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A Multifunctional Tetrametallic Ru–Pt Supramolecular Complex Exhibiting Both DNA Binding and Photocleavage

Ran Miao,[†] Matthew T. Mongelli,[†] David F. Zigler,[†] Brenda S. J. Winkel,[‡] and Karen J. Brewer*,[†]

Departments of Chemistry and Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

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A new tetrametallic supramolecular complex, $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)PtCl_2](PF_6)_6$ (bpy = 2,2′-bipyridine, dpp = 2,3-bis(2-pyridyl)-pyrazine), has been prepared and characterized. This supramolecular assembly is multifunctional, forming coordinate covalent bonds to DNA through its *cis*-$PtCl_2$ moiety and photocleaving DNA through its Ru polypyridine chromophores. Electronic absorption spectroscopy shows ligand-based $\pi\rightarrow\pi^*$ transitions in the UV with Ru-based metal-to-ligand charge transfer transitions throughout much of the visible. The Ru→dpp CT transitions center at 542 nm (ϵ = 35 000 M^{-1} cm^{-1}). This complex has a HOMO localized on peripheral Ru with $E_{1/2}^{oxd}$ = 1.58 V vs Ag/AgCl, and a LUMO based on the μ-dpp connecting Ru and Pt, $E_{1/2}^{red}$ = –0.40 V. Gel electrophoresis analysis shows the tetrametallic coordinates to pUC18 DNA and, when excited with visible light, cleaves DNA through an oxygen-mediated pathway.

Ru polyazine complexes show applications in diverse arenas due in part to their strong visible light absorbing ability, often emissive metal-to-ligand charge transfer (MLCT) excited states, and tunable properties.[1] The incorporation of polyazine bridging ligands has allowed for the construction of complex systems with diverse building blocks, taking advantage of the rapid intramolecular energy and electron-transfer processes in these assemblies. Supramolecular complexes incorporating Ru light absorbers (LAs) have been widely studied for potential applications in a variety of light-activated processes.[2,3] Despite the large number of Ru polyazine supramolecular assemblies developed to date, few couple reactive metals such as Pt to these LA units despite the promise such systems hold.[4−8]

A variety of metal complexes have the ability to target DNA, with many being of interest as anti-cancer agents.[4,9−20] Interactions of metal complexes with DNA have been shown to include both covalent binding and noncovalent interactions, such as intercalation or groove binding. Coordinate covalent binding of a metal complex to DNA often leads to inhibition of transcription and/or replication. Metal complexes are of interest as potential photodynamic therapy (PDT) agents, inhibiting cell function via light-mediated processes such as DNA cleavage.[9,10,15,18,19]

A well-known class of DNA-binding metal complexes is based on cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$.[4,11−14] The effectiveness of Pt in binding DNA led to the development of many cisplatin analogues.[6,7,14,15] Incorporation of LA metal units into this structural motif has been used to develop spectroscopic tags including complexes such as $[(tpy)RuCl(dpp)PtCl_2]^+$ (tpy = 2,2′:6′,2″-terpyridine, dpp = 2,3-bis(2-pyridyl)pyrazine),[4] which can bind DNA. The addition of LA units into this structural motif opens up the possibility of photoactivation. Other complexes such as

* To whom correspondence should be addressed. E-mail: kbrewer@vt.edu. Fax: (540) 231-3255. Phone: (540) 231-6570.
[†] Department of Chemistry.
[‡] Department of Biological Sciences.

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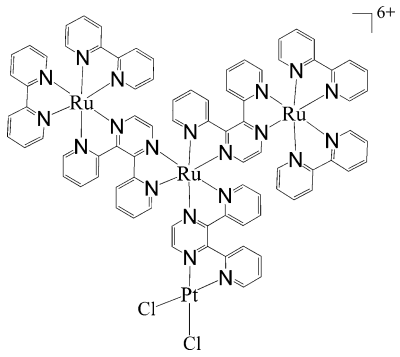

Figure 1. Tetrametallic supramolecular complex $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)PtCl_2]^{6+}$ (bpy = 2,2′-bipyridine, dpp = 2,3-bis(2-pyridyl)pyrazine).

$[(bpy)_2Ru(dpp)PtCl_2]^{2+}$ (bpy = 2,2′-bipyridine)[6,7] have been prepared without exploring DNA binding. The systems $[(bpy)_2Ru(dpq)PtCl_2]^{2+}$ and $[(bpy)_2Ru(dpb)PtCl_2]^{2+}$ have been shown to coordinatively bind DNA (dpq = 2,3-bis(2-pyridyl)quinoxaline and dpb = 2,3-bis(2-pyridyl)-benzoquinoxaline).[5]

Metal complexes capable of cleaving DNA using low-energy visible light are of particular importance as potential PDT agents.[15−19] Metal complexes directly photocleave DNA or more commonly sensitize molecular oxygen to cleave DNA. Ruthenium-based light absorbers with polyazine ligands typically photocleave DNA indirectly through oxygen-mediated pathways.[9,10,15,17−21]

Reported herein is the coupling of a *cis*-dichloroplatinum(II) DNA binding site to a three-metal Ru polyazine DNA photocleavage unit, $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)PtCl_2](PF_6)_6$ (**Ru_3Pt**), Figure 1. This new supramolecular complex has been synthesized, characterized, and shown to bind to and photocleave DNA. This supramolecular assembly represents the first such multifunctional DNA-binding and photocleavage agent constructed from this polyazine structural motif. A building block synthetic method is used to synthesize the trimetallic Ru chromophore $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)](PF_6)_6$[2,3,23] (**$Ru_3$**) prior to the final *cis*-$PtCl_2$ binding step. Detailed synthetic information is provided in the Supporting Information.

The **Ru_3Pt** supramolecular assembly was characterized by MS, electronic absorption spectroscopy, and electrochemistry. The FAB and MALDI-TOF MS are consistent with the supramolecular complex structure, and are included in the Supporting Information. The light-absorbing and redox properties of this supramolecular complex are indicative of their composition, displaying properties consistent with each structural subunit.

The electrochemical properties of supramolecular complexes of this type are indicative of the number and type of subunits with oxidations being metal based and reductions typically being ligand based.[1,3,6,7,23] When the ligand dpp is bridging between two electropositive metals like Ru(II) and Pt(II), the π^*-acceptor orbitals are stabilized and the dpp possesses two sequential one electron reductions prior to terminal bpy reductions.[3,5,6,22,23] The electrochemical properties of **Ru_3Pt** are summarized in Table 1 and are included in the Supporting Information. The oxidative couple at 1.58 V corresponds to the $Ru^{II/III}$ processes for the two peripheral metals in **Ru_3Pt**. By comparing the newly prepared **Ru_3Pt** and the trimetallic synthon, **Ru_3**, a shift is observed in the reduction potential for the formally terminal dpp from −1.08 to −0.40 V vs Ag/AgCl for the now bridging dpp. The two other bridging dpp ligands connecting the peripheral Ru to the central Ru reduce at −0.60 and −0.71 V, respectively.

Table 1. Electrochemical Properties for $[\{(bpy)_2Ru(dpp)\}_2Ru(\mathbf{dpp})PtCl_2](PF_6)_6$[a]

	$E_{1/2}$ (V)	
complex	oxidations	reductions
$[\{(bpy)_2Ru(dpp)\}_2Ru(\mathbf{dpp})](PF_6)_6$	1.58 $Ru^{II/III}$	−0.50 $dpp^{0/-}$ −0.64 $dpp^{0/-}$ −1.08 $\mathbf{dpp}^{0/-}$ −1.21 $dpp^{-/2-}$ −1.36 $dpp^{-/2-}$ −1.52 $\mathbf{dpp}^{-/2-}$
$[\{(bpy)_2Ru(dpp)\}_2Ru(\mathbf{dpp})PtCl_2](PF_6)_6$	1.58 $Ru^{II/III}$	−0.40 $\mathbf{dpp}^{0/-}$ −0.60 $dpp^{0/-}$ −0.71 $dpp^{0/-}$ −1.12 $\mathbf{dpp}^{-/2-}$ −1.22 $dpp^{-/2-}$ −1.30 $dpp^{-/2-}$

[a] Data measured in 0.1 M Bu_4NPF_6 acetonitrile, E vs Ag/AgCl. bpy = 2,2′-bipyridine and dpp = 2,3-bis(2-pyridyl)pyrazine.

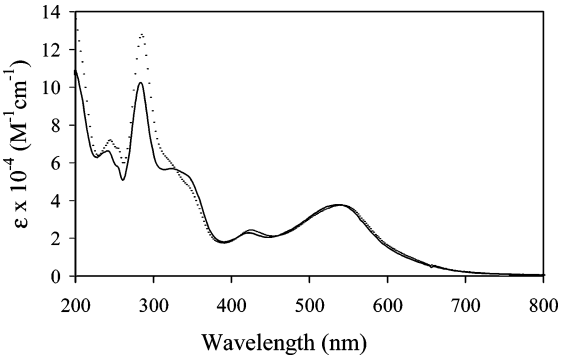

Figure 2. Electronic absorption spectra in CH_3CN at RT of $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)PtCl_2](PF_6)_6$ (− − −) and $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)](PF_6)_6$ (· · ·).

Electronic absorption spectroscopy is used to study the light-absorbing properties of Ru polyazine complexes.[1−3] Ru polyazine complexes usually show intense peaks in the UV region corresponding to a ligand-based $\pi\rightarrow\pi^*$ transition with overlapping MLCT transitions in the visible region. Figure 2 shows the electronic absorption spectrum for **Ru_3Pt** and **Ru_3** in acetonitrile. The **Ru_3Pt** tetrametallic is an efficient light absorber throughout the UV and visible regions. The UV contains $\pi\rightarrow\pi^*$ transitions for bpy (290 nm) and dpp (320 nm). The shoulder at 320 nm is characteristic of μ-dpp $\pi\rightarrow\pi^*$ transitions. The Ru(dπ)→bpy(π^*) CT transition occurs at 416 nm and peaks at ca. 520−540 nm correspond to the Ru(dπ)→μ-dpp(π^*) and Ru(dπ)→μ-dpp(π^*) CT

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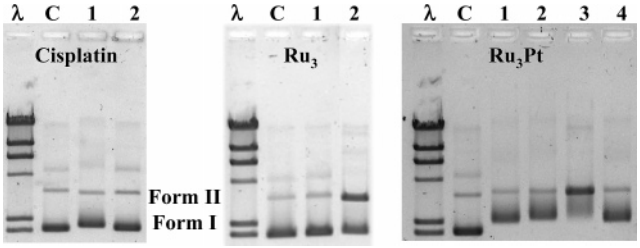

Figure 3. DNA binding and photcleavage by $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)PtCl_2](PF_6)_6$ (**Ru_3Pt**) assayed with 0.8% agarose gel electrophoresis post-stained with ethidium bromide using circular plasmid pUC18 DNA with comparison to cis-$[Pt(NH_3)_2Cl_2]$ (Cisplatin) and $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)](PF_6)_6$ (**Ru_3**). Lanes λ are the molecular weight standards (23, 9.4, 6.6, 4.4, 2.3, and 2.0 kb); lanes C are the DNA controls showing mostly form I and minor form II pUC18 DNA. The cisplatin lane 1 is 5:1 base pair (BP)/metal complex (MC), and lane 2 is 20:1 BP/MC incubated for 1 h at 37 °C showing the known coordinate covalent binder leads to an decrease in migration of form I supercoiled pUC18 plasmid through the gel at a 5:1 ratio. The **Ru_3** lane 1 is 20:1 BP/MC incubated for 1 h at 37 °C showing that the migration of pUC18 through the gel is not significantly impacted by addition of the **Ru_3** complex. Lane 2 of **Ru_3** is 20:1 BP/MC photolyzed with 450−1000 nm light for 1 h under atmospheric conditions showing photocleavage of the DNA by the **Ru_3** complex. The **Ru_3Pt** lane 1 is 20:1 BP/MC kept in the dark at room temperature, lane 2 is 20:1 BP/MC incubated for 1 h in the dark at 37 °C, lane 3 is 20:1 BP/MC photolyzed with 450−1000 nm light for 1 h under atmospheric conditions, lane 4 is 20:1 BP/MC photolyzed with 450−1000 nm light for 1 h under argon. This shows the **Ru_3Pt** coordinatively binds to DNA and in the presence of O_2 photocleaves DNA.

transitions. The extinction coefficient for the MLCT transitions at 542 nm is 35 000 M^{-1} cm^{-1}, consistent with the number of overlapping transitions in this region.

The DNA-binding and photocleavage ability of the title complex, **Ru_3Pt**, has been investigated using gel electrophoresis. The *cis*-dichloroplatinum(II) moiety is designed to allow the complex to coordinate to DNA. The Ru polyazine units are designed to allow the complex to undergo sensitization of molecular oxygen, leading to DNA photocleavage. The presence of the *cis*-dichloroplatinum(II) subunit delivers the Ru polyazine subunit to its target prior to optical excitation. The DNA binding and photocleavage of **Ru_3Pt** was assayed using gel electrophoresis with pUC18 (2686 BP, Bayou Biolabs) circular plasmid DNA post-stained with ethidium bromide.[4,5] DNA interactions of **Ru_3Pt** were compared to a binding analyses of **Ru_3** and cisplatin, Figure 3. Lanes λ are the molecular weight standards (23, 9.4, 6.6, 4.4, 2.3, and 2.0 kb). Lanes C are the pUC18 DNA controls, showing primarily the supercoiled (form I) and minor nicked (form II) forms of the circular pUC18 plasmid. Lane 1 for cisplatin is 5:1 base pairs (BP)/metal complex (MC) and lane 2 is 20:1 BP/MC solution incubated at 37 °C for 1 h. The migration of form I of the pUC18 with the 5:1 ratio is retarded by the known coordination of cisplatin to the DNA, while form II migration is slightly enhanced upon DNA binding. Lane 1 of **Ru_3** is a 20:1 BP/MC solution incubated at 37 °C for 1 h, showing the migration of the DNA is not significantly impacted by addition of the **Ru_3** complex, consistent with this complex's expected lack of coordination to DNA. Lane 2 of **Ru_3** shows the same 20:1 BP/MC solution photolyzed at $\lambda_{irr} > 450$ nm for 1 h under atmospheric conditions. Conversion of the form I plasmid to form II is observed, consistent with the ability of this complex to photocleave DNA. Lane 1 of **Ru_3Pt** is the 20:1 DNA BP/MC solution kept in the dark. This lane shows a decreased migration of the form I pUC18 through the gel, indicative of the coordination of **Ru_3Pt** to the DNA through the Pt site. Form II of pUC18 also shows slightly slowed migration consistent with coordination by this large, highly cationic supramolecular assembly increasing the size and decreasing the negative charge of the plasmid.[4,5] Lane 2 shows the same 20:1 BP/MC mixture incubated at 37 °C for 1 h, displaying a similar retardation of migration through the gel, showing no enhancement of covalent binding by incubation. Lane 3 shows the same 20:1 BP/MC solution photolyzed at $\lambda_{irr} > 450$ nm for 1 h under atmospheric conditions. There is an appreciable conversion of the supercoiled (form 1) to nicked (form II) DNA, indicative of DNA photocleavage. Lane 4 is a 20:1 BP/MC solution photolyzed at $\lambda > 450$ nm for 1 h under argon. In the absence of molecular oxygen, no DNA photocleavage is observed, consistent with our molecular design.

The multifunctional supramolecular complex $[\{(bpy)_2Ru(dpp)\}_2Ru(dpp)PtCl_2](PF_6)_6$ has been prepared and shown to display both Pt-based DNA binding and Ru-based DNA photocleavage. Mass spectrometry, electrochemistry, and electronic absorption spectroscopy are consistent with the composition of this tetrametallic assembly. This complex has the ability to modify DNA through two distinct pathways, using each of the two bioactive subunits. The complex can both coordinate to DNA through the *cis*-dichloroplatinum(II) unit and photochemically sensitize molecular oxygen to cleave DNA through the Ru polyazine unit. The ability to deliver and anchor the drug directly to the target can be useful in anti-cancer drug development. The coordinate covalent binding to DNA through Pt may also direct the cleavage to the binding sites and enhance efficiency by a prelocalization at the target. Studies are in progress to prepare a series of related analogues to study interactions of this type of multifunctional supramolecular complex with DNA in more detail.[25]

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Supporting Information Available: Full experimental details, a reaction scheme, mass spectrometry data, and electrochemical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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