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Cleavage of DNA by Proton-Coupled Electron Transfer to a Photoexcited, Hydrated Ru(II) 1,10-Phenanthroline-5,6-dione Complex

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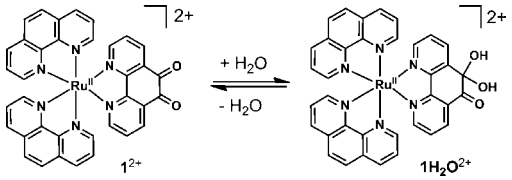
Supporting Information

ABSTRACT: Visible light irradiation of a ruthenium(II) quinone-containing complex, $[(phen)_2Ru(phendione)]^{2+}$ ($\mathbf{1}^{2+}$), where phendione = 1,10-phenanthroline-5,6-dione, leads to DNA cleavage in an oxygen independent manner. A combination of NMR analyses, transient absorption spectroscopy, and fluorescence measurements in water and acetonitrile reveal that complex $\mathbf{1}^{2+}$ must be hydrated at the quinone functionality, giving $[(phen)_2Ru(phenH_2O)]^{2+}$ ($\mathbf{1H_2O}^{2+}$, where $phenH_2O$ = 1,10-phenanthroline-6-one-5-diol), in order to access a long-lived $^3MLCT_{hydrate}$ state ($\tau \sim 360$ ns in H_2O) which is responsible for DNA cleavage. In effect, hydration at one of the carbonyl functions effectively eliminates the low-energy $^3MLCT_{SQ}$ state (Ru^{III} phen-semiquinone radical anion) as the predominant nonradiative decay pathway. This $^3MLCT_{SQ}$ state is very short-lived (<1 ns) as expected from the energy gap law for nonradiative decay,[1] and too short-lived to be the photoactive species. The resulting excited state in $\mathbf{1H_2O}^{2+*}$ has photophysical properties similar to the ^3MLCT state in $[Ru(phen)_3]^{2+*}$ with the added functionality of basic sites at the ligand periphery. Whereas $[Ru(phen)_3]^{2+*}$ does not show direct DNA cleavage, the deprotonated form of $\mathbf{1H_2O}^{2+*}$ does via a proton-coupled electron transfer (PCET) mechanism where the peripheral basic oxygen sites act as the proton acceptor. Analysis of the small molecule byproducts of DNA scission supports the conclusion that cleavage occurs via H-atom abstraction from the sugar moieties, consistent with a PCET mechanism. Complex $\mathbf{1}^{2+}$ is a rare example of a ruthenium complex which 'turns on' both reactivity and luminescence upon switching to a hydrated state.

A number of transition metal complexes have attracted attention as potential photodynamic therapy (PDT) agents for cancer treatment, as they often have a high affinity for DNA and accessible photoexcited states in the visible region.[2] Turro, Brewer, Dunbar, and others have shown that complexes of Ru(II), Os(II), and dinuclear Rh(III) cores can be made into effective DNA photocleavage agents.[3−5] Most commonly, the PDT agent works by activation of cellular O_2 to form reactive oxygen species (ROS), such as $^1O_2$ or ·OH, which are responsible for inducing apoptosis via damaging reactions such as DNA cleavage. Only a few of these transition metal complexes are able to cleave DNA upon photoexcitation without the need for O_2; however, it is clear that such an approach could offer some therapeutic advantages in that many cancer cells are under hypoxic stress.[6−9]

Herein, we describe the oxygen independent DNA cleavage activity of a ruthenium(II) complex, $[(phen)_2Ru(phendione)]Cl_2$ ($\mathbf{1}Cl_2$) upon visible light irradiation into the MLCT band at 470 nm. Complex $\mathbf{1}^{2+}$ is a commonly used synthon in ruthenium polypyridyl chemistry[10−12] and yet, only recently has its unusual solvent and temperature dependent paramagnetism,[13] and now photoreactivity, been described. An investigation into the mechanism of DNA cleavage reveals that complex $\mathbf{1}^{2+}$ is in equilibrium with a hydrated species $\mathbf{1H_2O}^{2+}$ (see Scheme 1) and that the latter is the photoactive species.

Scheme 1. Hydration Equilibrium for Complex $\mathbf{1}^{2+}$ in Water



The excited state photophysics of **1** and $\mathbf{1H_2O}^{2+}$ and the mechanism by which DNA cleavage is induced were probed by a combination of transient absorption and luminescence spectroscopy, electrochemistry, and product analysis as described below.

The DNA photocleavage activity of **1** under aerobic and anaerobic conditions was examined using a common plasmid cleavage assay. Because of the hydration reaction (vide infra), $\mathbf{1}^{2+}$ is present as an equilibrium mixture of $\mathbf{1}^{2+}$ and $\mathbf{1H_2O}^{2+}$ which is collectively referred to as **RuPD** in the following section. Supercoiled pUC18 plasmid DNA was incubated with **RuPD** (loading ratio: 1 Ru complex per approximately 6 DNA base pairs) and irradiated with 470 nm light for various lengths of time in the presence or absence of O_2. The plasmid cleavage products were visualized using agarose gel electrophoresis to separate supercoiled (Form I), circular (Form II) and linear

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(Form III) plasmid DNA, as shown in Figure 1. As can be seen in lane 2, no DNA cleavage is observed upon incubation of

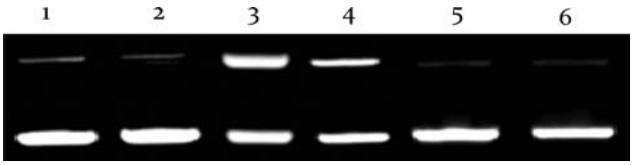

Figure 1. Ethidium bromide stained 1% agarose gel of photocleavage products of **RuPD** and pUC18: 1 μg/1 μL, 0.154 mM pUC18 DNA base pairs (4 mM phosphate, 50 mM NaCl, pH = 7.4) and 27 μM of the chloride salt of the ruthenium complex, irradiated (when applicable) at 470 nm for 2 h; lane 1, plasmid only; lane 2, **RuPD** dark in presence of O_2; lane 3, **RuPD** irradiated in presence of O_2; lane 4, **RuPD** irradiated, deaerated; lane 5, $[Ru(phen)_3]^{2+}$ irradiated, deaerated; lane 6, $[(phen)_2Ru(dppz)]^{2+}$ irradiated, deaerated.

DNA with **RuPD** in the dark, but upon irradiation, DNA cleavage to circular form DNA is observed under both aerobic (lane 3) and anaerobic (lane 4) conditions, with somewhat better cleavage yields observed with O_2 present. In the anaerobic experiment (lanes 4–6), samples were subjected to three consecutive freeze–pump–thaw cycles prior to experimentation in an O_2-free glovebox. To accentuate the unusual anaerobic photocleavage activity of **RuPD**, two closely related ruthenium complexes, $[Ru(phen)_3]^{2+}$, which lacks the phendione ligand, and $[(phen)_2Ru(dppz)]^{2+}$ (dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine), which is known to bind DNA tightly ($K_b \sim 10^7$ M^{-1})[14] via intercalation, were examined for anaerobic photocleavage activity in the same assay. Neither $[Ru(phen)_3]^{2+}$ (lane 5) nor $[(phen)_2Ru(dppz)]^{2+}$ (lane 6) is seen to cause any cleavage under these conditions, indicating the essential role of the phendione ligand.

The enhanced photocleavage activity of **RuPD** in the presence of oxygen (compare lanes 3 vs 4 in Figure 1) shows that photoexcited **RuPD** can also induce DNA damage by the generation of ROS, but the data in lane 4 reveals that an O_2 independent pathway also exists. Because it is very difficult to completely remove O_2 from aqueous solutions, several anaerobic DNA photocleavage experiments were also performed in the presence of ROS quenchers. Superoxide dismutase (SOD) effectively quenches superoxide while sodium azide is known to quench both singlet oxygen and $OH^{\cdot}$.[5] Addition of either SOD or sodium azide did not show any attenuation of the photocleavage reaction. (see Figure S3). However, addition of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) does attenuate the cleavage (Figure S3). TEMPO is a good quencher for metal- and carbon-based radical species,[15−18] suggesting that one or both of these species are generated upon photoexcitation of **RuPD** and are responsible for the cleavage activity.

The question now arises, what is the photoreactive species and what is the mechanism of DNA cleavage? NMR analysis of complex $\mathbf{1}^{2+}$ in CD_3CN reveals a spectrum consistent with the proposed structure; however, the NMR spectrum in D_2O shows substantial line broadening and complicated speciation (see Figure S4). The hydration reaction shown in Scheme 1 was implicated as the enhanced hydration of carbonyl moieties on coordinated ligands is a known process[19] and frequently reported for phendione ligands in particular.[20−23] Anson and co-workers report an equilibrium constant of 0.9 for hydration of $[(bpy)_2Os(phendione)]^{2+}$ at pH 5.2 in aqueous solution.[21] Integration leads to an estimated equilibrium constant of 0.5 and as expected, addition of DCl shifts the equilibrium toward the nonhydrated species (Figure S4). The observation of two distinct species (noncoalescence) and line broadening suggests that the hydration/dehydration process is on the slow end of the NMR time scale (ca. 0.25 s),[24] in good agreement with that estimated by Anson and co-workers using electrochemical modeling for the Os complex process (k_{dehyd} = 0.5 s).[16]

To determine the photophysical properties of the two complexes, we used a combination of transient absorption (TA) and fluorescence techniques in acetonitrile or water. TA data is perhaps the most revealing. In MeCN, where $\mathbf{1}^{2+}$ is undoubtedly the bulk species in solution, we observe a weak signal consistent with bleaching of the ground state MLCT bands (400–500 nm) in the 0.05–1 ns time regime (see Figure S5) but not at longer times, indicating a subnanosecond lifetime. A more pronounced MLCT bleach is observed in the excited state difference spectrum of $[Ru(phendione)_3]^{2+}$ (Figure S5) and a monoexponential fit of this decay data gave τ = 60 ps (Figure S6). The very short lifetime of the $^3MLCT_{SQ}$ state in $[Ru(phendione)_3]^{2+*}$ is anticipated on the basis of the energy gap law[1] and, by analogy, is anticipated for the $^3MLCT_{SQ}$ state in $\mathbf{1}^{2+*}$ (although we were not able to measure this directly). In water, the difference TA spectrum of the mixture of $\mathbf{1}^{2+*}$ and $\mathbf{1H_2O}^{2+*}$ (Figure S7) shows a bleach in the ground state MLCT region which can be fit (monoexponentially) to τ = 334 ns, a lifetime that correlates well to the observed luminescence decay data (Figure S8. λ_{em} = 610 nm; τ_{em} = 359 ns). Given the very short lifetime of the nonhydrated complex, this longer-lived excited state behavior can be assigned to the hydrate $\mathbf{1H_2O}^{2+*}$. Hydration is reported to render the quinone function electrochemically inactive in some studies or sluggish in others, as it is proposed that it is necessary to dehydrate and reform the quinone for electrochemical activity.[21,23,25] As this hydration-dehydration process is very slow compared to the lifetimes of the excited state species, it is clear that the lowest energy excited state in $\mathbf{1H_2O}^{2+*}$ is somewhat similar to that in $[Ru(phen)_3]^{2+*}$, which is reflected both in the emission energy and the lifetime.

This model is shown graphically in the Jablonski diagram (Figure 2). Both $\mathbf{1}^{2+}$ and $\mathbf{1H_2O}^{2+}$ absorb in the ^{1}MLCT band and intersystem cross to the $^3MLCT_{phen}$ state. For $\mathbf{1}^{2+}$, this rapidly deactivates to the $^3MLCT_{SQ}$ state. From ground state

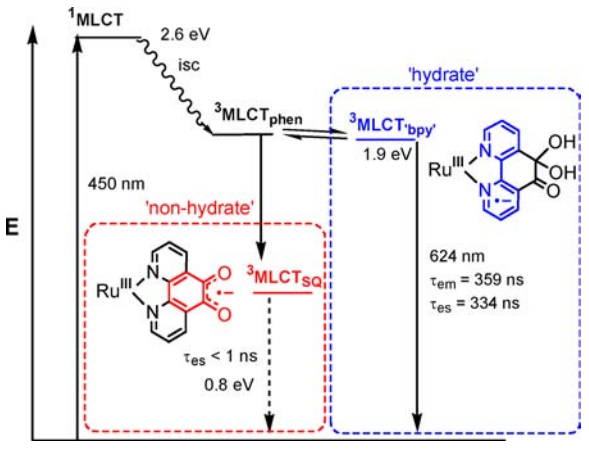

Figure 2. Proposed Jablonski diagram for $\mathbf{1}^{2+}$ in MeCN (red box) or $\mathbf{1H_2O}^{2+}$ in water (blue box). Deprotonation of $\mathbf{1H_2O}^{2+}$ to $\mathbf{1(OH)}^{+}$ shifts the emission maxima from 624 to 602 nm.

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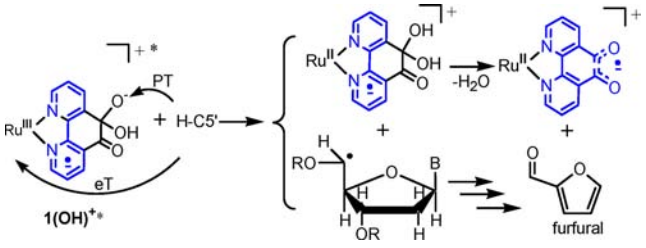
redox potentials and an estimate of 0.5 eV energy loss due to intersystem crossing, the energy of the $^3MLCT_{SQ}$ is estimated at 0.8 eV. For $\mathbf{1H_2O^{2+}}$, the SQ state is not available (on the time scale of the excited state process) and the 'bipyridine-like' portion of the phenH_2O ligand is the site of electron storage. This $^3MLCT_{bpy'}$ state is 1.9 eV above the ground state (from luminescence data) which is consistent with the considerably longer excited state lifetime.

In an effort to further establish the mechanism of action, we scaled up the anaerobic reaction and extracted the aqueous phase with CH_2Cl_2 to look for neutral, small-molecule DNA cleavage products characteristic of H-atom abstraction from either C1′ or C5′ of the deoxyribose units. The presence of furfural in the irradiated samples was confirmed by HPLC (Figure S9) and is indicative of H-atom abstraction from the C5′ position.[2,26,27]

Collecting all this data, we propose the mechanism shown in Scheme 2. It is clear that the oxygen independent cleavage of

Scheme 2. Proposed PCET Mechanism between $1(OH)^{+}$* and DNA



DNA by $\mathbf{1H_2O^{2+}}$* must involve the peripheral oxygens as otherwise we would expect to see similar photocleavage by complexes such as $[Ru(phen)_3]^{2+}$* and $[(phen)_2Ru(dppz)]^{2+}$*. Fluorescent pH titration (see Figure S10) established the pK_a and pK_a* for $\mathbf{1H_2O^{2+}}$ and $\mathbf{1H_2O^{2+}}$* at 5.8 and 7.1, respectively, indicating that the deprotonated complex, $\mathbf{1OH^{+}}$, is the proton acceptor, as shown in Scheme 2. We note that some of the increase in cleavage may also be due to an increase in the concentration of $\mathbf{1OH^{+}}$ as high pH favors formation of this species. Upon excitation, localization of the excited electron in the 'bpy-like' MO on the hydrated ligand would boost the basicity of the oxygen base which accepts a proton from the sugar C−H bond. At the same time, an electron is transferred to the Ru(III) site in a PCET mechanism, resulting in a Ru(II) semiquinone species (after dehydration) and a deoxyribose radical species. Formation of deoxyribose radical at any of the deoxyribose carbons is known to lead to strand scission by subsequent decay reactions.[22] The Ru(II) semiquinone species is likely to be unstable to disproportionation and yields $\mathbf{1^{2+}}$ and $[(phen)_2Ru(phendiol)]^{2+}$ as terminal products. We note that as the complex is bound to DNA, the short-lived $^3MLCT_{SQ}$ state cannot be ruled out as the reactive species at this point.

The PCET reaction between $[(bpy)_2Ru(phendione)]^{2+}$* and tetrachlorohydroquinone has been reported by Meyer and co-workers in a preliminary report, but no details or mention of the role of the hydrated species was included.[28−31] Similarly, Ru complexes with peripheral basic sites such as $[Ru(phen)_2(bpz)]^{2+}$ (bpz = 2,2′-bipyrazine) are also known to react with hydroquinones via essentially the same PCET mechanism as shown in Scheme 2. This is the first report to delineate the role of ligand hydration in this chemistry and to apply it toward a biological substrate. It is now clear that the aqueous photochemistry of $\mathbf{1^{2+}}$ is dominated by formation of the hydrate $\mathbf{1H_2O}$, which has the effect of turning off the redox activity of the quinone function leading to an excited state manifold similar to $[Ru(phen)_3]^{2+}$*, except that this complex also possesses peripheral basic sites. These two features combine to lead to effective DNA cleavage via a PCET mechanism. We note that when O_2 is not removed from the solution, DNA photocleavage is enhanced, presumably through the formation of ROS in addition to the O_2 independent mechanisms.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, tables, and figures regarding spectroscopic properties and HPLC chromatograms of the DNA small molecule products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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