Metal to Ligand Charge Transfer Induced DNA Photobinding in a Ru(II)-Pt(II) Supramolecule using Red Light in the Therapeutic Window: A New Mechanism for DNA Modification

Supporting Information

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Materials

The materials were used as received unless otherwise noted. The starting materials, $RuCl_3 \bullet 3H_2O$ and 4,7-diphenyl-1,10-phenanthroline were purchased from Alfa Aesar. The circular pUC18 plasmid DNA was purchased from Bayou Biolabs. The bridging ligand, 2,3-bis(2-pyridyl)pyrazine, and KPF_6 were obtained from Aldrich. Spectral grade acetonitrile was obtained from Burdick and Jackson. Tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) supporting electrolyte was purchased from Fluka. Sephadex LH-20 was purchased from GE Healthcare Biosciences Corporation. The calf thymus DNA (CT-DNA) was purchased from Rockland, Inc. and was dissolved in 0.1 M Phosphate buffer. The molecular weight marker, Lambda DNA/*Hin*dIII, was purchased from New England BioLabs. Electrophoresis grade agarose, molecular biology grade boric acid, molecular biology tris base, and molecular biology glycerol were purchased from Fisher Scientific. The Tris-Boric Acid (TB) buffer solution was prepared with 45 mM Tris base and 45 mM boric acid (pH = 7.4). The 0.1 M Phosphate Buffer (pH = 7.4)

was made using sodium phosphate monobasic dihydrate and sodium phosphate dibasic heptahydrate from Acros.

Preparation and analysis of $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$

The bimetallic complex, $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$ was prepared by reacting the monometallic precursor with a labile Pt(II) reagent.[1] The monometallic precursor, $[(Ph_2phen)_2Ru(dpp)]Cl_2$ and the labile Pt agent were synthesized using a modified methods that were previously reported.[2] $[(Ph_2phen)_2Ru(dpp)]Cl_2$ (0.50 g, 0.47 mmol) and $[PtCl_2(DMSO)_2]$ (1.0 g, 2.4 mmol) were heated at reflux in 25 mL of ethanol for ca. 2 hours in the dark. The reaction mixture was cooled to RT and the solid was collected by vacuum filtration. The solid was dissolved in a minimal amount of 2:1 ethanol:acetonitrile and was purified by LH-20 size exclusion chromatography using 2:1 ethanol:acetonitrile eluent. The first orange-red band was collected; solvent was removed under vacuum, and the complex was flash precipitated from dry acetone in diethyl ether, yield 75%. For the DNA studies, the $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$ product was used. However, for analysis the Cl^- salt was metathesized to the PF_6^- salt by dissolving $[(Ph_2phen)_2Ru(dpp)PtCl_2]Cl_2$ in a minimal amount of water and adding dropwise to a saturated aqueous solution of KPF_6. The solid was filtered, dried, washed with diethyl ether and flash precipitated from acetonitrile in diethyl ether. The ESI-MS of $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$ was consistent with the formulation, *m/z*: 1411 $[M\text{-}PF_6]^+$. The $^{195}Pt\{^1H\}$ NMR spectroscopy showed a single resonance, δ = -2170 ppm and no labile starting material. Electrochemisty, electronic absorption spectroscopy, emission spectroscopy, and excited state lifetime measurements were completed in spectral grade acetonitrile and are reported in Tables S1 and S2.

Table S1. Electrochemical data for $[(Ph_2phen)_2Ru(dpp)](PF_6)_2$ and $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$.

Complex[a]	$E_{½}^{ox}$ (V)[b]	$E_{½}^{red}$ (V)[b]
$[(Ph_2phen)_2Ru(dpp)](PF_6)_2$ [c]	+1.40 $Ru^{II/III}$	-1.02 $dpp^{0/-}$
		-1.37 $Ph_2phen^{0/-}$
		-1.56 $Ph_2phen^{0/-}$
$[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$	+1.57 $Ru^{II/III}$	-0.50 $dpp^{0/-}$
	+1.47 $Pt^{II/IV}$ [d]	-1.06 $dpp^{-/2-}$
		-1.37 $Ph_2phen^{0/-}$
		-1.56 $Ph_2phen^{0/-}$

[a]Ph_2phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine, [b]Recorded in 0.1 M Bu_4NPF_6 CH_3CN, with potential reported in V vs SCE, using a Ag wire pseudo reference electrode and ferrocene internal standard ($FeCp_2/FeCp_2^+$ = 0.43 V vs SCE)[3], [c]Redox potentials consistent with those in ref 2 (Mongelli), [d]E_p^a

Table S2. Spectroscopic and photophysical data for $[(Ph_2phen)_2Ru(dpp)](PF_6)_2$ and $[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$.

Complex[a]	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm) RT	Φ^{em} RT Ar[b]	τ(ns) RT Ar[c]	$\lambda_{max\ em}$ (nm) *77 K*[f]	τ (μs) 77 K
$[(Ph_2phen)_2Ru(dpp)](PF_6)_2$	474	664	$3.5x10^{-2}$	820	*607*	5.4
$[(Ph_2phen)_2Ru(dpp)PtCl_2](PF_6)_2$	517	740	$4.1x10^{-4}$	44	*694*	2.3

[a]Ph_2phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine, [b]Deoxygenated with Ar, CH_3CN solution, referenced to $[Os(bpy)_3]^{2+}$, Φ^{em} = 4.6 x 10^{-3}, and [c]Deoxygenated with Ar, CH_3CN solution.

Photobinding to pUC18 plasmid DNA and gel shift assay

Gel electrophoresis was used to assay photobinding of circular plasmid pUC18 DNA by $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$. The metal complex/DNA solution was prepared with ddH_2O (double deionized water) and 0.1 M NaH_2PO_4, forming a 5:1 BP:MC ratio (base pair:metal complex). A LED array, consisting of eight temperature-controlled cells using 5 W Luxeon Royal Blue Star LEDs (λ_{max} = 455 nm, total average flux = 2.0 x 10^{19} photons/min) or a Xe arc lamp, fitted with 455 and 590 nm cutoff filters ($\lambda_{max} \geq 590$ nm, total average flux = 6.0 x 10^{19} photons/min) and a temperature-controlled cell holder, were used for photolysis, profiles shown in supplementary figure 1A. The 3 mL samples were photolyzed for 0, 2.5, 5, 10, 20, 30, 45, and 60 minutes under air saturated conditions. The control samples were incubated at RT and 37 °C in the dark. To assay DNA photobinding, 10 µL of the sample solutions were mixed with 2 µL of glycerine based loading dye and loaded into the wells of a 30 mL gel prepared with 0.8% w/w agarose and 20% w/w TB buffer. Electrophoresis was performed using Owl Separation Systems, Inc., Model B1A electrophoresis state set at 105 V for 1.5 h. The gel was stained for 30 min in 0.5 µg/mL ethidium bromide (EtBr), destained for 15 min in ddH2O, and visualized on a Fisher Scientific FBTI-88 transilluminator. Photographs were taken using an Olympus E-320 digital camera equipped with a Peca Products Inc. EtBr filter. Control samples are shown in supplementary figures 2A and B.

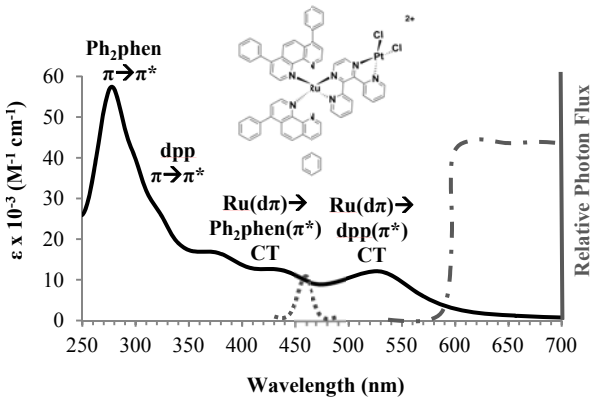

Figure S1: Structure of $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$, electronic absorption spectrum in H_2O at RT (black line), and the relative photon flux for the LED array (gray dotted line) and Xe arc lamp (gray dash-dot line).

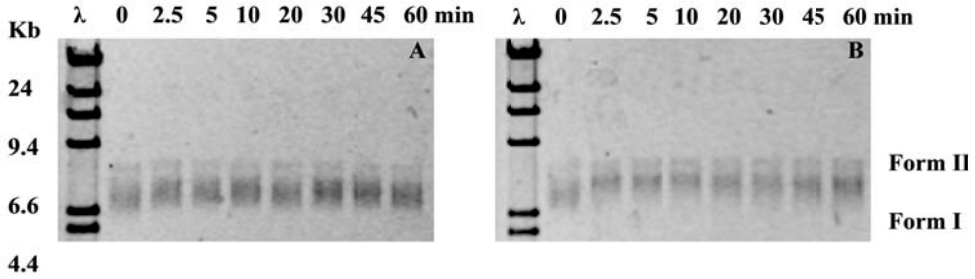

Figure S2: Gel electrophoresis shift assay showing the binding of $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$ to pUC18 plasmid DNA at RT (A) or 37 °C (B) in the dark. The lanes correspond to: λ) lambda molecular weight marker, C) pUC18 DNA plasmid control, and 0, 2.5, 5, 10, 20, 30, 45, and 60) 5:1 BP:MC solutions photolyzed for 20, 2.5, 5, 10, 20, 30, 45, and 60 minutes, respectively.

A second set of studies was completed to probe the photoinduced activity of the $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$ complex if photolyzed first in the absence of DNA and then added to a DNA buffer solution. The complex was photolyzed (λ_{irr} = 455 nm) in the presence and absence of DNA for 10 minutes, keeping the final phosphate buffer concentration and metal complex ratio the same before photolysis and adjusting the samples to the same 5:1 BP:MC ratio and final phosphate buffer concentrations. The first sample was photolyzed under normal conditions with a 0.02 M final phosphate buffer

concentration, and pUC18 plasmid at a 5:1 BP:MC ratio. The second sample was photolyzed under similar conditions without pUC18. The final sample was the Ru(II)-Pt(II) complex dissolved in 0.1 M phosphate buffer and photolyzed. Following photolysis, the samples were adjusted so the final phosphate buffer concentration was 0.02 M and the ratio of BP:MC was 5:1. The samples were assayed using gel shift assay and the gel was visualized as reported above, figure S3. The results indicate that the Ru(II)-Pt(II) bimetallic complex when photolyzed in the presence of DNA photobinds efficiently. Some enhancement in DNA binding is seen in lanes 2 and 3 indicative of formation of an aqua intermediate, which may facilitate DNA binding. The enhanced photobinding in the presence of DNA may suggest association of the complex with DNA provides for enhanced photosubstitution. This may occur by the proximity of the DNA bases or through lengthening of the excited state lifetime by solvation changes in the presence of the DNA.

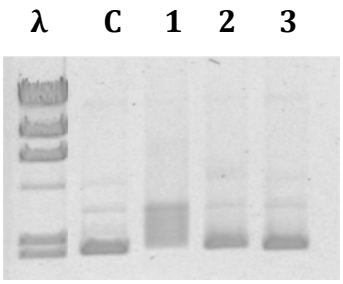

Figure S3: Gel electrophoresis shift assay showing the photoinduced binding activity by $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$ to pUC18 plasmid DNA. The lanes correspond to: λ) lambda molecular weight marker, C) pUC18 DNA plasmid control, 1) metal complex photolyzed in the presence of pUC18 plasmid, 2) metal complex photolyzed in 0.02 M phosphate buffer with pUC18 plasmid added following photolysis, and 3) metal complex photolyzed in 0.1 M phosphate buffer with pUC18 plasmid added following photolysis.

Photobinding to CT-DNA and selective precipitation assay

Calf thymus DNA (CT-DNA) that was exposed to $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$ was selectively precipitated from solution using an adapted version of a previously reported method.[4] The solid CT-DNA was hydrated in ddH_2O. The DNA container was placed on ice and the DNA solution was sonicated for three 30 s pulses. The concentration of the sonicated DNA was measured using the base pair molar absorptivity value $\varepsilon_{260} = 13{,}200$ $M^{-1}cm^{-1}$. The $[(Ph_2phen)_2Ru(dpp)PtCl_2]^{2+}$ metal complex was dissolved in water. A stock metal complex-DNA solution was prepared with ddH_2O and 0.1 M NaH_2PO_4, forming a 5:1 BP/MC ratio forming a total volume of 18 mL. The stock solution was split into six 3 mL quartz cells and was photolyzed using a LED array or Xe arc lamp. Both the dark controls (RT and 37 °C) and photolyzed samples (455 nm or ≥ 590 nm) were deoxygenated with Ar for 20 min prior to exposure to the experimental conditions through a septum. At each time point, 0, 2.5, 5, 10, 20, 30, 45, and 60 min, a 500 μL aliquot was removed. The DNA was precipitated using 20 μL 5 M NaCl and 2000 μL of 95% ethanol. The sample was centrifuged for 3 min at 13000 rpm.

The amount of metal complex that was unbound to DNA remained in the supernatant and the absorption at 525 nm was measured by an Agilent 8453 A spectrophotometer. A blank sample was prepared using the same components without metal complex. The supernatants of each of the samples were measured and the absorptivity at 525 nm was recorded. These experiments were repeated in triplicate. The change in absorptivity (A_t/A_0) vs. time was graphed.

Photobinding to CT-DNA and DNA melting point assay

Similar to the above preparation, stock metal complex-DNA solution was prepared with ddH_2O and 0.1 M NaH_2PO_4, forming a 5:1 BP/MC ratio and a total volume of 6 mL. The stock solution was split into two 3 mL cuvettes. The metal complex-DNA sample and DNA control sample were photolyzed using the reported LED array and conditions from the selective precipitation experiment. Separately a metal complex-DNA solution was purged with Ar and placed in the dark at 37 °C for 60 min. The melting point was studied using the change in A_{260} vs. temperature using a JASCO-815 CD instrument with a thermally controlled cell compartment. The temperature was equilibrated for 20 s prior to taking the absorption reading at 260 nm and readings were taken every 4 °C from 60-100 °C. The melting point was determined from the A_{260} vs. temperature graph. Reported melting point temperatures were similar to those previously reported.[5]

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