kumar, C. V.; Tan, W. B.; Betts, P. W. *J. Inorg. Biochem.* **1997**, *68*, 177.

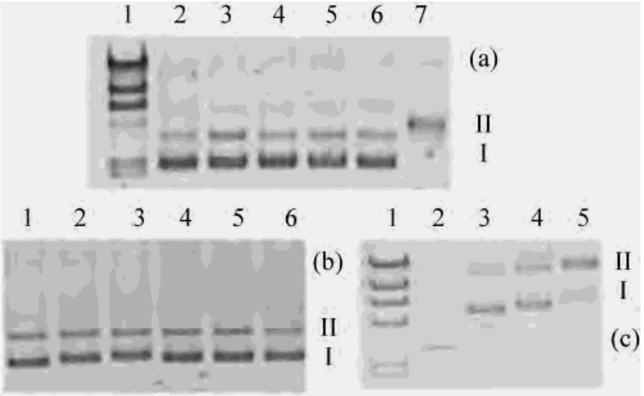


Figure 3. (a) Imaged agarose gel showing the photocleavage of pUC18 plasmid by $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ in the absence of molecular oxygen. Lane 1 λ molecular weight standard, lanes 2 and 3 plasmid controls, lanes 4 and 6 plasmid incubated at 37 °C (2 h) in the presence of $[(bpy)_2Ru(dpp)]^{2+}$ and $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$, respectively (1:5 metal complex/base pair), lanes 5 and 7 plasmid irradiated at $\lambda \geq 475$ nm for 10 min in the presence of $[(bpy)_2Ru(dpp)]^{2+}$ and $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$, respectively. (b) Lanes 1 and 2 plasmid controls, lanes 3 and 5 plasmid incubated at 37 °C (3 h) in the presence of $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ and $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$, respectively, lanes 4 and 6 plasmid irradiated at $\lambda \geq 475$ nm for 10 min in the presence of $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ and $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$, respectively. (c) Imaged agarose gel showing photocleavage of pBluescript plasmid in the absence of molecular oxygen by $[(bpy)_2Ru(dpp)RhCl_2(dpp)Ru(bpy)_2](PF_6)_5$. Lane 1 is the λ molecular weight standard, lane 2 is the control linearized DNA (cut with HindIII) with no metal present, lane 3 is the control circular DNA with no metal present, lane 4 is a 1:5 metal complex/base pair mixture of the plasmid with the metal complex incubated at 37 °C (4 h), and lane 5 is a 1:5 metal complex/base pair mixture of the plasmid with the metal complex photolyzed at 520 ± 5 nm for 4 h. All gels used 0.8% agarose, 90 mM Tris, and 90 mM boric acid buffer (pH = 8.2, ionic strength = 0.0043 M calculated using the Henderson−Hasselbalch equation).[42]

Lane 1 (Figure 3a) shows the λ molecular weight standard. Lane 2 (Figure 3a) indicates that pUC18 plasmid is found mostly as the supercoiled state (form I) with a small amount of nicked, circular DNA (form II). When irradiated ($\lambda_{irr} \geq 475$ nm) for 10 min, the plasmid alone (lane 3) does not cleave.[42] When incubated at 37 °C for 2 h in the presence of the monometallic precursor, $[(bpy)_2Ru(dpp)]^{2+}$ (lane 4), or in the presence of the trimetallic complex, $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ (lane 6), the plasmid DNA is not cleaved. When irradiated for 10 min in the presence of the monometallic precursor (lane 5), no evidence for DNA cleavage is observed. In the absence of molecular oxygen when the plasmid is irradiated for 10 min ($\lambda_{irr} \geq 475$ nm) in the presence of $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ at a 1:5 metal complex to base pair ratio (lane 7), conversion of the supercoiled DNA to the nicked form is observed. Figure 3c, lane 5, shows a similar cleavage of pBluescript plasmid using a narrow band excitation. These cleavage reactions are also observed in the presence of molecular oxygen. The photocleavage of DNA by $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ but not the monometallic ruthenium synthon illustrates the role of the supramolecular architecture, including Rh, on the desired photoreactivity. The cleavage product migrates slightly slower through the gel than native nicked plasmid, and similar results have been observed by Turro.[14]

To explore the role of the Rh LUMO, resulting in an MMCT excited state, on the DNA photocleavage, the bpm analogue $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ and the Ir analogue $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$, which contain inaccessible Rh($d\sigma^*$) and Ir($d\sigma^*$) orbitals,[39,40] were studied for their ability to photocleave DNA. The Ir analogue has nearly identical electronic absorption spectroscopy to that of the Rh complex. This allows it to function well as a control system possessing a lowest lying MLCT state. The results of this study are shown in Figure 3b. Lanes 1 and 2 (Figure 3b) are the plasmid controls. Lanes 3 and 5 reveal that when the plasmid is incubated at 37 °C in the presence of $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$ or $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$, respectively, at a 1:5 metal complex to base pair ratio, no DNA cleavage occurs. Similar solutions irradiated ($\lambda_{irr} \geq 475$ nm) for 10 min (lanes 4 and 6), in the absence of molecular oxygen, also do not result in DNA cleavage. Similar studies in the presence of oxygen also do not result in DNA cleavage.

These results indicate that our mixed-metal supramolecular complex, $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$, is capable of DNA photocleavage and similar systems without a Rh($d\sigma^*$) based LUMO do not display this behavior. This illustrates that our modifications of the coordination environment, yielding the desired orbital ordering, $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$, creates a system that photocleaves DNA via an MMCT excited state. Additionally, photocleavage can occur in the absence of molecular oxygen.

This study presents a new structural motif for DNA photocleavage agents, functioning from a previously unstudied excited state for this application. While the mode of DNA photocleavage is unclear, frank cleavage is observed consistent with reactivity arising from the photogenerated Rh(II) site. This supramolecular architecture allows for substitution of components to tune properties of these systems, allowing for the development of many new complexes that should display similar reactivity. Studies are underway to explore additional supramolecular complexes with this interesting photoreactivity and to probe this reaction in more detail.

Acknowledgment. The authors thank Prof. Brenda Winkel for her molecular biology contributions, the National Science Foundation (Grant CHE-9632713), and OSER/Carilion Biomedical Institute for their generous support of this research.

Supporting Information Available: Enlarged version of Figure 3. This material is available free of charge via the Internet at http://pubs.acs.org.

IC0257726

---

(42) pUC18 plasmid is 2686 bp (Bayou Biolabs). Irradiation used a 1000 W xenon arc lamp, a water IR filter, and a 475 nm cut off filter. Solutions were 3.5 μM in metal complex and 6.9 mM in phosphate buffer (pH = 7) and allowed for ionic association of the cationic metal complexes with DNA. Dexoygenation was accomplished by bubbling with Ar for 30 min prior to the photolysis of the samples in an airtight cell blanketed with Ar.