

Photoinitiated DNA Binding by *cis*-[Ru(bpy)₂(NH₃)₂]²⁺

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Received July 13, 2004

The ligand-loss photochemistry of *cis*-[Ru(bpy)₂(NH₃)₂]²⁺ (bpy = 2,2'-bipyridine) was investigated in water and in the presence of added ligands such as bipyridine and chloride. Irradiation of the complex results in the covalent binding to 9-methyl- and 9-ethyl-guanine, as well as to single-stranded and double-stranded DNA. This photoinduced DNA binding is not observed for the control complex [Ru(bpy)₂(en)]²⁺ (en = ethylenediamine) under similar irradiation conditions. The results presented here show that octahedral Ru(II) complexes with photolabile ligands may prove useful as photoactivated cisplatin analogs.

Cisplatin, *cis*-Pt(NH₃)₂Cl₂, and related Pt(II) complexes are anticancer agents in the treatment of various cancers, however, their toxicity toward healthy cells and acquired resistance remain serious problems that still must be overcome.^{1–4} The action of cisplatin relies on the formation of *cis*-[Pt(NH₃)₂(OH)₂]²⁺ through sequential thermal ligand exchange, followed by the binding of this species to GpG DNA sequences, thus resulting in intrastrand DNA cross-links which disrupt cellular transcription.^{1–5} The activation of a molecule with low energy light provides a means to localize the action of a given drug to the irradiated area, a field generally known as photodynamic therapy (PDT).^{6,7} PDT is successful in the treatment of lung and esophageal cancers, however, O₂ is necessary for the typical PDT drugs to function.^{6–8} This oxygen requirement represents a drawback, since many malignant cancer cells are hypoxic.^{6–8} The present work combines the mode of action of cisplatin with PDT utilizing the photoaquation of *cis*-[Ru(bpy)₂(NH₃)₂]²⁺ (**1**) (bpy = 2,2'-bipyridine) and its subsequent binding to oligonucleotides. The present work describes a means to

photoactivate the covalent binding of a transition metal complex, a cisplatin analog, to DNA.

Owing to the numerous Ru(II) amines that are known to undergo ligand-loss photochemistry and the *cis*-disposition of the complex,^{9,10} **1** was chosen for our initial studies on photoinitiated DNA binding. Removal of the chlorides from *cis*-Ru(bpy)₂Cl₂ with AgBF₄ followed by the reaction with NH₃ in methanol resulted in the formation of **1**.¹¹ For comparison, the related ethylenediamine (en) complex [Ru(bpy)₂(en)]²⁺ (**2**) was synthesized following a reported procedure.¹²

The electronic absorption spectrum of **1** in water exhibits bpy ππ* transitions at 290 nm (ε = 55 500 M⁻¹ cm⁻¹) and 243 nm (ε = 20 600 M⁻¹ cm⁻¹), which are observed at 243 nm (ε = 19 600 M⁻¹ cm⁻¹) and 291 nm (ε = 57 500 M⁻¹ cm⁻¹) in **2**. In aqueous solutions of **1**, the Ru–bpy MLCT transitions are observed at 345 nm (ε = 7340 M⁻¹ cm⁻¹) and 490 nm (ε = 8210 M⁻¹ cm⁻¹), which shift to 344 nm (ε = 7580 M⁻¹ cm⁻¹) and 485 nm (ε = 9750 M⁻¹ cm⁻¹) in **2**. The position, intensities, and assignments of the absorption maxima in **1** and **2** are consistent with those previously reported for these and related systems.^{13,14} Complexes **1** and **2** were shown to exhibit weak MLCT emission at 741 nm (Φ = 0.002, τ = 52 ns) and 715 nm (Φ = 0.003, τ = 173 ns), respectively, in EtOH/MeOH glasses at 157 K.¹⁵

The photolysis of **1** in water under an argon atmosphere results in the sequential loss of the NH₃ ligands and the formation of the corresponding bis-aqua complex in acid

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- (12) Complex **2** was prepared from *cis*-Ru(bpy)₂Cl₂ and en by a reported procedure (ref 13). The reaction mixture was dried, and the red product was precipitated from acetone/ether. ¹H NMR in DMSO-*d*₆, δ (int, mult): 9.3 (2H, d), 8.8 (2H, d), 8.6 (2H, d), 8.3 (2H, t), 7.9 (4H, t), 7.6 (2H, d), 7.3 (2H, t), 4.8 (2H, t). ES-MS: *m/z* = 235.0 for [Ru(bpy)₂(en)]²⁺.
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solution and *cis*-[Ru(bpy)₂(OH)₂(OH)]⁺ (**3**) in water ($\Phi = 0.024(2)$ with $\lambda_{\text{irr}} = 350$ nm and $\Phi = 0.018(2)$ with $\lambda_{\text{irr}} = 400$ nm),¹⁶ with the characteristic absorption at 490 nm ($\epsilon = 9300$ M⁻¹ cm⁻¹).¹⁷ Similar results are observed in air. No chemical changes are observed for aqueous solutions of **1** upon irradiation with $\lambda_{\text{irr}} > 450$ nm or when kept in the dark.¹⁶ The wavelength dependence and quantum yields of NH₃ photoaquation are similar to those reported for numerous related complexes with low energy MLCT transitions,^{9,10} including *cis*-[Ru(NH₃)₄(isn)₂]²⁺ (isn = isonicotinamide), with $\Phi = 0.029(1)$ ($\lambda_{\text{irr}} = 365$ nm), $\Phi = 0.010(1)$ ($\lambda_{\text{irr}} = 436$ nm), and $\Phi = 0.00045(3)$ ($\lambda_{\text{irr}} = 480$ nm).¹⁸ The irradiation time dependence for the formation of **3** from **1** is biphasic, which indicates that the reaction proceeds through an intermediate, possibly *cis*-[Ru(bpy)₂(NH₃)(OH)]⁺, formed following the initial loss of a single NH₃ ligand. The formation of the bis-aqua product from the intermediate requires irradiation, consistent with one photon necessary to remove each NH₃ ligand.¹⁹ *Cis*-*trans* isomerization of the bis-aqua complex can be ruled out, since no changes are observed in the dark following photolysis for a period of 24 h.¹⁷

The changes in the electronic absorption spectrum of 25 μM **1** upon irradiation ($\lambda_{\text{irr}} > 345$ nm) in CH₂Cl₂ in the presence of 50 μM (*t*-Bu)₄NCl (TBACl) are consistent with the formation of *cis*-Ru(bpy)₂Cl₂ with its characteristic maximum at 553 nm ($\epsilon = 8910$ M⁻¹ cm⁻¹) in CH₂Cl₂.¹⁴ The photolysis of **2** with $\lambda_{\text{irr}} > 345$ nm in water or in CH₂Cl₂ in the presence of TBACl does not result in ligand exchange under these experimental conditions, likely due to the bidentate nature of the en ligand. Similar photoreactivity is observed for **1** with $\lambda_{\text{irr}} > 375$ nm, but not in the dark. An intermediate is observed in the photochemistry of **1** with TBACl in CH₂Cl₂ described above with maximum at 540 nm, which may be attributed to the monosubstituted product, *cis*-[Ru(bpy)₂(NH₃)Cl]⁺, at ~ 5 min irradiation. Interestingly, this intermediate is not observed with lower energy irradiation ($\lambda_{\text{irr}} > 375$ nm), where after 2 min only the appearance of the final product, *cis*-Ru(bpy)₂Cl₂, is evident. A possible explanation for these observations is that high energy excitation of *cis*-[Ru(bpy)₂(NH₃)Cl]⁺ results in preferential and direct chloride dissociation, whereas lower energy irradiation results in loss of NH₃ (or both Cl⁻ and NH₃). The ligand loss in Ru(II) ammine complexes is known to arise from a ligand field (LF) state with (t_{2g})⁵(e_g)¹ electron configuration.^{9,10} The lack of photoaquation with $\lambda_{\text{irr}} > 450$ nm is consistent with a LF state that is thermally inaccessible from the low-lying MLCT state(s) in **1**.

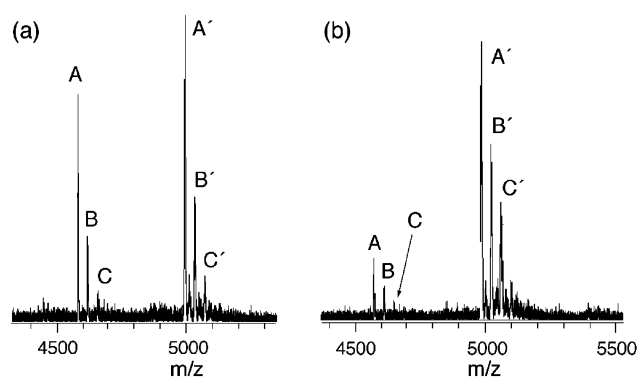


Figure 1. Electro-spray mass spectra of solutions of 10 μM **1** photolyzed with $\lambda_{\text{irr}} > 345$ nm (10 min) in the presence of 10 μM ss-15-mer (a) strand 1 and (b) strand 2 in 5 mM Tris (pH = 7.5, 50 mM NaCl).

In the presence of 1 equiv of free bpy ligand in solution, photolysis of **1** ($\lambda_{\text{irr}} > 375$ nm) results in the formation of [Ru(bpy)₃]²⁺, characterized by the growth in absorption at 452 nm and emission at 620 nm.¹⁴ The formation of [Ru(bpy)₃]²⁺ following photoaquation clearly shows the ability of the labile water ligands in *cis*-[Ru(bpy)₂(OH)₂]²⁺ to be replaced by a strong ligand, such as bipyridine. The photolysis of 5 mM **1** ($\lambda_{\text{irr}} > 345$ nm, 18 h) in the presence of 2.5 equiv of 9-methylguanine (9-MeG) or 9-ethylguanine (9-EtG) in water results in the formation of *cis*-[Ru(bpy)₂(9-MeG)]²⁺ and *cis*-[Ru(bpy)₂(9-EtG)]²⁺, for which the parent ion peaks were detected at $m/z = 578.2$ and $m/z = 592.2$, respectively, using electro-spray mass spectrometry (ESMS). In addition, the photochemistry was followed by ¹H NMR, and it parallels the thermal reactions previously reported for *cis*-[Ru(bpy)₂(H₂O)₂]²⁺ with 9-MeG and 9-EtG.^{20–22}

The products of the photolysis of 10 μM **1** in the presence of 10 μM bases (0.67 μM strands) single-stranded 15-mer oligonucleotides, 5'-AGTGCCAAGCTTGCA-3' (strand 1) and 5'-TGCAAGCTTGGCACT-3' (strand 2), with $\lambda_{\text{irr}} > 345$ nm (10 min, 5 mM Tris, pH = 7.5, 50 mM NaCl) were monitored by ESMS following repeated washings with 1 M TEAA, 50% CH₃CN and H₂O. The peaks labeled A in Figure 1, observed at m/z values of 4578.8 and 4570.3, correspond well to the calculated masses of strand 1 ($m/z = 4575$) and strand 2 ($m/z = 4566$), respectively. The relative intensity pattern and position of the peaks labeled A, B, and C in Figure 1 are also observed in the ESMS collected for each strand alone, and peaks B and C correspond to addition of one and two CH₃CN molecules, respectively. The peaks labeled A', B', and C' in the spectra in Figure 1 show the covalent binding of the *cis*-Ru(bpy)₂ fragment to each single strand peak, A, B, and C, respectively, upon photolysis. In contrast, when the solutions containing **1** and either strand 1 or strand 2 are kept in the dark for several hours and subjected to purification, which removes complex that is not covalently bound, no evidence of the Ru(II) complex or

(16) Irradiation with 150 W Xe arc lamp (PTI). The wavelength was controlled either with high-pass colored glass filters or using 350 nm (10LF10, Newport) and 400 nm (57510, Oriel) band-pass filters for the quantum yield experiments. Quantum yields were measured relative to ferrioxalate (Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993).

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Ru(bpy)₂ fragment bound to the oligonucleotide is detected in the ESMS. Furthermore, no binding of the Ru(II) complex to either 15-mer strand is observed when 10 μM of **2** is photolyzed with 10 μM bases of strand 1 and strand 2 with λ_{irr} > 345 nm (10 min, 5 mM Tris, pH = 7.5, 50 mM NaCl) or when kept in the dark. These results are consistent with the lack of photoreactivity of **2** in water.

It should be noted that **1** is in 15-fold excess in the photolysis experiments, however, only one covalently bound Ru(bpy)₂ unit is detected per strand. In addition, greater binding of Ru(bpy)₂ following the photolysis of **1** is observed in strand 2 (82% bound) compared to strand 1 (58% bound). Strand 2 possesses a GG step, which is known to be the preferential binding site for cisplatin.^{22–25} Cisplatin has been shown to covalently bind to adjacent purines in ds-DNA, primarily effecting intrastrand cross-links at GpG (65%) and ApG (25%) sites.^{24,25} However, at the present time the position of the binding of the Ru(bpy)₂ unit on either strand remains unknown.

Annealing of the two complementary strands, strands 1 and 2, results in the formation of a double-stranded duplex (ds-15-mer),²⁶ with which photoinduced binding experiments were conducted. A shift in the melting temperature of the ds-15-mer (50 μM) to lower temperature (Δ*T* = –5 °C) is observed following its photolysis with 50 μM *cis*-[Ru(bpy)₂-(NH₃)₂]²⁺, and no shift is measured when solutions containing **1** and ds-15-mer are kept in the dark or when solutions of **2** are photolyzed with the ds-15-mer under similar experimental conditions. The shift measured for the ds-15-mer upon photolysis with **1** is consistent with intrastrand covalent binding of the complex to the duplex, since a Δ*T* = –8 °C was previously reported for a 20-mer duplex upon covalent binding of cisplatin.²⁷ In contrast, formation of interstrand cross-links by mono- and dinuclear Ru(II) complexes has been shown to result in positive shifts of the melting temperature, and complexes with a single labile ligand have little effect on the thermal denaturation of calf-thymus DNA.²⁸

Additional evidence for the photoinduced binding of **1** to ds-DNA is shown in Figure 2 for linearized pUC18.²⁸ It is well known that the covalent binding of cisplatin to ds-DNA results in reduced mobility of linearized plasmid on agarose gels. This reduction in mobility as a function of increasing cisplatin concentration is shown in Figure 2a. Photolysis of

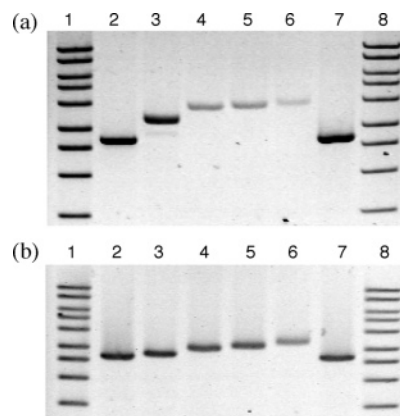


Figure 2. Ethidium bromide stained agarose gels of 50 μM linearized pUC18 plasmid (10 mM phosphate, pH = 7.5) in the presence of various ratios of (a) cisplatin incubated for 4 h at 37 °C and (b) **1** irradiated (λ_{irr} > 345 nm) for 15 min at 25 °C. Lanes 1 and 8: DNA molecular weight standard (1 kb, Sigma). Lanes 2 and 7: linearized plasmid alone, Lanes 3–6: [DNA bp]/[Complex] = 100, 20, 10, 5.

1 in the presence of linearized plasmid also results in decreased mobility of the DNA (Figure 2b). In contrast, no change in mobility is observed for samples exposed to similar concentrations of **1** in the dark. These mobility assays are consistent with the photoinduced binding of **1** to ds-DNA. It should be noted that photocleavage of supercoiled pUC18 plasmid by **1** is not observed under these photolysis conditions.

Previous work has shown that *cis*-[Ru(bpy)₂(H₂O)₂]²⁺ and Ru(phen)₂Cl₂ (phen = 1,10-phenanthroline) covalently bind to DNA,^{20,29–31} however, this binding, like that of cisplatin, is thermally activated and difficult to control. Although photoactivated DNA binding and cytotoxicity by octahedral Rh(III) complexes has been previously reported, it requires the use of UV light.³² Similarly, the photoinduced ligand exchange of square planar Pt(en)Cl₂ is only accessible with λ_{irr} = 310 nm.³³ The results presented here show that octahedral Ru(II) complexes are able to covalently bind to ss-DNA and ds-DNA following photoinduced ligand loss. Although the photoactivated DNA binding by complex **1** requires near-UV light, this work shows that new Ru(II) complexes can be designed to access the ligand-loss photochemistry with visible light. Such complexes may prove useful as photoactivated cisplatin analogs.

Acknowledgment. C.T. thanks the National Institutes of Health (RO1 GM64040-01) for their generous support. The authors thank Dr. Kari Green-Church for performing the ESMS experiments with the single-stranded oligonucleotides.

IC049075K

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