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cis-$[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ as a Photoactivated Cisplatin Analog

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Cisplatin, *cis*-$Pt(NH_3)_2Cl_2$, has been a successful antitumor agent against various cancers.[1−5] Although many related drugs have been developed, their toxicity towards healthy cells and acquired cellular resistance still require improvement.[1−5] The ligand exchange of the coordinated chloride ions for water molecules in cisplatin produces a diaqua Pt(II) species, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, that binds covalently to DNA and disrupts cellular transcription.[1−5] Limitations of ciplatin and related drugs are their activation through thermal ligand exchange, which can take place in both healthy and cancerous cells, and the cellular resistance developed towards these agents.

Selectivity toward tumor tissue can be achieved through the activation of drugs by light, such that a molecule that has no or low toxicity in the dark becomes highly toxic upon irradiation with low energy light. This mode of drug activation provides a means to localize the action of the drug to the irradiated area and is commonly known as photodynamic therapy (PDT). PDT has been used successfully in the treatment of lung and esophageal cancers,[6−8] however, a drawback of the PDT drugs currently in use is that their action is mediated by O_2. Since malignant cancers are often hypoxic,[6−8] new O_2-independent PDT agents must be sought. Recently, *cis*-$[Ru(bpy)_2(NH_3)_2]^{2+}$ was shown to covalently bind to DNA upon irradiation with near-UV light ($\lambda_{irr} \geq 345$ nm).[9] The present work focuses on the photoinitiated binding of *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_6]^{2+}$ (**1**) (structure shown in Figure 1a) to ds-DNA initiated with visible light ($\lambda_{irr} \geq 455$ nm).

The electronic absorption spectrum of **1** in CH_3CN exhibits peaks with maxima at 363 nm ($\epsilon = 420\ M^{-1}\ cm^{-1}$) and 525 nm ($\epsilon = 218\ M^{-1}\ cm^{-1}$). A TDDFT calculation on the model complex *cis*-$[Rh_2(\mu\text{-}O_2C_2H)_2(CH_3CN)_6]^{2+}$ (**1a**) in the gas phase predicts transitions at 387 and 501 nm, where the lowest energy transition is predicted to be metal-centered with $Rh_2(\pi^*) \rightarrow Rh_2(\sigma^*)$ parentage. The transition at higher energy arises from a combination of filled molecular orbitals (MOs) localized on the dirhodium core to orbitals with contributions both from the $Rh_2(\sigma^*)$ MO and from a set of two equatorial $RhL_{eq}(\sigma^*)$ MOs (L_{eq} = O atoms on acetate ligands and N atoms on equatorial CH_3CN ligands). These assignments are in agreement with those of related dirhodium(II,II) paddlewheel complexes, such as $Rh_2(\mu\text{-}O_2CCH_3)_4$.[10,11]

It was previously established that the axial CH_3CN ligands of **1** exchange readily with coordinating solvent molecules (Figure 1a), whereas the equatorial CH_3CN ligands are not labile at room temperature in the dark.[12] The lowest energy electronic transition of **1** shifts from 525 nm in CH_3CN to 555 nm ($\epsilon = 160\ M^{-1}\ cm^{-1}$) in H_2O.[13] This shift is attributed to the exchange of the axial CH_3CN ligands in **1** for solvent H_2O molecules to form *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_4(H_2O)_2]^{2+}$ (**2**), since it is well known that the energies of the $Rh_2(\sigma)$ and $Rh_2(\sigma^*)$ molecular orbitals (MOs) in dirhodium complexes are dependent on the nature of the axial ligand(s).[10,11,14] The red shift in the absorption in water is supported

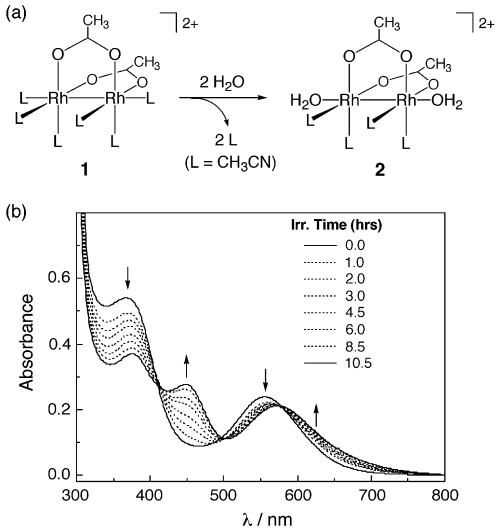

Figure 1. (a) Thermal axial ligand exchange of **1** to generate **2** in aqueous media and (b) photolysis of **2** in H_2O ($\lambda_{irr} \geq 345$ nm).

by gas-phase TDDFT calculations on the two model complexes, **1a** and *cis*-$[Rh_2(\mu\text{-}O_2C_2H)_2(CH_3CN)_4(H_2O)_2]^{2+}$ (**2a**), which predict $Rh_2(\pi^*) \rightarrow Rh_2(\sigma^*)$ transitions at 501 nm ($f = 0.0003$) and 556 nm ($f = 0.0004$), respectively (f = oscillator strength). DFT calculations also show a decrease in the HOMO−LUMO gap of 0.23 eV when the axial ligands are varied from CH_3CN in **1a** to H_2O in **2a**. The ^1H NMR spectrum of **1** in D_2O provides further evidence of the exchange of the two axial CH_3CN ligands in this solvent (Supporting Information).[13]

Following the initial axial ligand exchange of **1** in water to generate **2**, no further spectral changes were noted at room temperature after 5 days or after incubation at 60 °C for 6 h in the dark. Changes in the electronic absorption and ^1H NMR spectra of **2** were observed upon photolysis ($\lambda_{irr} \geq 455$ nm, 5 h) in H_2O and D_2O, respectively.[15] The ^1H NMR spectrum of **2** in D_2O shows the loss of two additional CH_3CN ligands from the complex upon irradiation ($\lambda_{irr} \geq 455$ nm, 1−5 h, Supporting Information), pointing at the dissociation of two equatorial CH_3CN ligands. The changes in the electronic absorption spectrum of **2** observed upon irradiation in H_2O are shown in Figure 1b. The peak at 373 nm decreases in intensity with the concomitant appearance of a band at 450 nm. In addition, the peak at 555 nm shifts to 573 nm ($\epsilon = 136\ M^{-1}\ cm^{-1}$) and decreases in intensity. The exchange of two equatorial CH_3CN ligands for H_2O molecules in **2** can give rise to three different isomers of *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(CH_3CN)_2(H_2O)_4]^{2+}$ (**3a**−**3c**), complexes with two axial and two equatorial H_2O ligands. Although it is currently unknown if one, two, or all three isomers are generated upon photolysis, TDDFT calculations predict the lowest energy transition of all three species to be red-shifted relative to that in **2** (Supporting Information).

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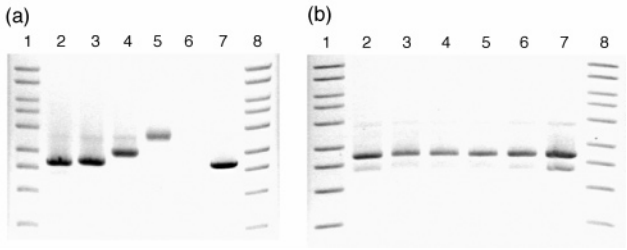

Figure 2. Imaged ethidium bromide stained agarose gel of 50 μM linearized pUC18 plasmid (10 mM phosphate, pH = 7.5) in the presence of various concentrations of **2** (a) irradiated (λ > 455 nm) and (b) incubated in the dark at 25 °C for 20 min. Lanes 1 and 8: DNA molecular weight standard (1kb, Sigma). Lanes 2 and 7: linearized plasmid alone. Lanes 3−6: [DNA bp]/[Complex] = 100, 20, 10, 5.

The power dependence of the photolysis of **2** ($\lambda_{irr} \geq 455$ nm and $\lambda_{irr} \geq 345$ nm, 1 h) followed by UV−vis and ^{1}H NMR in H_2O and D_2O, respectively, is consistent with the formation of the product, **3**, via a one-photon process.[16] These results point at the initial photoaquation of one equatorial CH_3CN ligand, followed by the loss of a second equatorial CH_3CN ligand in a dark reaction. The quantum yield for the formation of **3** is dependent on the irradiation wavelength, with $\Phi_{355} = 0.37$ and $\Phi_{509} = 0.09$ at 355 and 509 nm, respectively.[17] Such dependence of the photolysis quantum yield on wavelength has been previously shown for transition metal complexes to be associated with the photochemistry taking place from excited states that lie above the ground state.[18] As discussed above, the excited state of **1** that absorbs at 363 nm has been calculated to possess contributions from $RhL_{eq}(\sigma^*)$ molecular orbitals. The set of $RhL_{eq}(\sigma^*)$ orbitals are composed of the in-phase and out-of-phase combinations of the $d_{x^2-y^2}$ orbitals of the Rh atoms, each with σ^* character to the equatorial ligands, L_{eq}, including both the bridging acetate and acetonitrile ligands. Placing electron density on the MO with σ^* character of the RhL_{eq} orbitals in the excited state can result in the dissociation of equatorial ligands upon irradiation with light.

Irradiation of **2** in water ($\lambda_{irr} \geq 455$ nm) in the presence of bpy (bpy = 2,2'-bipyridine) or 9-Et-G (9-Et-G = 9-ethyl guanine) results in the coordination of bpy and 9-Et-G to the dirhodium core (Supporting Information). Similar results were reported for related dirhodium complexes in the presence of bpy in solution upon refluxing over several days,[19] as well as their thermally activated coordination to nucleobases and oligonucleotides.[20] Although coordination of the dirhodium complex to ligands and nucleobases in solution is promising, in order for **2** to act as a photo-cisplatin analog, the complex must be able to bind to double-stranded DNA (ds-DNA) upon irradiation.

Photolysis of **2** in the presence of linearized pUC18 plasmid results in decreased mobility of the ds-DNA on an agarose gel (Figure 2a).[21] In contrast, no change in the mobility was observed for samples of **2** incubated with plasmid in the dark at 25 °C for 20 min (Figure 2b) or at 37 °C for 4 h (Supporting Information). Such decrease in plasmid mobility on agarose gels has been previously shown to occur upon the covalent binding of cisplatin to ds-DNA owing to the kinking of the DNA induced by the drug.[9] This effect is also shown in the Supporting Information.

A requirement of a potential PDT agent is low cytotoxicity in the dark and increased toxicity upon irradiation. The cytotoxicity of **2** towards Hs-27 human skin cells increases from an LC_{50} of 410 ± 9 to 12 ± 2 μM when irradiated with visible light (400−700 nm, 30 min).[22] This 34-fold increase in toxicity is significantly greater than the 5-fold increase measured for hematoporphyrin, a key component in the commercially available PDT agent Photofrin, under similar experimental conditions. In addition, the toxicity of **2** towards Hs-27 cells is lower than that of hematoporphyrin (LC_{50} = 21 ± 1 μM) by a factor of ∼20 in the dark. Cationic dirhodium complexes with bidentate chelating ligands occupying the four equatorial positions taken up by CH_3CN in **2**, such as *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2(H_2O)_2]^{2+}$, do not exhibit an increase in cytotoxicity towards Hs-27 cells upon irradiation. These properties make **2** a promising photo-cisplatin analog and a potential PDT agent. To our knowledge, **2** is the first metal−metal bonded complex to bind to DNA upon irradiation with visible light.

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Supporting Information Available: Gel mobility assays, DFT and TDDFT calculations, photolysis in D_2O followed by NMR, and photolysis in the presence of 9-Et-G and bpy. This material is available free of charge via the Internet at http://pubs.acs.org.

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