PLATINUM, RHODIUM, AND RUTHENIUM COMPLEXES AS POTENTIAL PDT AGENTS

DISSERTATION

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By

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* * * * *

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Photodynamic Therapy (PDT) is a method to selectively kill cancer cells through the absorption of visible light by a photosensitizer. Currently used PDT agents are oxygen-dependent, however, there is a need to develop new drugs that are oxygen-independent. Metal complexes represent a new promising class of PDT agents that can work well in the presence and absence of oxygen.

The complex Pt(dppz)Cl₂ (dppz = dipyrido[3,2-a:2’,3’-c]phenazine) was synthesized and characterized, and its DNA binding and photocleavage properties are reported and compared to those of cisplatin, Pt(NH₃)₂Cl₂, and the related complex Pt(bpy)Cl₂ (bpy = 2,2’-bipyridine). It was shown that Pt(dppz)Cl₂ intercalates between the DNA bases, while Pt(NH₃)₂Cl₂ and Pt(bpy)Cl₂ bind covalently to the duplex. Upon irradiation (λ_{irr} > 395 nm, 20 min) Pt(dppz)Cl₂ is able to photocleave plasmid DNA both in air and in the absence of oxygen, resulting in the nicked form. In contrast, cisplatin and Pt(bpy)Cl₂ are photochemically inactive towards DNA under these irradiation conditions.

[Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂]²⁺ (R₁ = R₂ = NO₂, Cl, H, CH₃, OCH₃, 15-crown-5, 18-crown-6 or R₁ = H, R₂ = Cl, CH₃, OCH₃) complexes were also investigated for their photoreactivity toward DNA. The complexes do not intercalate into the DNA duplex, but they cleave DNA upon irradiation with λ > 500 nm. The percent photocleavage decreases
with the increase in the reduction potential of the \( R_1, R_2 \)-dppz ligand (as the ligand easier to reduce). A linear relationship between \( 1/|\text{DNA}_{\text{nicked}}| \) and the free energy \( \Delta G^0 \) for charge recombination was established. It was also shown that free axial positions are required for dirhodium complexes to be photoactive.

Many ruthenium complexes exhibit DNA light switch behavior, where they are non-emissive in water and become luminescent upon intercalation into the DNA duplex. These complexes may act as probes of DNA structures or sensors. The emission intensity of \( [\text{Ru(bpy)}_2\text{(tpphz)}]^2^+ \) (tpphz = tetrapyrido[3,2-a:2’,3’-c:3’’, 2’’-h:2’’, 3’’-j] phenazine) and \( [\text{Ru(bpy)}_2\text{(taptp)}]^2^+ \) (taptp = 4,5,9,18-tetraazaphenanthreno[9,10-b] triphenylene) increases by factors 50- and 4-fold upon the addition of DNA, respectively. The emission from intercalated \( [\text{Ru(bpy)}_2\text{(tpphz)}]^2^+ \) is quenched statically in the presence of Co\(^{2+}\) and Zn\(^{2+}\) ions, and the emission can be fully restored by the addition of EDTA. The emission of \( [\text{Ru(bpy)}_2\text{(tpphz)}]^2^+ \) can be reversibly turned “on” and “off” over the several cycles. Owing to the absence of additional coordination sites, the emission of DNA-intercalated \( [\text{Ru(bpy)}_2\text{(taptp)}]^2^+ \) is not quenched by transition metal ions in solution.
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CHAPTER 1

INTRODUCTION

1.1. Chemotherapy

Cancer is a disease caused by the uncontrolled division of abnormal cells. Currently the three classical approaches that are typically used to fight cancer are surgery, chemotherapy, and \( \gamma \)-radiation, and in some cases, a combination of these treatments. Modern chemotherapy drugs possess various different modes of action. For example, bleomycin inhibits the function of the enzyme DNA polymerase by causing single-stranded DNA breaks.\(^1\) Other drugs, such as the Vinca alkaloids, bind to tubulin and inhibit the metaphases of mitosis,\(^2\) and adriamycin induces free radical generation and inhibits DNA synthesis.\(^3\) Cisplatin, \( \text{cis-Pt(NH}_3\text{)}_2\text{Cl}_2 \), binds covalently to DNA producing intra-strand cross-links,\(^4,5\) which results in the inhibition of DNA replication and transcription by RNA polymerase II, thus inducing apoptosis.\(^6\) Chemotherapy drugs taken orally, by injection, or intravenously are distributed throughout the body, and although they selectively kill the rapidly proliferating cancerous cells, they still exhibit significant toxicity towards healthy cells. Chemotherapeutic agents also target the rapidly dividing healthy cells in bone marrow,\(^7\) hair follicles,\(^8\) and the reproductive system,\(^9\) producing severe side effects that often have a substantial impact on a patient’s life. In
addition, the development of tumors resistant to various drugs is one of the major obstacles in chemotherapy that needs to be addressed.

1.2. Photodynamic Therapy

Photodynamic therapy (PDT) permits the localization of the action of the anti-cancer drug (photosensitizer) to the cancerous area through the selective irradiation of the tumor tissue with visible or near IR light. Since at the concentrations used the drug is not toxic in the absence of light, this technique circumvents the death of healthy tissue. Due to the low tissue penetration (~1 cm, 600 < \( \lambda \) < 800 nm) of light,\(^{10}\) PDT may be used only for the treatment of small cancers localized on or under the skin or in accessible locations,\(^{11}\) such as the esophagus or lungs.\(^{12,13}\) The light sources used for PDT are typically light emitting diodes (LED) to treat skin cancers\(^{14}\) or laser light directed through fiber optics to reach the lungs or esophagus.\(^{15}\)

Currently four compounds have passed clinical trials and are used for PDT.\(^{16}\) Photofrin\(^{\circledR}\), a mixture of hematoporphyrin and its derivatives, was the first PDT agent approved by the FDA and is currently in use (Figure 1.1). Photofrin\(^{\circledR}\) produces \(^1\)O\(_2\) upon excitation with \( \lambda > 630 \) nm (50-500 J/cm\(^2\)), which is the species that induces cell death (Figure 1.2). However, the key drawback of this drug is its inability to destroy the cell in the absence of oxygen, since malignant tumors are often hypoxic. Furthermore, Photofrin\(^{\circledR}\) is composed of approximately 60 compounds and, therefore, the specific mixture is difficult to reproduce. Owing to the low absorption coefficient of the drug, \( \sim 1170 \) M\(^{-1}\)cm\(^{-1}\) at \( \lambda = 630 \) nm, high concentrations of Photofrin\(^{\circledR}\) have to be administered.\(^{16}\)
Foscan® (tetra(m-hydroxyphenyl)chlorin), is another oxygen-dependent photosensitizer that was approved by the European Union for the treatment of head and neck cancers in 2001. Relatively low concentrations of Foscan® (0.1 mg/kg of body weight) are required for PDT compared to those of Photofrin® (2 mg/kg of body weight). The drug is activated with longer wavelength (λ > 659 nm) and lower light intensity (10 J/cm²) compared to Photofrin®.17 Levulan® (5-aminolevulinic acid) and Metvix® (5-aminolevulinic methylate) are currently used for treatment of actinic keratosis and basal-cell carcinoma. It should be noted, however, that all current PDT agents are oxygen dependent, and are not effective against anoxic or hypoxic areas of the cancer tissues.

Therefore, there is a need for the development of PDT drugs that are independent of oxygen. In addition, to be useful in PDT, the photosensitizer must absorb light in the therapeutic window between 600 and 800 nm and have low cytotoxicity in the dark.

Metal complexes represent a promising field in the discovery of new PDT agents since they may react with double-stranded DNA directly from their excited state18,19 or via the production of various reactive species, such as OH•, O²⁻ or ¹O₂.20,21,22 Some examples of metal complexes that cleave DNA with similar efficiency under aerobic and anaerobic conditions upon irradiation have been reported recently. Those examples include mononuclear Re(I),23 Ru(II),18 and Rh(III) complexes,24 enediyne analogs of Cu(I) and Cu(II),25 dinuclear rhodium(II,II),26 and trinuclear Ru(II)-Rh(III)-Ru(II) complexes.27
**Figure 1.1.** The molecular structure of hematoporphyrin, an agent currently used in PDT.

**Figure 1.2.** Schematic representation of the production of $^{1}\text{O}_2$ by energy transfer from Photofrin $^{\circ}$, $S$, following intersystem crossing from the short-lived singlet excited state, $^{1*}\text{S}$, to the long-lived triplet, $^{3*}\text{S}$, upon absorption of a photon.
Bibliography


CHAPTER 2

EXPERIMENTAL SECTION

2.1. Materials

Pt(bpy)Cl₂, ethidium bromide, Hoechst 33258, disodium terephthalate, sodium salicylate, CoCl₂ • 6 H₂O, ZnCl₂, KCl, CsCl, BaCl₂, AgBF₄ and DMSO were purchased from Aldrich and used without further purification. Pt(NH₃)₂Cl₂ was purchased from Strem Chemicals. NaCl, Na₂HPO₄, NaH₂PO₄, sodium acetate, trizma base, trizma HCl, EDTA, boric acid, CaCl₂ • 2 H₂O, molecular weight standards, and the SmaI restriction enzyme kit were purchased from Sigma and used as received. All components for the gel loading buffer, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF, and glycerol, were purchased from Sigma and used as received. Calf thymus (CT) DNA was purchased from Sigma and was dialyzed against 5 mM tris, 50 mM NaCl (pH = 7.5) buffer three times during a 48 hour period prior to use. pUC18 plasmid was purchased from Bayou Biolabs and purified using the Concert Miniprep System from Life Technology. Sonicated herring sperm DNA was purchased from Invitrogen and used without purification. The substituted dppz ligands, R₁,R₂-dppz, Pt(dppz)Cl₂, cis-[Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂](O₂CCH₃)₂, 2-hydroxyterephthalic acid, [Ru(bpy)₂(tpphz)]Cl₂ and [Ru(bpy)₂(tptap)]Cl₂ were obtained the group of Prof. Kim Dunbar at Texas A&M
University and used as received, however, the preparation and characterization of the various complexes is briefly discussed below.

2.2. Synthesis

2.2.1. Pt(dppz)Cl₂

Dppz (dppz = dipyrido[3,2-a:2',3'-c]phenazine) ligand (74.8 mg, 0.26 mmol) in acetone (15 ml) was added to a red slurry of K₂PtCl₄ (106.7 mg, 0.26 mmol) in acetone/water (15 ml / 20 ml). The mixture was stirred and refluxed for 16 h. The reaction mixture was allowed to cool to room temperature. The yellow-greenish precipitate was filtered out, washed several times with hot water and hexanes, and dried under vacuum. Yield 88.3 mg (62 %). ¹H NMR in DMSO-d₆, δ / ppm (multiplicity, integration): 9.03 (d, 2H), 8.65 (d, 2H), 7.92 (m, 2H), 7.90 (m, 2H), 7.70 (m, 2H). Anal. Calcd for PtC₁₈H₁₀N₄Cl₂: C, 39.49; N, 10.24; H, 1.84. Found C, 39.42; N, 10.27; H, 1.93. Electrospray mass-spectrometry analysis of Pt(dppz)Cl₂ detected Pt(dppz)Cl₂(DMSO) (627, 100 %).

2.2.2. [Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂](O₂CCH₃)₂

The compounds Rh₂(μ-O₂CCH₃)₄( CH₃CN)₂ and dppz were synthesized by previously reported methods.¹ ² ³ ⁴ A solution of Rh₂(μ-O₂CCH₃)₄( CH₃CN)₂ (100 mg, 0.19 mmol) in acetonitrile was treated with solid dppz (107.7 mg, 0.38 mmol), and the suspension was heated to reflux for 24 h. After this time period, the red mixture was cooled to room temperature, filtered, and washed with CH₃CN to afford a red solid (93%
yield). Subsequent crystallization from a methanol/benzene/diethyl ether solution in an excess of NaBF₄ produced crystals suitable for X-ray characterization. FAB mass spectrum: \( m/z = 443.98 \) ([Rh₂(µ-O₂CCH₃)₂(dppz)₂]²⁺). \(^1\)H NMR of the diacetate salt in CDCl₃ / CD₃OD (1:1 v:v), \( \delta \) / ppm (multiplicity, integration, assignment): 1.75 (s, 6H, CH₃CO₂), 2.67 (s, 6H, CH₃CO₂), 7.73 (m, 4H, dppz), 7.81 (m, 4H, dppz), 7.90 (m, 4H, dppz), 8.64 (d, 4H, dppz), 9.05 (d, 4H, dppz).

[Rh₂(µ-O₂CCH₃)₂(R₁,R₂-dppz)₂](O₂CCH₃)₂ complexes were synthesized from Rh₂(µ-O₂CCH₃)₄(CH₃CN)₂ and the corresponding R₁,R₂-dppz ligand using similar procedures to that described above.

2.2.3. [Ru(bpy)₂(tpphz)]Cl₂

The ligand tpphz (tpphz = tetrapyrido[3,2-a:2′,3′-c:3″,2″-h:2′′,3″-f]phenazine) and [Ru(bpy)₂(tpphz)](PF₆)₂ (bpy = 2,2′-bipyridine) were prepared by following a literature procedure.\(^5\) [Ru(bpy)₂(tpphz)]Cl₂ was precipitated from a solution of the PF₆⁻ salt of the complex by the addition of 10 M lithium chloride to an acetone solution. The solid was filtered, washed with acetone, diethyl ether, and dried under vacuum.

2.2.4. [Ru(bpy)₂(tatp)]Cl₂

The ligand tatp (tatp = 4,5,9,18-tetraazaphehanthreno[9,10-b] triphenylene) was prepared by a reported method,\(^6\) and [Ru(bpy)₂(tatp)](PF₆)₂ was prepared by a modified literature procedure.\(^7\) A mixture of tatp (100 mg, 0.26 mmol) and [Ru(bpy)₂Cl₂] • 2H₂O (136 mg, 0.61 mmol) was heated in a microwave oven under N₂ for 30 min 6 mL ethylene glycol.\(^8\) After cooling, the mixture was poured into 30 mL of H₂O, 2 mL of
aqueous NH₄PF₆ (98 mg, 0.6 mmol) was then added, and the solution was stirred for 15 min. The precipitate was filtered, dried, and then eluted from an alumina column with toluene/CH₃CN (1:1) to provide an orange solid (186 mg, 66%) with mp > 280 °C. ¹H NMR in CD₃CN, δ / ppm (multiplicity, integration, J / Hz): 9.87 (d, 1H, 8.1), 9.58 (d, 1H, 7.7), 8.76 (d, 1H, 8.1), 8.57 (d, 1H, 8.5), 8.52 (d, 1H, 8.1), 8.20 (d, 1H, 6.0), 8.12 (t, 1H, 7.3), 8.01 (t, 1H, 7.3), 7.96-7.86 (m, 4H), 7.74 (d, 1H, 6.0), 7.47 (t, 1H, 6.9), 7.25 (t, 1H, 6.8). MS m/z = 941 (100%) corresponding to [Ru(bpy)₂(taptp)](PF₆)⁺.

2.2.5. 2-Hydroxyterephthalic Acid

2-Hydroxyterephthalic acid was synthesized according to a literature procedure.⁹ FAB mass spectrum: m / z = 90 corresponding to C₆H₃(OH)(COO)₂⁻.

2.3. Methods

All DNA titration experiments were conducted in 5 mM Tris buffer, pH = 7.5, 50 mM NaCl. Owing to their low water solubility, Pt(bpy)Cl₂ and Pt(dppz)Cl₂ were first dissolved in DMSO, then 7.7 µL of this stock solution was added to 1.5 mL of buffer, since such a low percentage of DMSO has been shown to not interfere with nucleic acids.¹⁰ All solutions were incubated for 3 hours at room temperature prior to each titration in order to ensure that the coordinated chloride ions completely exchanged with H₂O molecules in the solvent.¹¹ [Ru(bpy)₂(tpphz)]Cl₂ and [Ru(bpy)₂(taptp)]Cl₂ stock solutions were prepared directly in H₂O and were added to the buffer, resulting in 9-30 µM complex concentration. These solutions were titrated with DNA without any incubation. The DNA binding constant, Kₘ, of Pt(dppz)Cl₂, [Ru(bpy)₂(tpphz)]Cl₂ and
[Ru(bpy)$_2$(tapt)]Cl$_2$ were obtained from changes in the absorption spectra of each complex and fits of the titration data to eq 1, which is typically utilized for intercalators with $K_b > 10^5$ M$^{-1}$.\textsuperscript{12}

\begin{equation}
\frac{\varepsilon_a - \varepsilon_o}{\varepsilon_f - \varepsilon_o} = \frac{b - (b^2 - 2 K_b^2 C_t [DNA]_t / s)^{1/2}}{2 K_b C_t}
\end{equation}

In eq 1, $b = 1 + K_b C_t + K_b [DNA]_t / 2 s$, $C_t$ and [DNA] represent the total complex and DNA concentrations, respectively, $s$ is the base pair binding site size, and $\varepsilon_a$, $\varepsilon_o$, and $\varepsilon_f$ represent the apparent, free, and bound complex molar extinction coefficients, respectively. For complexes with lower DNA binding constants ($K < 10^4$ M$^{-1}$), such as Pt(bpy)Cl$_2$, eq 2 was utilized.\textsuperscript{13}

\begin{equation}
\frac{[DNA]_t}{\varepsilon_a - \varepsilon_o} = \frac{[DNA]_f}{\varepsilon_f - \varepsilon_o} + \frac{1}{K_b (\varepsilon_f - \varepsilon_o)}
\end{equation}

Emission titrations were performed in 5 mM Tris, pH 7.5, 50 mM NaCl. The excitation wavelength, $\lambda_{exc}$, for both Ru(II) complexes was set at 450 nm. The intensity of the emission of [Ru(bpy)$_2$(tphh)]$^{2+}$ and [Ru(bpy)$_2$(tpt)]$^{2+}$ was measured at 634 nm and 623, respectively, and fitted to eq 3 to obtain DNA binding constants.\textsuperscript{14}

\begin{equation}
\frac{1 - I_o}{I_f - I_o} = \frac{b - (b^2 - 2 K_b^2 C_t [DNA] / s)^{1/2}}{2 K_b C_t}
\end{equation}

In eq 3, $b = 1 + K_b C_t + K_b [DNA] / 2 s$, $I$ is the emission intensity in the presence of a given DNA concentration, $I_o$ is the emission intensity in the absence of DNA, $I_f$ is the
final maximum emission intensity of completely bound complex, $K_b$ is the equilibrium binding constant, $C_t$ is the total metal concentration, and $s$ is the binding site size. All titrations were repeated at least twice.

The light-switch titrations were carried out in a fluorescence cuvette. In a typical titration experiment, 2 mL of $\text{[Ru(bpy)}_2\text{L}]^{2+}$ ($\text{L} = \text{tpphz, taptp}$) and CT DNA ([DNA]:[Ru] = 10:1, $\text{[Ru(bpy)}_2\text{L}]^{2+} = 3.7 \, \mu\text{M} – 12 \, \mu\text{M}$) in 5 mM Tris, 50 mM NaCl, pH = 7.5 buffer solution was placed in a 1×1 cm quartz fluorescence cell. The UV-visible absorption and fluorescence spectra were repeatedly recorded at room temperature in air after the addition of aliquots of stock solutions of $\text{Co}^{2+}$, $\text{Zn}^{2+}$, or EDTA.

Equilibrium dialysis experiments were performed against 100 $\mu$M calf-thymus DNA in 5 mM Tris buffer, pH = 7.5, 50 mM NaCl. The complex concentration was varied from 10 to 20 $\mu$M. Owing to the low solubility of $\text{Pt(dppz)Cl}_2$ in water, DMSO stock solutions were utilized. In the equilibrium dialysis experiments small volumes of the DMSO stock solution of the complex (4 mL) were added to the buffer (496 mL), resulting in 0.8 % volume DMSO. CT DNA was placed in a dialysis bag and was dialyzed against 200 mL of the complex for 2-5 days. Equilibrium dialysis experiments were conducted at least twice. The DNA binding constant, $K_b$, was determined from $K_b = C_b / [C_t(S_{total} – C_b)]$, where $C_b$, $C_t$, and $C_f$ represent the concentrations of bound, total, and free complex, respectively, and $S_{total}$ is the total DNA concentration. $C_t$ and $C_f$ were measured spectroscopically before and after dialysis, respectively, using an extinction coefficient of $5.4 \times 10^3 \, \text{M}^{-1}\text{cm}^{-1}$ at 375 nm for $\text{Pt(dppz)Cl}_2$, $14.7 \times 10^3 \, \text{M}^{-1}\text{cm}^{-1}$ at 450
nm for $[\text{Ru(bpy)}_2(\text{tptap})]^2^+\text{ and } 20.0 \times 10^3\text{ M}^{-1}\text{cm}^{-1}\text{ at } 450\text{ nm for } [\text{Ru(bpy)}_2(\text{tpphz})]^2^+$. $C_b$ was calculated from the mass balance, $C_b = C_t - C_f$.

For the relative viscosity measurements with Pt(dppz)Cl$_2$, Pt(bpy)Cl$_2$, Hoechst 33258, cisplatin, and EtBr, 200 µM sonicated herring sperm DNA was used (5 mM Tris, pH = 7.5, 50 mM NaCl), and the concentration of the each probe was varied from 10 to 60 µM. The stock solutions of all the complexes possessed the same concentration of DMSO, such that the same amount of DMSO was present at a given complex concentration measured in the viscosity experiments. The highest complex concentration resulted in 2.5% volume of DMSO. As a control, DMSO alone was added to DNA and the relative viscosity was calculated as $\eta_{\text{DMSO}} = (t_{\text{DMSO}} - t_o) / t_o$, where $t_{\text{DMSO}}$ is the flow time of DNA with added DMSO, and $t_o$ is the flow time of buffer alone. The relative viscosity data for the metal complexes are plotted as $(\eta / \eta_o)^{1/3} / (\eta_{\text{DMSO}} / \eta_o)^{1/3}$ vs R, where R = [probe] / [DNA]. The relative viscosity $\eta$ was calculated as $\eta = (t - t_o) / t_o$, where t is the flow time of DNA solution with added probe. $\eta_0$ is the correction for the relative viscosity of DNA alone, $\eta_0 = (t_{\text{DNA}} - t_o) / t_o$.

The relative viscosity of DNA solutions upon the addition of $[\text{Ru(bpy)}_2(\text{tphhz})]\text{Cl}_2$ and $[\text{Ru(bpy)}_2(\text{tptap})]\text{Cl}_2$ was measured using 1 mM herring sperm DNA (5 mM Tris, pH 7.5, 50 mM NaCl). EtBr, Hoechst 33258 and $[\text{Ru(bpy)}_2\text{L}]^{2+}$ (L = tpphz, tapt) complexes were added to the DNA solutions with final concentrations of 0.05, 0.1, 0.2, and 0.3 mM. Relative viscosity data are plotted as $(\eta / \eta_o)^{1/3}$ vs R, where $\eta = t - t_o$, $\eta_o = t_{\text{DNA}} - t_o$. When needed, Co$^{2+}$ was added to DNA solution along with the Ru(II) complexes maintaining the [Co]:[Ru] ratio at 1:2, 1:1 and 2:1.
For plasmid linearization, 100 µM pUC18 plasmid DNA was mixed with 10 units of SmaI in 20 µL total volume, the solutions were incubated for 1 h at 40 °C, and were then lyophilized and resuspended in water. Plasmid concentrations were measured spectroscopically using an extinction coefficient of 6,600 M⁻¹cm⁻¹ at 260 nm. For gel mobility assays, 50 µM linearized plasmid was typically mixed with solutions of metal complex so that the [base pair]/[complex] ratio ranged from 5 to 100. Each solution was then incubated at 40 °C for 4 h in 5 mM phosphate buffer, pH 7.2. To each 20 µL sample, 4 µL of 6x loading dye (0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF, and 30% glycerol in water) was added prior to loading onto the agarose gel (0.8% agarose, 0.09 M Tris, 0.002 M disodium salt of EDTA, 0.09 M boric acid, pH 8.3). The electrophoresis was allowed to run at 108 V for 1.5 h. The gels were stained with 0.5 µg/mL EtBr solution for 30 min and were then kept in a water bath for 15 min.

For the DNA photocleavage experiments, typically 100 µM pUC18 plasmid was mixed with a given concentration of metal complex (5 mM Tris, 50 mM NaCl, pH 7.5) in a total volume of 20 µL, and each solution was either kept in the dark or irradiated. The samples were loaded onto a 1% agarose gel with EtBr incorporated within the gel at final concentration of 5 mg/L.

2.4. Instrumentation

The ¹H NMR spectra were recorded on a DPX-250 NMR spectrometer. Absorption measurements were performed on a Hewlett-Packard diode array
spectrometer (HP 8453) equipped with an HP 89090A temperature controller and HP 8453 Win System software.

The corrected steady-state luminescence spectroscopy was carried out on a SPEX Fluoromax-II spectrofluorimeter. When required, deaeration of the solution was carried out by bubbling with argon or nitrogen for 20 min. Luminescence quantum yields were measured using dilute solutions and [Ru(bpy)$_3$]$^{2+}$ in oxygen-free CH$_3$CN ($\Phi_{\text{em}} = 0.06$) as the actinometer.\textsuperscript{15} Emission lifetime measurements were conducted either on an Edinburgh nF900 single photon counting instrument with fitting software or on a home-built time-resolved system using the frequency doubled (532 nm) or tripled (355 nm) output of a Spectra-Physics GCR-150 Nd:YAG laser (fwhm ~8 ns, ~5 mJ / pulse) as the excitation source. The emission wavelength was controlled using a Jobin Yvon monochromator and was detected with a red-sensitive PMT (Hamamatsu R928). The signal was digitized on a Tektronics 400 MHz oscilloscope (TDS 380), and the data were collected on a PowerMac 7600/132 (Apple) equipped with a National Instruments GPIB interface (NI-488.2) and a National Instruments data acquisition board (PCI-1200) programmed with Labview 4.1 software.

A 150 W Xe lamp (PTI LPS220) housed in a Milliarc compact arc lamp housing and powered by a Model LPS-220 lamp power supply was used in the DNA photocleavage experiments. The wavelength of the light reaching the sample was controlled with long pass colored glass filters (CVI) and a 10 cm water cell in the light path. The EtBr stained agarose gels were imaged using a GelDoc 2000 transilluminator (BioRad) equipped with Quality One software.
Viscosity measurements were carried out in a Cannon-Manning Microviscometer, immersed in a temperature-controlled water bath (Neslab RTE - 100) maintained at 24.0 ± 0.1°C. The flow time was recorded using a digital stopwatch, and each sample was measured three times and an average flow time was calculated.

Electrochemical measurements were performed in dry CH$_3$CN or DMSO with 0.1 M [Bu$_4$N][BF$_4$] as the electrolyte on a BAS CV-50W voltammetric analyzer (100 mV/s) in a three-electrode cell with a platinum disk working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl electrode separated from the bulk solution by a frit, as a quasi-reference electrode. Ferrocene (E$_{1/2}$(FeCp$^{+/0}$) = 0.425 V vs SCE) was added as an internal standard following each experiment.
Bibliography


CHAPTER 3

PHOTOPHYSICAL PROPERTIES, DNA BINDING, AND DNA PHOTOCLEAVAGE BY Pt(dppz)Cl₂

3.1. Introduction

It was discovered serendipitously by Rosenberg¹ in 1964 that cisplatin, cis-Pt(NH₃)₂Cl₂, inhibits cell proliferation. Today cisplatin is a well-known antitumor drug used for treatment of ovarian and testicular cancers. Cisplatin covalently binds to double-stranded DNA following the exchange of the coordinated chloride ions with water molecules in aqueous media with a half-time of 3-4 h under physiological conditions (Figure 3.1).²,³,⁴ This DNA modification results in the disruption of cellular processes, including transcription, and culminates in cell death.⁵ Cisplatin has been shown to bind primarily to adjacent guanine nucleobases (G’s) in the DNA sequence through coordination to the N7 of each guanine, thus producing intrastrand cross-links that result in the distortion of the DNA structure due to unwinding, bending, and shortening the DNA.⁶,⁷ Owing to the toxicity of cisplatin towards healthy cells and the resistance developed by some tumors towards the drug, many new Pt(II) complexes have been prepared and their DNA binding and anti-tumor efficacy tested in order to circumvent these problems.³,⁸,⁹,¹⁰
Many intercalating transition metal complexes have been investigated as photonucleases, probes of DNA structure, and in long-range charge transfer through DNA. The intercalating dppz ligand (dppz = dipyrido[3,2-a:2’-3’-c]phenazine) has been utilized for many of these studies, and include complexes of Ru(II), Rh(II), Re(I), Cu(II), Co(III), and Pt(II). In [Ru(bpy)$_2$(dppz)]$^{2+}$ (bpy = 2,2'-bipyridine) and [Ru(NH$_3$)$_4$(dppz)]$^{2+}$, for example, DNA binding constants of $1 \times 10^6$ M$^{-1}$ and $1.24 \times 10^5$ M$^{-1}$ were reported, respectively. In general, the magnitude of the binding constant, together with the hypochromic and bathochromic shifts observed in the absorption spectra of these complexes as a function of DNA concentration, are consistent
with the intercalation of the dppz ligand.\textsuperscript{23,24} In addition, DNA photocleavage has been reported for some of these mononuclear systems,\textsuperscript{16,19,25} as well as new dirhodium complexes possessing dppz ligands.\textsuperscript{15}

The present study combines the photoactive dppz ligand, which is expected to intercalate between the DNA bases, with a Pt(II) center with labile chloride ligands for the potential covalent binding to purine nucleobases. The DNA binding and photocleavage by Pt(dppz)Cl\textsubscript{2} was investigated and the results are compared to cisplatin and the related complex Pt(bpy)Cl\textsubscript{2} (Figure 3.2).

![Molecular structures of Pt complexes](image)

**Figure 3.2.** Molecular structures of (a) cisplatin, (b) Pt(bpy)Cl\textsubscript{2}, and (c) Pt(dppz)Cl\textsubscript{2}.
3.2. Results and Discussion

Owing to the presence of both the known intercalating dppz ligand and labile chloride ligands in Pt(dppz)Cl₂, both intercalative and covalent binding modes to double-stranded DNA (ds-DNA) by the complex are possible. Various methods were utilized to ascertain the mode of binding of Pt(dppz)Cl₂ to DNA, including the changes in the absorption spectrum of the complex as a function of DNA concentration, the variation in relative viscosity, and gel mobility assays. The binding constant of the complex to DNA was also measured by equilibrium dialysis. The results from each of these methods for Pt(dppz)Cl₂ are discussed below and are compared to those obtained for cisplatin and Pt(bpy)Cl₂.

3.2.1. Electronic Absorption and Emission

The electronic absorption spectrum of cisplatin in water consists of two peaks with maxima at 275 nm (\(\varepsilon = 110 \text{ M}^{-1}\text{cm}^{-1}\)) and 300 nm (\(\varepsilon = 170 \text{ M}^{-1}\text{cm}^{-1}\)) previously assigned to spin-allowed Pt(II)-centered dd ligand-field (LF) transitions, \(1^A_1 \rightarrow \{1^A_2,1^B_1\}\) and \(1^A_1 \rightarrow 1^B_2\), respectively. A weak shoulder is also observed at ~350 nm (\(\varepsilon = 25 \text{ M}^{-1}\text{cm}^{-1}\)) in the spectrum of cisplatin, which is believed to arise from the spin-forbidden \(1^A_1 \rightarrow 3^B_2\) LF transition.

The absorption spectrum of Pt(bpy)Cl₂ exhibits peaks at 261 nm (\(\varepsilon = 1.5 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}\)), 312 nm (\(\varepsilon = 7.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}\)), and 322 nm (\(\varepsilon = 1.1 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}\)) in water, with a shoulder at ~350 nm (\(\varepsilon = 2.7 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}\)). Owing to the high extinction
coefficients the peaks at 312 nm and 322 nm were assigned to the ligand-centered (LC)
$^{1} \pi \pi^* \pi$ transition from the bpy ligand, since solvent-insensitive diimine-centered transitions
in the 300-322 nm region are characteristic for platinum diimine complexes and were
also observed in the spectrum of Ir(bpy)$_3^{2+}$.\textsuperscript{31,32} Owing to the solvent-dependence of the
shoulder at $\lambda > 350$ nm, it was assigned by others to a spin-allowed MLCT transition.\textsuperscript{31,33}

The prominent features in the absorption spectrum of Pt(dppz)Cl$_2$ in water are
peaks with at 270 nm ($\varepsilon = 1.1 \times 10^4$ M$^{-1}$cm$^{-1}$), 362 nm ($6.1 \times 10^3$ M$^{-1}$cm$^{-1}$), and 380 nm
($6.0 \times 10^3$ M$^{-1}$cm$^{-1}$), and a shoulder at 420 nm ($\varepsilon = 4.6 \times 10^3$ M$^{-1}$cm$^{-1}$) that extends into
the visible region. From comparison to the absorption spectra of other dppz complexes,
the peaks at 362 and 380 nm can be assigned to dppz-centered $^{1} \pi \pi^*$ LC transitions.\textsuperscript{15,23,24}

The maximum of the shoulder at 420 nm is solvent-dependent (400 nm in DMF or
DMSO), and likely results from a MLCT transition.

No emission was detected from degassed H$_2$O, DMSO, or CH$_2$Cl$_2$ solutions of
Pt(dppz)Cl$_2$ at room temperature or at 77 K. In contrast, both cisplatin and Pt(bpy)Cl$_2$
are emissive in glasses at 77 K and as solids at room temperature.\textsuperscript{30,33} The emission from
both cisplatin and Pt(bpy)Cl$_2$ has been assigned as arising from a ligand field state due to
the absence of vibronic structure and the insensitivity to the solvent. The absence of
luminescence in Pt(dppz)Cl$_2$ points at the presence of low-lying non-emissive state
involving the dppz ligand, such as $^3$MLCT or $^3 \pi \pi^*$. 
3.2.2. Chloride / Water Exchange

It is known from previous studies that the absorption spectrum of cisplatin in water changes upon the exchange of the coordinated chloride ions with water molecules, resulting in the growth of the 276 nm peak and the decrease of the absorption at 300 nm.\(^2\) Pt(bpy)Cl\(_2\) and Pt(dppz)Cl\(_2\) were placed in water to effect chloride-water exchange and the progress was monitored by UV-vis spectroscopy. Characteristic changes in the electronic absorption spectrum of Pt(bpy)Cl\(_2\) are the growth of the peak at 305 nm and the decrease of the MLCT transition at \(\lambda > 345\) nm (Figure 3.3). The 322 nm peak blue-shifts by 2 nm upon the completion of the water / chloride exchange while preserving a similar intensity.

Silver tetrafluoroborate, AgBF\(_4\) was added in 4-fold excess to an aqueous solution of Pt(bpy)Cl\(_2\) to form [Pt(bpy)(H\(_2\)O)\(_2\)]\(^2+\) (eq 4).

\[
\text{Pt(bpy)Cl}_2 + 2 \text{AgBF}_4 \rightarrow [\text{Pt(bpy)}(\text{H}_2\text{O})_2]^{2+} + 2 \text{BF}_4^- + 2 \text{AgCl(s)} \quad (4)
\]

As shown in Figure 3.3 for Pt(bpy)Cl\(_2\), the spectra of the species obtained after the incubation of the complex in water for 3 hrs at 25 °C, and after the addition of AgBF\(_4\) are identical. These results are consistent with the complete exchange of chloride ions for water molecules in both cases, thus generating the di-aqua species [Pt(bpy)(H\(_2\)O)\(_2\)]\(^2+\). An overall decrease in the absorption spectrum of Pt(dppz)Cl\(_2\) is observed upon chloride / water exchange, which is complete after 3 hrs in pure water and after the addition of 4-fold excess of AgBF\(_4\) (Figure 3.4). However, the intensity of the dppz-based transitions in the 250 – 400 nm region decrease by the factor of ~2 after 3 hrs in pure water. Since
the free dppz ligand itself is stable in water over this time period, the changes in absorption cannot be attributed to ligand decomposition. It is also evident in Figure 3.4 that the intensity of the MLCT transition decreases when the complex remains in H₂O for 3 hrs, while after the addition of AgBF₄ the MLCT intensity does not decrease. Dppz ligand loss might be responsible for the more pronounced intensity decrease. However, the absorption spectrum of the free dppz ligand in water does not match to that in Figure 3.4 after the completion of chloride / water exchange. Besides, the absorption coefficient at 270 nm of the free dppz ligand is 4 times greater than that of Pt(dppz)Cl₂, not consistent with the overall decrease in the intensity. Most likely π-stacking aggregation of the dppz ligands in the complex is responsible for decrease of the absorption intensity observed over time in water following or during the Cl⁻ / H₂O exchange.

**Figure 3.3.** Electronic absorption spectra of (a) 81µM [Pt(bpy)L₂]²⁺ in water before (—) L = Cl⁻, and after (······) L = H₂O the addition of excess AgBF₄ in pure water, and (b) 31 µM [Pt(bpy)L₂]²⁺ in water (−−−−−) L = Cl⁻ at t = 0 min, and (·−·) L = H₂O at t = 3 hrs.
3.2.3. DNA Binding Studies

The changes in the absorption spectra of Pt(bpy)Cl₂ and Pt(dppz)Cl₂ in buffer (5 mM Tris, pH = 7.5, 50 mM NaCl) over a period of 3 hrs are consistent with the exchange of the chloride ligands with H₂O to generate the chloride salt of the respective diaqua activated complexes, [Pt(bpy)(H₂O)₂]²⁺ and [Pt(dppz)(H₂O)₂]²⁺. Owing to this chloride exchange over time, the optical titrations with DNA were conducted both immediately after the addition of each complex to buffer, as well as and with the activated complexes generated after the incubation of the corresponding dichloride complex in buffer for 3 hrs prior to the experiment.
Modest changes in the absorption of 20 µM [Pt(bpy)(H₂O)₂]²⁺ were observed upon addition of 200 µM DNA (5 mM Tris, pH = 7.5, 50 mM NaCl) (Figure 3.5a). The modest hypochromicity in the absorption of [Pt(bpy)(H₂O)₂]²⁺ throughout the near-UV and visible region as the DNA concentration is increased and can be fit to eq 2 to yield

\[ K_b = 6.6(7) \times 10^3 \text{ M}^{-1} \] (Figure 3.6). Hypochromic and bathochromic shifts are observed in the near-UV and visible regions of the absorption spectrum of 20 µM [Pt(dppz)(H₂O)₂]²⁺ upon the addition of DNA with a final DNA concentration of 200 µM (5 mM Tris, pH = 7.5, 50 mM NaCl), resulting in 10% hypochromicity and 1.5 nm bathochromic shift at 380 nm (Figure 3.5b, inset). Fits of the absorption changes at 380 nm to eq 1 as a function of DNA concentration results in a binding constant, \( K_b \), of 4.5(8) \times 10^5 \text{ M}^{-1} (s = 1.0) (Figure 3.7). This value is in good agreement with binding constants of other dppz transition metal complexes where the dppz ligand intercalates between the DNA bases.¹⁹,²² The value of \( K_b \) measured for Pt(dppz)Cl₂ upon addition of DNA before and after complete chloride / water exchange is within experimental error, indicating that intercalation is likely the only binding mode for this complex. Similarly, fits of the changes in the absorption at 480 nm of 20 µM solutions of the intercalator ethidium bromide (EtBr) upon addition of DNA (5 mM Tris, pH = 7.5, 50 mM NaCl) to eq 1 results in \( K_b = 1.1(5) \times 10^6 \text{ M}^{-1} \) (s = 1.74), which is similar to values previously reported.²⁸

The binding constants of Pt(dppz)Cl₂, Pt(bpy)Cl₂, and EtBr were also measured by equilibrium dialysis, and were determined to be 7.6(7) \times 10^5 \text{ M}^{-1}, 5.8(6) \times 10^3 \text{ M}^{-1},
and $3.8(6) \times 10^6 \text{ M}^{-1}$, respectively. The binding constants of all the molecules are in a good agreement with those obtained from the absorption titrations.

**Figure 3.5.** Absorption changes upon titration of (a) 20 µM [Pt(bpy)$_2$(H$_2$O)$_2$]$^{2+}$ and (b) 20 µM [Pt(dppz)(H$_2$O)$_2$]$^{2+}$ with 16.8 - 200.4 µM DNA with 16.8 µM increments (5 mM Tris, pH 7.5, 50 mM NaCl). The titration was performed immediately after dissolving complexes in water. The inset shows the absorption changes before and after the addition of 200.4 µM DNA.
**Figure 3.6.** Fit of the absorption changes of $[\text{Pt(bpy)}(\text{H}_2\text{O})_2]^{2+}$ at 322 nm upon addition of DNA to eq 2 (5 mM Tris, pH 7.5, 50 mM NaCl).

**Figure 3.7.** Fit of the absorption changes of $[\text{Pt(dppz)}(\text{H}_2\text{O})_2]^{2+}$ at 380 nm upon addition of DNA to eq 1 (5 mM Tris, pH 7.5, 50 mM NaCl).
3.2.4. Relative Viscosity Changes

The relative viscosity changes to DNA solutions have been shown to be a reliable means to determine the DNA binding mode of compounds.\textsuperscript{34} Intercalators, such as EtBr, result in an increase in the relative viscosity due to the DNA lengthening, whereas minor groove binders like Hoechst 33258 have little effect on the relative viscosity of DNA solutions.\textsuperscript{34} The changes in the relative viscosity of 200 \(\mu\text{M}\) DNA upon the addition of various concentrations of EtBr, Hoechst 33258, Pt(dppz)Cl\(_2\), Pt(NH\(_3\))\(_2\)Cl\(_2\), and Pt(bpy)Cl\(_2\) are shown in Figure 3.8. It is evident from Figure 3.8 that the viscosity changes observed upon addition of Pt(dppz)Cl\(_2\) parallel those measured for the intercalator EtBr. In contrast, increasing concentrations of Pt(bpy)Cl\(_2\) do not result in an increase in the relative viscosity, a result that is inconsistent with intercalative binding and is similar to that observed for the minor-groove binder Hoechst 33258. Cisplatin is known to have a “shortening effect” on DNA induced by covalent binding, which decreases the viscosity.\textsuperscript{35,36} The results shown in Figure 3.8 for cisplatin are consistent with previous reports with a decrease in viscosity from \(R = 0.0\) to 0.1,\textsuperscript{35} followed by a slight increase at \(R > 0.1\). It should be noted that there was no incubation of Pt(dppz)Cl\(_2\), cisplatin, or Pt(bpy)Cl\(_2\) with DNA prior to the viscosity measurements.
3.2.5. Covalent Binding to DNA

The binding to DNA can also be probed using agarose gel electrophoresis owing to the reduced mobility of linearized plasmid upon covalent binding of metal complexes.\textsuperscript{29} Cisplatin is known to crosslink DNA, thus creating a kinked DNA structure. Figure 3.9a shows the decrease in the mobility of 50 µM linearized pUC18 plasmid with increasing concentrations of cisplatin. Lanes 1 and 8 are the molecular weight standards from 1000 bp to 10,000 bp in increments of 1,000 bp. Lanes 2 and 7 represent linearized plasmid not treated with metal complex. Lanes 3 – 6 show the mobility of 50 µM linearized plasmid incubated with 0.5, 2.5, 10, and 50 µM cisplatin for 4 h at 37 °C. The lower intensity of the lanes with higher complex concentration is probably due to the pronounced structural changes in the DNA structure upon cisplatin.
coordination, resulting in reduced intercalation of EtBr and lower emission enhancement. Similar results were obtained with Pt(bpy)Cl$_2$ (Figure 3.9b), where greater concentrations of the metal complex results in decreased mobility of DNA. However, the mobility shift gel assay for Pt(dppz)Cl$_2$ resulted the same mobility of the pure linearized plasmid at similar concentrations of metal complex (Figure 3.10). It appears that Pt(dppz)Cl$_2$ binds to DNA via an intercalative mode even after complete chloride / water exchange.

**Figure 3.9.** EtBr stained agarose gels (0.8%) of 50 µM linearized pUC18 plasmid (5 mM phosphate, pH = 7.5) in the presence of various concentrations of (a) cisplatin and (b) Pt(bpy)Cl$_2$ incubated for 4 h at 37 °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.
3.2.6. DNA Photocleavage

As shown in Figure 3.11, 20 μM Pt(dppz)Cl₂ is able to photocleave 100 μM plasmid DNA (5 mM Tris, pH = 7.5, 50 mM NaCl) upon irradiation with visible light (λ<sub>irr</sub> > 395 nm, 20 min), resulting in circular, nicked DNA. It is evident from lanes 4 and 5 (Figure 3.11) that the photocleavage by the Pt(dppz)Cl₂ complex is oxygen-independent. The DNA photocleavage was undertaken using 2 mM sodium azide, a known scavenger of ¹⁰O₂ and OH•, and 2 mM DMSO and mannitol, which are known scavengers of OH•. The scavengers did not have any impact on the photocleavage, therefore Pt(dppz)Cl₂ likely cleaves DNA directly from its excited state. When 0.1 M
OH• scavengers like NaN₃, sodium formate, and mannitol were added, the DNA photocleavage decreased from 18% to 11%, 4%, and 10%, respectively.

No detectable DNA cleavage was observed upon irradiation of plasmid in the presence of cisplatin or Pt(bpy)Cl₂ under similar experimental conditions (Figure 3.12). This dramatic difference in the ability of Pt(dppz)Cl₂ to photocleave DNA is likely due to the presence of dppz ligand. The results obtained for Pt(dppz)Cl₂ are similar to those reported for [Re(dppz)(CO)₃(py)][O₃SCF₃]. The latter cleaves plasmid upon irradiation with λ > 350 nm (30 min) to produce nicked DNA under aerobic and anaerobic conditions, and it was shown that singlet oxygen and hydroxyl radical were not involved in the process.

Figure 3.11. EtBr stained agarose gel (2%) showing the photocleavage (λIRR > 395 nm, 20 min) of 100 µM pUC18 plasmid by 20 µM Pt(dppz)Cl₂ (5 mM Tris, pH 7.5, 50 mM NaCl). Lane 1, DNA only, dark; lane 2, linearized plasmid; lanes 3-5, DNA + Pt(dppz)Cl₂: in the dark (lane 3), irradiated in air (lane 4), irradiated under argon (lane 5).
3.3. Concluding Remarks

The DNA binding and photocleavage by Pt(dppz)Cl₂ was investigated and compared to those of cisplatin, Pt(NH₃)₂Cl₂, and the related complex Pt(bpy)Cl₂. The hypochromicity of absorption spectrum of Pt(dppz)Cl₂ observed upon addition of DNA is consistent with an intercalative binding mode, from which a DNA binding constant of $K_b = 4.0(8) \times 10^5 \text{ M}^{-1}$ was calculated. A similar binding constant, $7.6(7) \times 10^5 \text{ M}^{-1}$, was determined for the complex from equilibrium dialysis. Intercalation of the complex is also supported by the increase in relative viscosity in the presence of Pt(dppz)Cl₂, which is not observed for cisplatin or Pt(bpy)Cl₂ under similar experimental conditions. Mobility shift assays show that Pt(bpy)Cl₂ covalently binds to DNA upon incubation at 37 °C for 4 hrs, however, no covalent binding is observed for Pt(dppz)Cl₂ under similar conditions.
experimental conditions. Upon irradiation ($\lambda_{\text{irr}} > 395$ nm, 20 min) Pt(dppz)Cl$_2$ is able to photocleave plasmid DNA both in air and in the absence of oxygen, resulting in the nicked form. In contrast, cisplatin and Pt(bpy)Cl$_2$ are photochemically inactive towards DNA under these irradiation conditions.
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4.1. Introduction

The agents currently used in photodynamic therapy, PDT, damage cells via the generation of singlet oxygen upon irradiation, and become inactive after the oxygen in the vicinity of the drug is consumed. New systems need to be explored to find drugs that can damage tumors under both aerobic and anaerobic conditions. Recently, dirhodium complexes were explored for their photoinduced DNA damage, and were shown to produce nicked plasmid DNA upon irradiation in the presence and absence of oxygen.\(^1\)\(^2\)

The DNA photocleavage by \(\textit{cis}^{-}\left[Rh_2(\mu-O_2CCH_3)_{2}\left(R_1,R_2\text{-dppz}\right)_{2}\right]^{2+}\) and \(\textit{cis}^{-}\left[Rh_2(\mu-O_2CCH_3)_{2}\text{-dppz}\right]_{2}^{2+}\) has already been shown to be oxygen-independent and it is believed to take place from a \(\text{Rh}_2 \rightarrow \text{dppz MLCT excited state.} \) It is expected that the ability of the complexes to photocleave DNA should be affected by the lifetime of the MLCT excited state, \(^{*}[\text{Rh}_2^{+}\text{-dppz}^{-}]\). In order to increase the reactivity, various substituents were introduced on the dppz ligand in order to tune the reduction potential of the ligand and increase the MLCT lifetime.
4.2. Background

After the formulation of the energy gap law,\(^3,4\) a relationship between the non-radiative decay and electron transfer theory was established. Multiple investigations of metal polypyridyl complexes revealed a linear relationship between the logarithm of non-radiative decay rate and the energy gap between the ground and excited state.\(^4,5,6,7\) Here we show that the amount of DNA photocleavage can be related to the lifetime of the reactive excited state in a series of non-emissive dirhodium complexes, which in turn depends on the excited state energy according to the energy gap law.

4.2.1. Electron Transfer Theory

Electron Transfer (ET) represents an important process in chemistry and biology. In 1992, the Nobel prize in chemistry was awarded to Prof. Rudolf Marcus for his development of ET theory, where he established the relationship between \(\Delta G^0\), the reaction free energy, and \(k_{\text{ET}}\), the rate constant of ET (eq 5).\(^8\)

\[
k_{\text{ET}} = k_{\text{ET}} (0) \exp \left\{ -\frac{(\lambda + \Delta G^0)^2}{4 \lambda k_B T} \right\}
\]

In eq 5, \(k_{\text{ET}}(0)\) is the activationless ET rate constant at the point where \(\lambda = -\Delta G^0\). The total reorganizational energy, \(\lambda\), consists of the sum of the inner- and outer-sphere reorganizational energies, \(\lambda_{\text{in}}\) and \(\lambda_{\text{out}}\), respectively.\(^8\) The energy from the structural changes within the molecule (e.g., bond lengths) necessary to accommodate the shift in
charge following ET is taken into account in $\lambda_{in}$. The second term, $\lambda_{out}$, is the solvent reorganizational energy and is given by eq 6.

$$
\lambda_{out} = e^2 \left( \frac{1}{2r_1} + \frac{1}{2r_2} - \frac{1}{r_{12}} \right) \left( \frac{1}{\varepsilon_{op}} - \frac{1}{\varepsilon_s} \right)
$$

(6)

In eq 6, $\varepsilon_{op}$ and $\varepsilon_s$ are the optical and static dielectric constants, respectively, and $r_{12} = r_1 + r_2$ is the sum of the molecular radii of the reactants, $r_1$ and $r_2$.

The dependence of $\ln (k_{ET})$ vs $\Delta G^0$ in eq 5 predicts an increase in the ET rate when the driving force increases for $|\Delta G^0| < \lambda$ (normal region) (Figure 4.1). At the point where $|\Delta G^0| = \lambda$, the fastest, activationless $k_{ET}$ value is expected. As the driving force is increased further, such that $|\Delta G^0| < \lambda$, $k_{ET}$ decreases with driving force, resulting in the Marcus inverted region. Eq 5, therefore, predicts a parabolic dependence of $\ln (k_{ET})$ vs $\Delta G^0$ (Figure 4.1). Figure 4.2 shows the three regimes of ET depicting the differences in activation energy, $E_a$, and $\Delta G^0$.

Since Marcus’ predictions of the driving force dependence on ET rates in 1968, the normal region was observed for bimolecular reactions, however, the inverted region was only observed in 1986 for unimolecular donor-acceptor systems. In those systems the donor (D) and acceptor (A) were incorporated in frozen glasses or polymers, were covalently bound, or were held at a constant distance within proteins in order to eliminate diffusion. Only in the early 90’s was the inverted region observed for bimolecular reactions owing to the leveling-off effect afforded by the rate of diffusion of the reactants. The first observation of bimolecular ET in the inverted region was
demonstrated for the back ET between the one-electron oxidized \([\text{Ir}(1,5\text{-cyclooctadiene})(\mu\text{-pyrazolyl})]_2^+\) complex and a series of reduced pyridinium salts in 1992.\(^{15}\) The next direct observation of the inverted region in ET was possible between cytochrome c and Ru(II) diimine complexes by choosing bimolecular systems in which the rate of the activationless ET process is lower than the rate of diffusion.\(^{16}\)

The dependence of \(k_{ET}\) on various factors was investigated in numerous systems with electron donors and electron acceptors covalently linked through spacers. It was found that \(k_{ET}\) falls exponentially (eq 7) as the distance between D and A increases; this dependence was confirmed by multiple ET systems that include proteins and metal complexes, among others, and follow eq 7.\(^{17,18,19}\)

\[
k_{ET} = k_o \exp \{-\beta (R - R_o)\}
\]

In eq 7, \(\beta\) is the damping constant, \(R_o\) is the edge-to-edge van der Waals closest contact between D and A, and \(R\) is the distance between D and A.\(^{20}\)

The solvent polarity has a strong effect on ET rates, since the charge separated ion pairs are better stabilized by polar solvents. It was experimentally shown that a decrease in solvent polarity results in a decrease in \(\lambda\) which results in changes to the ET rate.\(^{21}\) The effect of the ionic strength on the \(k_{ET}\) was also studied and the ET rates for neutral reactants measured in polar solvents do not change as a function of the ionic strength. In contrast, \(k_{ET}\) increases in non-polar solvents with the ionic strength of the solution.\(^{22}\)
After the publication of Marcus ET theory, multiple reports were devoted to the study of a relationship between $k_{ET}$ and $\Delta G^0$. According to the classic eq 5, the curve $k_{ET}$ vs $\Delta G^0$ is bell-shaped with the maximum at $\Delta G^0 = \lambda$ for systems with adiabatic ET (Figure 4.1). Recently the first direct observation of the bell-shaped energy law in normal, activationless, and inverted regions in intramolecular ET process within covalently linked Zn-porphyrin-imide systems was presented.\(^{23}\) However, the photoinduced ET behavior in several systems that include organic geminate ion pairs,\(^{24}\) and transition metal complexes\(^{5,25}\) were found to deviate from the behavior predicted by the classical ET theory. In those experiments, the normal region was not observed and the logarithm of the charge recombination rate was found to change linearly with the free energy, $\Delta G^0$.\(^{26,27,28}\) In addition, there was little solvent polarity effect on $k_{ET}$.\(^{24}\) despite predictions of the Marcus theory.

**Figure 4.1.** Plot of $\ln(k_{ET})$ vs the free energy driving force, $|\Delta G^0|$. 

4.2.2. Energy Gap Law

After the publication of Marcus ET theory, multiple reports were devoted to the study of a relationship between $k_{ET}$ and $\Delta G^0$. According to the classic eq 5, the curve $k_{ET}$ vs $\Delta G^0$ is bell-shaped with the maximum at $\Delta G^0 = \lambda$ for systems with adiabatic ET (Figure 4.1). Recently the first direct observation of the bell-shaped energy law in normal, activationless, and inverted regions in intramolecular ET process within covalently linked Zn-porphyrin-imide systems was presented.\(^{23}\) However, the photoinduced ET behavior in several systems that include organic geminate ion pairs,\(^{24}\) and transition metal complexes\(^{5,25}\) were found to deviate from the behavior predicted by the classical ET theory. In those experiments, the normal region was not observed and the logarithm of the charge recombination rate was found to change linearly with the free energy, $\Delta G^0$.\(^{26,27,28}\) In addition, there was little solvent polarity effect on $k_{ET}$.\(^{24}\) despite predictions of the Marcus theory.
Figure 4.2. Potential energy surfaces showing the three regimes of ET: normal, activationless, and inverted regions for a D-A pair with varying driving force ($E_a$ = activation energy).
The energy gap law was derived and investigated in detail for the non-radiative decay of emissive MLCT excited states of polypyridyl complexes of Ru(II), Os(II), and Re(I). Several assumptions were made for those systems to be applicable to the energy gap law. The nonradiative decay is dominated by a series of medium-frequency ring stretching modes with energy spacing between 1000 and 1600 cm\(^{-1}\). Those vibrations can be treated as single harmonic oscillator with averaged energy spacing of \(\hbar \omega\) and electron-vibrational coupling constant \(S_M\) (eq 8).

\[
S_M = \frac{1}{2} \frac{M \omega}{\hbar} (\Delta Q_e)^2
\]

In eq 8, \(M\) is the reduced mass, \(\Delta Q_e\) is the change in equilibrium displacement between ground and excited states. Non-radiative decay occurs at \(E_o >> S_M \hbar \omega\) (\(E_o\) is the energy gap) at the weak electronic coupling limit, and at \(\hbar \omega >> k_B T\), when the contribution from the upper vibronic states to the \(\nu^* = 0\) is negligible. In this case, the dependence of the \(\ln (k_{nr})\) on \(E_o\) is linear instead of parabolic as given by eq 9.²⁶

\[
\ln \tau^{-1} \approx \ln k_{nr} \propto -\frac{\nu E_o}{\hbar \omega_M}
\]

The non-radiative decay value depends on the square of the vibrational overlap integral between \(\nu^* = 0\) level in the excited state and \(\nu = 0, 1, 2,..\) the levels of the acceptor in the ground state. As \(E_o\) decreases, the overlap between those two vibrational wave functions increases in the weak coupling limit, thus resulting in shorter lifetimes.
4.2.3. Typical Mechanisms of DNA Cleavage

Metal complexes are able to cleave single-stranded DNA (ss-DNA), ds-DNA, and RNA by various oxidative mechanisms which may target the nucleobases or sugar moieties. Nucleic acids can be targeted by agents following photoexcitation by direct ET from guanine (G), the most easily oxidized base, resulting in a base-sensitive lesion. Singlet oxygen, $^1\text{O}_2$, formed by energy transfer from the excited agent forms products such as 8-oxo-guanine upon its reaction with G and the generation of OH• radicals target the nucleobases or the sugars. In addition, electron transfer to O$_2$ can result in the production of reactive O$_2^-$ and covalent adducts can also be formed between the agent and the nucleobases. Additional DNA damage following irradiation is also possible through the generation of other radicals, reactive excited states, or intermediates.

Deoxyribose oxidation results from H-atom abstraction from the deoxyribose ring producing H$_2$O and a sugar-centered radical, which in turn rearranges to generate unique products that depend on which H atom was removed. If the products are identified, one can deduce which H atom was abstracted from the sugar. In principle, all H atoms are available for abstraction (Figure 4.3), but H-4´ and H-5´ are the most accessible from the minor groove in B-form ds-DNA. It was determined that for non-specific oxidants the theoretical probability of abstraction correlates with C-H bond strength, however, the accessibility of the hydrogen atom remains critical for abstraction. The H-5´ position is the most prone to abstraction by free radicals diffusing along the helix since it is the H-atom most exposed to the solvent in ds-DNA. Furfural is a unique product that results
from H-5′ abstraction. It was previously shown that preference for H-atom abstraction by OH• radical decreases in the order H-5′ > H-4′ > H-2′ ~ H-3′ > H-1′.

Figure 4.3. Molecular structure of deoxyribose ring.

4.2.4. Dirhodium Tetraacetate and Related Complexes

Dinuclear systems with d7-d7 and d8-d8 electron configuration are known to produce diradical excited states upon photoexcitation (Figure 4.4). These excited states resemble the activated form of known enediyne antitumor agents. The enediynes are able to effect double-stranded cleavage of ds-DNA from the diradical intermediate, which is able to abstract two H-atoms simultaneously from nearby sugars. The d8-d8 bimetallic complex [Pt2(pop)4]4+ [pop = (HO)(O)POP(O)(OH)2] was found to undergo direct hydrogen abstraction from alcohols and ds-DNA from its excited state.42 The mechanism for hydrogen abstraction is believed to proceed through the formation of a long-lived triplet excited state of the complex, *[Pt2(pop)4]+.42 However, the negative charge of [Pt2(pop)4]4+ precludes the binding of the complex to the anionic DNA. This electrostatic repulsion results in low efficiency of DNA photocleavage.
Dirhodium decaacetonitrile(II,II) was shown to form two mononuclear metal-centered radicals upon irradiation with $\lambda > 435$ nm, which eventually recombine to regenerate the starting material (Figure 4.5a). In $[\text{Rh}_2(\text{CH}_3\text{CN})_{10}]^{4+}$, the initially formed radicals can diffuse away from each other in solution, and therefore would be unlikely to effect double strand cleavage of ds-DNA. The $d^7-d^7$ dirhodium tetraacetate complex, $\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_4$ (Figure 4.5b), possesses four bridging ligands that keep the rhodium nuclei together, thus making diradical photochemistry possible upon excitation.

\[ \text{Enediyne} \quad \Delta, \text{UV } h\nu \quad \text{vis } h\nu \quad \text{vis } h\nu \]

\[ M = \text{Pt(III), Rh(II)} \quad d^7-d^7 \quad M = \text{Pt(II)} \quad d^8-d^8 \]

**Figure 4.4.** Photoinduced and thermal Bergman cyclization of enediyines and photogenerated radical excited states in $d^7-d^7$ and $d^8-d^8$ complexes.

48
Figure 4.5. Molecular structures of (a) $[\text{Rh}_2(\text{CH}_3\text{CN})_{10}]^{2+}$ ($\text{AN} = \text{CH}_3\text{CN}$) and (b) $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4$.

Variation of the axial ligand $L$ can lead to dramatic changes in the electronic absorption spectra of dirhodium complexes such as $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4(L)_2$, resulting from energy changes in orbitals that interact strongly with the symmetric and antisymmetric linear combinations of the axial ligand orbitals. In particular, the metal-metal $\sigma$ and $\sigma^*$ MO’s, which are involved in low-energy transitions, are affected significantly by axial coordination. From EPR spectra, electrochemical measurements, crystal structure, and electronic structure calculations of dirhodium tetracarboxylate complexes, their electronic structure was elucidated.\(^{44,45}\) The electron configuration was shown to be $\sigma^2 \pi^4 \delta^2 \delta^* \pi^*^4$ for $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4$ with weakly $\sigma$-donating axial ligands ($\text{H}_2\text{O}$, THF, alcohols, nitriles, chloride) and $\pi^4 \delta^2 \pi^*^4 \delta^* \sigma^2$ for strong $\sigma$-donating ligands such as phosphines and phosphates.\(^{46}\) Recent research showed that the excited state of $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4(L)_2$ is long-lived (4-5 $\mu$s) and its lifetime and spectral features are independent of $L$ ($L = \text{py}$, THF, PPh$_3$, CH$_3$OH).\(^{47}\) The electron configuration of the excited state is believed to be $\sigma^2 \pi^4 \delta^2 \delta^* \pi^*^3 \sigma^1$ in $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4(\text{PPh}_3)_2$.\(^{48}\)
Dirhodium complexes have been shown to possess anti-cancer activity in a manner similar to cisplatin.\textsuperscript{49} \( \text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4(\text{CH}_3\text{OH})_2 \) has been shown to bind covalently to ss-DNA and to inhibit transcription.\textsuperscript{50} Several covalent Rh-DNA products have been reported including DNA interstrand cross-links.\textsuperscript{51} The complex was shown to bind to AA sites of ss-DNA through the binding to the axial sites of \( \text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4 \).\textsuperscript{52} In general, the lability of the axial ligand drives the product formation.

It was shown that \( \text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4 \) is not able to cleave DNA upon excitation, but the photochemically generated \([\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4]^+\) cation is able to effect single strand photocleavage of ds-DNA.\textsuperscript{53} The reactivity of the cation is believed to arise from the photocatalytic production of OH• from \( \text{H}_2\text{O} \) as shown in Figure 4.6. Once excited, \( ^*\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4 \) transfers an electron to an acceptor to form a cation. DFT calculations have shown that after deprotonation of an axial \( \text{H}_2\text{O} \), two resonance forms of the \([\text{Rh}_2]-\text{OH}\) adduct are possible.\textsuperscript{54} One of those forms, with OH• radical in the axial position, is likely responsible for the H-atom abstraction from ds-DNA. Once the H atom is abstracted, the \( \text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4 \) complex returns to its ground state, which makes this cycle photocatalytic.\textsuperscript{47}

\( \text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4 \) was shown to abstract H-atoms from i-PrOH and ds-DNA upon irradiation with visible light in the presence of \( N\)-methyl-3-CN-pyridinium tetrafluoroborate, an external electron acceptor, to produce acetone and nicked DNA, respectively. When the DNA photolysis products were analyzed, furfural was found suggesting that DNA nicking occurs from H-5 abstraction. This result is consistent with H-atom abstraction by a diffusible radical in solution.
However, the requirement of an external electron acceptor makes the use of \( \text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_4 \) as a PDT agent impractical. To circumvent this problem, new dirhodium complexes (Figure 4.7) with one or two photoactive dppz ligands serving as internal electron acceptor were synthesized.

![Figure 4.6](image)

**Figure 4.6.** Photocatalytic cycle of \( \text{OH}^\bullet \) generation via the oxidation of the \( \text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_4(\text{H}_2\text{O})_2 \) following electron transfer to an electron acceptor A.

![Figure 4.7](image)

**Figure 4.7.** Molecular structures of (a) \( \text{cis-[Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{dppz})(\eta^1\text{-O}_2\text{CCH}_3)\text{L}]^+ \) (L = \( \text{CH}_3\text{OH} \)) and (b) \( \text{cis-[Rh}_2(\mu-\text{O}_2\text{CCH}_3)_4(\text{dppz}_2)]^{2+} \) (\( R_1 = R_2 = \text{H} \)).
4.2.5. Preliminary Studies on Dirhodium dppz Complexes

The DNA photocleavage and binding of $\text{cis-}[\text{Rh}_2(\mu-O_2\text{CCH}_3)_2(\text{dppz})(\eta^1-O_2\text{CCH}_3)(\text{CH}_3\text{OH})]^+\,$ (mono-dppz) and $\text{cis-}[\text{Rh}_2(\mu-O_2\text{CCH}_3)_2(\text{dppz})_2]^{2+}\,$ (bis-dppz) were previously investigated.$^{1,2}$ Both complexes showed negative deviation from Beer’s Law due to $\pi$-stacking aggregation of the hydrophobic dppz ligands in water.

The DNA binding constants, $K_b$, for the mono-dppz and bis-dppz complexes were found to be $1 \times 10^3$ and $4 \times 10^3$ M$^{-1}$, respectively, measured by equilibrium dialysis. Positive shifts in the relative viscosity of DNA indicated intercalation of the mono-dppz complex, while the bis-dppz complex did not affect the relative viscosity of DNA solutions. The absence of DNA intercalation by the bis-dppz complex is believed to arise from the presence of the two dppz ligands in an almost parallel orientation (Figure 4.8).

The distance between the DNA bases in ds-DNA is 3.4 Å.$^{55}$ Intercalation of an aromatic molecule causes the DNA bases to spread apart, however, they cannot accommodate two ligands that are already 3.494 Å apart.

Both the mono- and bis-dppz complexes produce nicked DNA upon irradiation with visible light in the absence of an external electron acceptor. Greater DNA photocleavage was observed for the mono-dppz complex, which is probably due its intercalation and greater binding constant compared to the bis-dppz complex. Both compounds effect single-stranded cleavage of ds-DNA under aerobic and anaerobic conditions.
The cytotoxicity, LD\textsubscript{50} (LD\textsubscript{50} represents the concentration of the agent that results in 50 % cell death), toward human skin cells of the mono-dppz complex is 27 ± 2 μM, which is significantly higher than that of bis-dppz complex (135 ± 8 μM). This difference is likely due to the DNA intercalation of the former. Irradiation with 400 < λ < 700 nm (5 J / cm\textsuperscript{2}) results in an increase in the toxicity to 21 ± 2 μM and 39 ± 1 μM for mono- and bis-dppz complexes, respectively. This small increase in cytotoxicity of \textit{cis}-[Rh\textsubscript{2}(\mu-O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}(dppz)(η\textsubscript{1}-O\textsubscript{2}CCH\textsubscript{3})(CH\textsubscript{3}OH)]\textsuperscript{2+} upon irradiation does not make it a desirable PDT agent. In contrast, the low cytotoxicity of \textit{cis}-[Rh\textsubscript{2}(dppz)\textsubscript{2}(\mu-O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}]\textsuperscript{2+} in the dark and decrease of LC\textsubscript{50} upon irradiation makes it an attractive target for further investigation as a potential PDT agent. Therefore, a new series of compounds with general formula \textit{cis}-[Rh\textsubscript{2}(\mu-O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}(R\textsubscript{1},R\textsubscript{2}-dppz)\textsubscript{2}]\textsuperscript{2+} was synthesized and their DNA photocleavage was investigated.
4.2.6. Bis-crown Ethers

Since the discovery of the ability of neutral crown ethers to bind alkali and alkaline ions\textsuperscript{56} a novel avenue of supramolecular and host-guest chemistry has continued to develop. Different cyclic and cage organic systems were synthesized\textsuperscript{57,58} and numerous studies on the incorporation of metal ions inside cavity were reported. Some dyes, derivatives of benzene-15-crown-5-ether, were found to be sensitive ionophores that change color upon binding of a specific ion.\textsuperscript{59} Derivatives of the aza-18-crown-6 ethers have demonstrated excellent “on-off” fluorescence switching driven by probe binding.\textsuperscript{60} A coumarin-based macrocycle is currently used as a chemosensor for the detection of the marine toxin saxitoxin.\textsuperscript{61} Many crown-based sensors are currently used in ion-selective electrodes,\textsuperscript{60} and recently researchers turned their attention to inorganic hosts due to the possible photochemical properties associated with MLCT excited state.\textsuperscript{62} Multiple Ni(II), Ru(II), Re(I), Cu(I) and Zn(II) complexes with crown ethers attached to ligand moiety have been investigated.\textsuperscript{62,63,64,65,66}

The binding affinities of crown ethers to alkali and alkaline metal ions are dependent on by the cavity size of the crown ether.\textsuperscript{56a} Bis-15-crown-5 ethers (Figure 4.9) have shown selectivity to form 1:1 complexes with K\textsuperscript{+}, Rb\textsuperscript{+}, Cs\textsuperscript{+}, and NH\textsubscript{4}\textsuperscript{+} possessing sandwich-like structure. Due to the presence of the two crown ethers, metal ions whose diameter is larger than the crown ether cavity are bound more selectively. This phenomenon is called the “bis crown effect”. Smaller cations, like Na\textsuperscript{+}, form 2:1 complexes, with one Na\textsuperscript{+} residing on each crown ether.\textsuperscript{67,68,69} The stability constants measured for bis-15-crown-5 with a trianisyl bridge decrease as K\textsuperscript{+} > Rb\textsuperscript{+} > Cs\textsuperscript{+} \approx \text{NH}_4\textsuperscript{+}, in agreement with the relative size of the ion and the crown ether cavity.\textsuperscript{70}
Due to the possible effect of the cations incorporated into crown systems on the properties of the complex, the electrochemical potentials and DNA photocleavage activity of $\text{cis-[Rh}_2(\mu-O_2CCH_3)_2(15\text{-crown-5-dppz})_2]^{2+}$ (10) and $\text{cis-[Rh}_2(\mu-O_2CCH_3)_2(18\text{-crown-6-dppz})_2]^{2+}$ (11) were investigated in the presence of different alkali and alkaline metal ions.

\textbf{Figure 4.9.} Schematic illustration of bis(crown ethers). Two crown subunits are connected through the bridge X (X = alkene or alkane chain, metallocene, trianisyl) (Adapted from reference 70).
4.3. Results and Discussions

4.3.1. Ground State Properties of cis-[Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂]²⁺

Several dirhodium complexes with the general formula cis-[Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂](O₂CCH₃)₂ were synthesized by a method analogous to that previously reported,² and the structures of the substituted dppz ligands are shown in Figure 4.10. Complexes with electron-withdrawing groups on the dppz ring (1-3) are moderately soluble in DMSO and DMF, while complexes with electron-donating groups (4-11) are soluble in H₂O, CH₃CN, DMSO, and DMF.

The electronic absorption spectra of complexes 1-11 are shown in Figure 4.11, and Table 4.1 lists the maxima along with the corresponding ε values. The bands centered at ~280 nm and ~370 nm are likely associated with intraligand ππ* transitions of the dppz ligand. Those transitions were also reported for [Rh₂(μ-O₂CCH₃)₂(dppz)(η¹-O₂CCH₃)(CH₃OH)]⁺ and dppz-containing Ru(II) complexes.¹,⁷¹,⁷² A shoulder at ~420 nm was also observed in cis-[Rh₂(μ-O₂CCH₃)₂L₂]²⁺ (L = bpy, 1,10-phenanthroline) (ε ~ 2 × 10³ M⁻¹ cm⁻¹)³ and Rh₂(μ-O₂CCH₃)₄ (ε ~ 112 M⁻¹ cm⁻¹) spectra.⁷⁴,⁷⁵ The 20-fold difference in ε values suggests that this transition may involve polypyridyls and is LMCT in nature.⁷³ The lowest energy transition at λ > 500 nm is common for dirhodium complexes and is centered on the bimetallic core, previously assigned as arising from Rh₂(π*) → Rh₂(σ*).
Figure 4.10. Molecular structures of the $R_1,R_2$-dppz ligands and the numbering scheme for the $cis$-$[\text{Rh}_2(\mu_2\text{O}_2\text{CCH}_3)_2(R_1,R_2\text{-dppz})_2]^{3+}$ complexes.
Figure 4.11. Electronic absorption spectra of cis-[Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂](O₂CCH₃)₂ in water: (----) 1, (······) 2, (−−−) 7, and (-----) 8.

Table 4.1. Reduction potentials and absorption data of 1-11 (continued).

<table>
<thead>
<tr>
<th>Complex</th>
<th>E₁/₂, V vs NHE</th>
<th>λ₁ absorb, nm (×10⁻³ OD, M⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.61, -1.09, -1.56</td>
<td>280 (57.6), 375 (25.4), 490sh (9.5)</td>
</tr>
<tr>
<td>2</td>
<td>-0.74, -0.97, -1.36, -1.49</td>
<td>277 (60.4), 375 (17.8), 393 (17.0), 440sh (5.3), 580 (1.0)</td>
</tr>
<tr>
<td>3</td>
<td>-0.81, -1.11, -1.36, -1.63</td>
<td>278 (83.7), 370 (18.6), 383 (18.1), 420sh (6.7), 520sh (1.8)</td>
</tr>
<tr>
<td>4</td>
<td>-0.84, -1.03, -1.63</td>
<td>278 (57.9), 360 (11.7), 363 (15.2), 434 (5.5), 590 (0.4)</td>
</tr>
<tr>
<td>5</td>
<td>-0.84, -1.10, -1.41, -1.61</td>
<td>283 (67.4), 375 (13.3), 420sh (5.5), 520sh (1.2)</td>
</tr>
<tr>
<td>6</td>
<td>-0.83, -1.21, -1.40, -1.53</td>
<td>280 (67.0), 384 (11.5), 431sh (2.2), 550sh (0.2)</td>
</tr>
<tr>
<td>7</td>
<td>-0.87, -1.16, -1.71</td>
<td>285 (67.4), 386sh (14.3), 431sh (2.5), 530sh (0.4)</td>
</tr>
</tbody>
</table>
The oxidation potential of the precursor, Rh$_2$(μ-O$_2$CCH$_3$)$_4$ was measured to be 1.41 V vs NHE in CH$_3$CN as was previously reported. E$_{1/2}$(Rh$_2$$^{+}$/$^{0}$) was obtained only for 11 in CH$_3$CN due to the limited solubility of the other complexes in this solvent and was found to be 1.04 ± 0.05 V vs NHE. Better stabilization of the oxidized [Rh$_2$] core is consistent with an increase of electron density on [Rh$_2$] from the nucleophilic nitrogen atoms and π-backbonding from dppz that stabilize Rh(III) state. The dppz-centered reduction potentials listed in Table 4.1 were obtained in DMSO and are composed of three or four reversible one-electron waves, which are possibly consecutive one-electron reductions of the dppz ligands. It is evident from Table 4.1 that ligands with electron-withdrawing groups are easiest to reduce. The dppz-centered reduction potentials obtained here are similar to those for mononuclear complexes, where for [Co(phen)$_2$(dppz)]$^{3+}$ and [Ni(phen)$_2$(dppz)]$^{2+}$ reduction potentials at −0.93 and −1.05 V vs NHE were reported, respectively. For [Ru(bpy)$_2$(dppz)]$^{2+}$ and [Ru(bpy)$_2$(di-CH$_3$-dppz)]$^{2+}$ the first dppz-based reduction potentials are −0.73 and −0.81 V vs NHE, respectively.
4.3.2. DNA Binding

Since the 1-11 complexes are structurally similar, complex 3 was chosen as a representative member of the series for the measurement of the changes in the relative viscosity of the DNA solutions. The stock solutions of the probes were prepared in CH₃OH. As shown in Figure 4.12, the relative viscosity of DNA increases upon addition of EtBr, a typical intercalator. In contrast, no changes in the relative viscosity of DNA were observed as a function of increasing concentration of 3. Therefore, intercalation can be excluded as a binding mode of 3, as was also shown for bis-dppz.² By analogy, it is expected that cis-[Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂]²⁺ complexes do not intercalate into DNA.

![Figure 4.12](image)

**Figure 4.12.** Relative viscosity plot as a function of R = [complex]/[DNA] for (•) EtBr and (■) 3.
4.3.3. DNA Photocleavage

Due to the potential use of the cis-[Rh\(_2\)(μ-CO\(_2\)CCH\(_3\))\(_2\)(R\(_1\),R\(_2\)-dppz)\(_2\)]\(^{2+}\) complexes as potential PDT agents, the wavelength dependence of the DNA photocleavage was investigated (Figure 4.13). The photocleavage of ds-DNA by 4 is efficient upon irradiation with light in the 395-495 nm range (Lanes 2-4). However, upon irradiation with λ > 550 nm (Lane 5) no photocleavage was observed. These results limit the use of complex 4 as a PDT agent, since the therapeutic window is between 600 and 800 nm.

In order to elucidate the mechanism of DNA photocleavage, the reaction of 4 with pUC18 plasmid was performed under aerobic, anaerobic conditions and in the presence of various scavengers. It is evident from Figure 4.14 the amount of photocleaved DNA is similar under anaerobic (58%) and aerobic conditions (54%), thus ruling out possible deactivation of the *[Rh\(_2\)] by energy or electron transfer to O\(_2\). Mannitol and HCOONa, which are hydroxyl radical scavengers, did not affect the photocleavage activity of the complex (60% and 53%, respectively), while NaN\(_3\), which is a \(^1\)O\(_2\) and OH• scavenger, reduced the amount of damaged DNA to 25%. This result may be indicative of the potential activity of OH• in DNA damage, since O\(_2\) is not involved in DNA photocleavage. However, NaN\(_3\) is also reactive with many other species.
Figure 4.13. EtBr imaged 1% agarose gel showing photocleavage of 100 μM pUC18 plasmid by 20 μM 4 irradiated for 20 min (5 mM Tris, pH 7.5, 50 mM NaCl). Lane 1, plasmid only, dark; lanes 2-4, pUC 18 + 3, irradiated in air: λ > 395 nm (lane 2), λ > 435 nm (lane 3), λ > 495 nm (lane 4), λ > 550 nm (lane 5).

The DNA photocleavage of 1-11 exhibit a strong dependence on the substituents, while the free dppz ligands did not photocleave DNA under similar experimental conditions (Figure 4.16). Table 4.2 lists the percent of DNA photocleavage by 1-11 relative to 4. Table 4.2 shows that complexes with electron-withdrawing groups in the dppz ligand are less effective photocleavage agents than those with electron-donating groups. As shown in Figure 4.16, 9 cleaves 95% of pUC18, while 4 is nearly half as active (58%). Moreover, the DNA photocleavage by 1 is very low (5%) under similar irradiation conditions (Figure 4.17).
Figure 4.14. EtBr imaged 1% agarose gel showing the photocleavage results of pUC18 (100 μM) by 5 μM 4 (λ > 395 nm, 15 min). Lane 1, plasmid only, dark; lane 2-7, pUC 18 + 3: dark in air (lane 2), irradiated in air (lane 3), irradiated under argon (lane 4), irradiated with 2 mM mannitol in air (lane 5), irradiated with 2 mM HCOONa in air (lane 6), irradiated with 2 mM NaN₃ in air (lane 7).

Figure 4.15. EtBr imaged 1% agarose gel showing the photocleavage results (λ > 395 nm, 15 min) of 100 μM DNA by 5 μM R₁,R₂-dppz ligands. Lane 1: DNA only, dark; lane 2: DNA + di-OMe-dppz, dark; lane 3: DNA + di-OMe-dppz, irr.; lane 4: DNA + OMe-dppz, dark; lane 5: DNA + OMe-dppz, irr.; lane 6: DNA + di-Me-dppz, dark; lane 7: DNA + di-Me-dppz, irr.; lane 8: DNA + di-Cl-dppz, dark; lane 9: DNA + di-Cl-dppz, irr.; lane 10: DNA + di-NO₂-dppz, dark; lane 11: DNA + di-NO₂-dppz, dark.
<table>
<thead>
<tr>
<th>% Cleavage Relatively to 4</th>
<th>-ΔG°, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>0.70</td>
</tr>
<tr>
<td>3</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>1.11</td>
</tr>
<tr>
<td>6</td>
<td>1.15</td>
</tr>
<tr>
<td>7</td>
<td>1.03</td>
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<tr>
<td>8</td>
<td>1.35</td>
</tr>
<tr>
<td>9</td>
<td>1.60</td>
</tr>
<tr>
<td>11</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Table 4.2. Percent of nicked DNA calculated relatively to 4 and -ΔG° calculated from eq 18.

Figure 4.16. EtBr stained 1% agarose gel showing the results (λ > 395 nm, 15 min) of pUC18 photocleavage (100 μM) by 5 μM [Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂]²⁺ in air. Lane 1: DNA only, dark; lane 2: DNA + 4, dark; lane 3: DNA + 4, irr.; lane 4: DNA + 7, dark; lane 5: DNA + 7, irr.; lane 5: DNA + 9, dark.; lane 6: DNA + 9, irr.
Figure 4.17. EtBr stained 1% agarose gel showing the results (λ > 395 nm, 15 min) of pUC18 photocleavage (100 μM) by 5 μM [Rh₂(μ-O₂CCH₃)₂(R₁,R₂-dppz)₂]²⁺ in air. Lane 1: DNA only, dark; lane 2: DNA + 4, dark; lane 3: DNA + 4, irr.; lane 4: DNA + 2, dark; lane 5: DNA + 2, irr.; lane 5: DNA + 1, dark.; lane 6: DNA + 1, irr.

Figure 4.18. Proposed DNA photocleavage from the charge-separated state of cis-[Rh₂(μ-O₂CCH₃)₂(dppz)₂]²⁺.

The presence the dppz ligands in complexes 1-11 and the known requirement of the presence of an electron acceptor for the DNA photocleavage by Rh₂(O₂CCH₃)₄
suggests that the dppz ligand may act as an electron acceptor as shown in Figure 4.18. Once excited, \([\text{Rh}_2(\mu-O_2CCH_3)_2(\text{dppz})_2]^{2+}\) may undergo intramolecular ET from the dirhodium core to the dppz ligand with the accommodation of the electron on dppz moiety to form the charge-separated species \([\text{Rh}_2^+-\text{dppz}^-]\). Time-resolved experiments performed with \([\text{Ru(bpy)}_2(\text{dppz})]^{2+}\) show that in its \(^3\text{MLCT}\) excited state the electron on the dppz ligand is localized on the phenazine part of the ligand.\(^{81,82}\) It may be postulated that the charge-separated species \([\text{Rh}_2^+-\text{dppz}^-]\) can either return to its ground state via charge recombination, CR, or perform the H-atom abstraction from DNA following OH\(^-\) generation by the oxidized Rh\(_2\) core. Therefore, according to the scheme in Figure 4.18, the effectiveness of the DNA photocleavage depends on CR rate constant, \(k_{\text{CR}}\) (eq 10-13).

\[
\frac{d[\text{DNA}]_{\text{nicked}}}{dt} = k_{\text{DNA}}[\text{DNA}][\text{Rh}_2^+-\text{dppz}^-] \tag{10}
\]

\[
\frac{d[\text{Rh}_2^+-\text{dppz}^-]}{dt} = k_{\text{ET}} \cdot *[\text{Rh}_2-\text{dppz}] - k_{\text{CR}}[\text{Rh}_2^+-\text{dppz}^-] - k_{\text{DNA}}[\text{DNA}][\text{Rh}_2^+-\text{dppz}^-] \tag{11}
\]

Since the photon density does not change in time, the concentration of \(*[\text{Rh}_2-\text{dppz}]\) in the solution, and, consequently, that of \([\text{Rh}_2^+-\text{dppz}^-]\) is constant, so the steady-state approximation can be applied (eq 12).

\[
\frac{d[\text{Rh}_2^+-\text{dppz}^-]}{dt} = 0 \tag{12}
\]

\[
[\text{Rh}_2^+-\text{dppz}^-] = \frac{k_{\text{ET}} \cdot *[\text{Rh}_2-\text{dppz}]}{k_{\text{CR}} + k_{\text{DNA}}[\text{DNA}]} \tag{13}
\]

Combination of eq 10 and 13 results in eq 14.

66
Assuming that the initial charge separation is much faster than CR and similar in all complexes, all the terms in eq 14 should be constant from one complex to another with the exception of $k_{CR}$, which is unique for each complex. Accordingly to this scheme, the DNA photocleavage should be proportional to $[Rh_2\textsuperscript{2+}-dppz]$ and inversely proportional to $k_{CR}$. It should be noted that the compound with the lowest CR rate should exhibit the greatest lifetime of the charge-separated state, since $\tau = 1 / k_{CR}$.

4.3.4. Relationship Between CR and DNA Photocleavage

As was shown above, the DNA photocleavage should depend on $k_{CR}$ and, therefore, on the reduction potential of the dppz ligand of the complex involved in the reaction. It is expected that the return to the ground state will be the faster as the energy difference between two states decreases according to the energy gap law (eq 9).\textsuperscript{83,84,85}

All dirhodium complexes investigated are non-emissive at room temperature or at 77 K.\textsuperscript{86} In this limit, equations 15-17 can be written.\textsuperscript{26}

$$\tau^{-1} = k_r + k_{nr}$$ \hspace{1cm} (15)

$$\Phi_{em} = \pi k_r$$ \hspace{1cm} (16)

$$\tau^{-1} \approx k_{nr}$$ \hspace{1cm} (17)

Since the rate of the non-radiative decay, $k_{nr}$, is much greater then the rate of radiative decay, it can be assumed that $k_{CR} \sim k_{nr}$. Varying the substituents $R_1$ and $R_2$ on...
the dppz ligand results in driving forces for charge recombination, $\Delta G^o$, from -2.00 V to -1.65 V. $\Delta G^o$ can be calculated from the electrochemical data for 1-11 listed in Table 4.2, and eq 18, using $E_{1/2}(\text{Rh}_2^{+/0}) = 1.04$ V vs NHE.

$$\Delta G^o = E_{1/2}(R_1,R_2\text{-dppz}^{0/-}) - E_{1/2}(\text{Rh}_2^{+/0})$$  \hspace{1cm} (18)

It is expected that the energy of the charge-separated state, [Rh$_2^{+}$-dppz$^{-}$], is smallest for [Rh$_2$(μ-O$_2$CCH$_3$)$_2$(di-NO$_2$-dppz)$_2$]$^{2+}$ owing to the electron-withdrawing groups on the dppz ring. The charge recombination (CR) is highly exergonic and is expected to lie in the inverted region, therefore $k_{CR}$ should decrease as the driving force, $|\Delta G^o|$, increases. As was shown above, [DNA]$_{nicked}$ should be inversely proportional to $k_{CR}$ (or $k_{nr}$) (eq 14), therefore, ln(1/[DNA]$_{nicked}$) should be directly proportional to $|\Delta G^o|$ according to the energy gap law (eq 9). The results are plotted in Figure 4.19 for the DNA photocleavage of complexes 1-11.

The plot in Figure 4.19 shows that there is a linear correlation between ln(1/[DNA]$_{nicked}$) and $|\Delta G^o|$. In this series of complexes, the ET distance between acceptor and donor is similar and there are likely only small changes in the vibrational structure caused by the substituents on the dppz ligand.

Previous examples of back intramolecular ET in [(4,4'-X)$_2$-bpy)Re(CO)$_3$(py-PTZ)]$^+$ (X = CO$_2$Et, C(O)NEt$_2$, H, CH$_3$,OCH$_3$) and [(bpz)Re(CO)$_3$(py-PTZ)]$^+$ (bpz = 2,2'-bipyrazine) have also shown perfect linear correlations of ln($k_{CR}$) vs $\Delta E_{1/2}$ in accordance with eq 9, where with $k_{nr}$ varied from $2.6 \times 10^5$ to $2.8 \times 10^8$ s$^{-1}$.\textsuperscript{22} In these systems, the $S_M$ and $h\omega$ values were obtained from the time-resolved Raman IR spectroscopy and Frank-
Condon analyses of the emission profiles. The presence of the medium and high frequency vibrational modes of the acceptor are critical, since it allows nuclear tunneling (vibrational overlap) between the states.

![Figure 4.19](image)

**Figure 4.19.** Plot of the ln (1/[DNA]\textsubscript{nicked}) vs energy gap, |ΔG|, V, for the DNA photocleavage by complexes 1-11. The corresponding complexes are indicated in the plot.

4.3.5. Requirement of Free Axial Positions for DNA Photocleavage

In the scheme for the production of OH\textsuperscript{•} shown in Figure 4.6, the accessibility of the axial position to H\textsubscript{2}O is critical for H-atom abstraction to take place. The coordination of labile molecules at the axial sites, like water or CH\textsubscript{3}OH, makes H-atom abstraction possible. [Rh\textsubscript{2}(μ-O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}(np)\textsubscript{2}](BF\textsubscript{4})\textsubscript{2} (np = 1,8-naphthyridine) and the
related complex \( [\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{pynp})_2](\text{BF}_4)_2 \) (pynp = 2-(2-pyridyl)-1,8-naphthyridine) shown in Figure 4.20 were utilized to compare the DNA photocleavage by complexes with the open and blocked axial positions.

The DNA photocleavage by \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{np})_2]^{2+} \) and \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{pynp})_2]^{2+} \) complexes was performed in the presence of an electron acceptor \( \text{py}^+ \) (\( \text{py}^+ = \text{N}-\text{methyl-3-CN-pyridinium tetrafluoroborate} \)) (Figure 4.21). Neither complex photocleaves DNA in the dark in the presence of \( \text{py}^+ \) (Lanes 2 and 5), and \( \text{py}^+ \) alone is not photoactive under the present experimental conditions (Lane 8). Upon irradiation, \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{np})_2]^{2+} \) (Lanes 6 and 7) cleaves DNA more effectively than \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{pynp})_2]^{2+} \) (Lanes 3 and 4) under anaerobic and aerobic conditions. Some inhibition of the photocleavage activity of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{np})_2]^{2+} \) was observed in air (Lane 6) compared to the photocleavage under nitrogen (Lane 7), which may be due to the deactivation of *\( [\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{np})_2]^{2+} \) by oxygen. Inhibition of the DNA photocleavage in air was also observed for \( \text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_4 \). These results show that the presence of available axial coordination sites in dirhodium complexes are a desirable structural characteristic for DNA photocleavage.
4.3.6. Generation of Hydroxyl Radical

The detection of the OH• is an important problem in biological, analytical, and physical chemistry. Several methods, such as...
chemiluminescence, EPR, HPLC, and GCMS have been utilized to detect various products after trapping of OH• by different substrates. Fluorescence from 2-hydroxyterephthalate is a very sensitive and specific method of OH• detection (eq 19). Terephthalic acid (TA) is non-emissive itself, but the reaction product generated following the Fenton’s reaction is highly emissive at 438 nm. The reported scavenging rate of OH• by TA is \(3.3 \times 10^9\) M\(^{-1}\)s\(^{-1}\), which is close to the diffusion limit.

\[
\begin{align*}
\text{COOH} & \quad + \quad \cdot\text{OH} \\
\text{COOH} & \quad \text{terephthalic acid} \\
\text{COOH} & \quad \text{2-hydroxyterephthalic acid}
\end{align*}
\]

In order to verify OH• production coupled with the charge separated excited states of the [Rh\(_2(\mu-O_2CCH_3)_2(R_1,R_2-dppz)_2\)]\(^{2+}\) complexes in H\(_2\)O, 75 \(\mu\)M of complex 9 was irradiated (\(\lambda_{\text{irr}} > 395\) nm) with 100 mM TA in 25 mM Tris, pH 7.5. Initially, at \(t = 0\) min no emission was observed, but the luminescence (\(\lambda_{\text{em}} = 438\) nm) increased as a function of irradiation time (Figure 4.22). After 40 min the concentration of 2-hydroxyterephthalate has 0.4 \(\mu\)M, as was calculated from comparison to a calibration graph (Figure 4.23). The calibration graph was constructed by measuring the emission intensity of dilute stock solutions at various concentrations of authentic 2-hydroxyterephthalate.
Figure 4.22. Fluorescence increase ($\lambda_{\text{exc}} = 320 \text{ nm}; \lambda_{\text{em}} = 438 \text{ nm}$) of 2-hydroxyterephthalate upon irradiation of 75 $\mu$M 9 and 100 mM terephthalic acid in aqueous solution (25 mM Tris, pH 7.5, 450 W short-arc lamp used as a light source, $\lambda_{\text{irr}} > 395 \text{ nm}$): (⋯) $t = 0 \text{ min}$; (——) $t = 40 \text{ min}$.

Figure 4.23. Calibration plot of the emission intensity of 2-hydroxyterephthalate in 5mM Tris (pH 7.5) vs its concentration.
The characteristic emission did not appear after the irradiation of \([\text{Rh}_2(\mu-O_2CCH_3)_2(\text{di-OMe-dppz})]^2+\) (9) alone under similar experimental conditions, or after the irradiation of \([\text{Rh}_2(\mu-O_2CCH_3)_2(\text{phen})_2]^2+\) (phen = 1,10-phenanthroline) with TA. The appearance of emission at 438 nm is consistent with the nucleophilic attack by OH• of TA.

4.3.7. Complexation Studies

Several methods, including NMR, conductivity, voltammetry, polarography, and electrospray ionization mass spectrometry are typically utilized to measure the binding constants of metal ions to crown ethers. Binding constants along with the number of ions bound to the ethers can also be obtained from the absorption changes upon addition of the ions and are derived below.\

\[
[\text{Rh}_2] + n\text{M}^+ \rightleftharpoons K_b \cdot [\text{Rh}_2 \bullet \text{nM}]^n+ \quad (19)
\]

\[
K_b = \frac{[\text{Rh}_2 \bullet \text{nM}^+]^n}{[\text{Rh}_2]^* \cdot [\text{M}^+]^n} \quad (20)
\]

\[
[\text{Rh}_2] = [\text{Rh}_2]^o - [\text{Rh}_2 \bullet \text{nM}]^{n-} \quad (21)
\]

In eq 20 and 21, \([\text{Rh}_2]^o\) is the initial concentration of the dirhodium complex, \([\text{Rh}_2]\) is the concentration of the free dirhodium complex after the addition of the cations, \([\text{Rh}_2 \bullet \text{nM}]^+\) is the concentration of the complex bound to the cations, \(K_b\) is the binding constant of the metal ion to dirhodium complex. Eq 22 is the combination of eq 20 and 21.
\[ K_b = \frac{[Rh_2 \cdot nM]^{n+}}{([Rh_2]_o - [Rh_2 \cdot nM]^{n+})[M^{+}]^n} \]  

(22)

\[ A_o = \varepsilon_o [Rh_2]_o \]  

(23)

\[ A = \varepsilon_o ([Rh_2]_o - [Rh_2 \cdot nM]^{n+}) + \varepsilon_b [Rh_2 \cdot nM]^{n+} \]  

(24)

\[ A = A_o + (\varepsilon_b - \varepsilon_o) [Rh_2 \cdot nM]^{n+} \]  

(25)

\[ [Rh_2 \cdot nM]^{n+} = \frac{A_o - A}{\varepsilon_o - \varepsilon_b} \]  

(26)

In eq 22–26, \( A_o \) and \( \varepsilon_o \) are the absorbance and extinction coefficients of the complex at the absence of cations, respectively, \( M^+ \), \( A \) is the absorbance of the complex at the presence of \( [M^+] \), \( \varepsilon_o \) is the extinction coefficient of the bound species; \( n \) is the number of metal ions bound to metal complex. Eq 22 can be easily transformed into eq 27. Eq 28 is a result of logarithm operation of eq 27 after the plugging of the meanings of \([Rh_2]_o\) and \([Rh_2\cdot nM]^{n+}\) from eq 23 and 26.

\[ \frac{[Rh_2]_o}{[Rh_2 \cdot nM]^{n+}} - 1 = \frac{1}{K_b [M^+]^n} \]  

(27)

\[ \log \left( \frac{A_o}{A_o - A} \cdot \frac{\varepsilon_o - \varepsilon_b}{\varepsilon_o} - 1 \right) = -n \log [M^+] - \log K_b \]  

(28)

A plot of \( \{A_o (\varepsilon_o - \varepsilon_b) / \varepsilon_o / (A_o - A) - 1\} \) vs \( \log [M^+] \) should result in a straight line with a slope of \( n \), and \( \log K_b \) can be obtained from the ordinate intercept of the straight line.
Absorption titrations of 10 and 11 with Na\(^+\), K\(^+\), Ca\(^{2+}\), Cs\(^+\), and Ba\(^{2+}\) were performed in water. Each titration was repeated twice and the results were averaged. No shift in peaks positions was observed upon coordination of the metal ions, however, the intensity of the transition at 293 nm gradually decreased. It is evident from Figure 4.24 that decrease in absorption does not result from the dilution of the sample by the addition of [M\(^+\)], but rather from the coordination of cation to the crown ether. If the decrease in absorption resulted from the dilution of the sample, the curve in Figure 4.24 would have been a straight line.

After fitting the absorption titration data to eq 28 (Figure 4.25), log \(K_b\) and \(n\) can be obtained and are listed in Table 4.3. The binding affinity for the studied cations are decreasing in a row Ba\(^{2+}\) > K\(^+\) > Cs\(^+\) ≥ Ca\(^{2+}\) ~ Na\(^+\). In general, the binding constants of metal ions to 10 and 11 parallel to those of 15-crown-5 and 18-crown-6, respectively. Ba\(^{2+}\) and K\(^+\), possessing almost the same radii, exhibit the highest binding constants according to their size match to the crown cavity. The stoichiometry of the binding of the metal ions, M, to 10 and 11 is 1:2 [Rh\(_2\)]:[M] (M = Ba\(^{2+}\), K\(^+\)). Such a binding mode was previously reported for Na\(^+\), K\(^+\) and Rb\(^+\) with bis-18-crown-6 ethers connected through the bridge of maleic and succinic acids.\(^{102}\) However, Cs\(^+\) prefers the formation of sandwiched structure with those systems in 1:1 ratio.\(^{102}\)

Somewhat different results were reported for bis-15-crown-5 ethers when the binding stoichiometry was 1:2 bis-crown:Na\(^+\) and 1:1 bis-crown:M\(^+\) (M = K, Rb, NH\(_4\)).\(^{70}\) The stoichiometry of the Na\(^+\), Ca\(^{2+}\), and Cs\(^+\) binding to 10 and 11 is 1.5:1 [M]:[Rh\(_2\)] ratio (M = Na\(^+\), Ca\(^{2+}\), and Cs\(^+\)), what might implicate 3:2 stoichiometry or the presence of 1:1 and 1:2 adducts.
Figure 4.24. Typical absorption titration curve of 6.4 μM 11 with Ba\(^{2+}\) in water monitored at 293 nm.

Figure 4.25. Logarithmic plot of changes of absorption upon titration of 6.4 μM 10 with 125 mM BaCl\(_2\) in water monitored at 294 nm.
Table 4.3. Calculated $n$ and $K_b$ for 10 and 11 obtained from the optical titrations in water.

4.3.8. DNA Photocleavage in the Presence of Alkali and Alkaline Metal Ions

It was anticipated that binding of alkali and alkaline metals by crown ethers would affect electron density of the dirhodium complex, and, consequently, the percentage of the DNA photocleavage. 25 mM KCl, CaCl$_2$, CsCl, and BaCl$_2$ along with 25 mM NaCl were added to DNA solutions and irradiated in the presence of 5 μM 10 or 11. In the presence of mono-valent metal ions (K$^+$, Cs$^+$) the photocleavage is slightly greater (120%), while in the presence of divalent metal ions (Ca$^{2+}$, Ba$^{2+}$) the photocleavage is lower (60%) compared to that in the presence 100% Na$^+$ ions (Figure 4.26). These results are not related to the binding affinities of the cations to 10 or 11 (Table 4.3). For example, Ba$^{2+}$ and Ca$^{2+}$ have the same impact on DNA photocleavage, while the binding affinity of the Ba$^{2+}$ to crowns is ~100 times greater than that of Ca$^{2+}$.

\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
M$^+$ & r$_{M^+}$ Å & $n$ & log $K_b$ & $n$ & log $K_b$ & log $K_b$ \\
\hline
Na$^+$ & 1.02 & 1.5 & 4.7 & 1.5 & 4.9 & 0.70 & 0.8 \\
K$^+$ & 1.38 & 2.1 & 6.2 & 1.9 & 6.1 & 0.74 & 2.03 \\
Ca$^{2+}$ & 1.00 & 1.6 & 4.9 & 1.5 & 4.8 & 0.3 & 0.48 \\
Cs$^+$ & 1.67 & 1.6 & 4.9 & 1.5 & 5.2 & 0.8 & 0.99 \\
Ba$^{2+}$ & 1.35 & 1.9 & 6.6 & 2.0 & 6.7 & 1.71 & 3.87 \\
\hline
\end{tabular}
\caption{Adapted from ref 70.}
\end{table}
Therefore, effect of the metal ions on the DNA photocleavage is not caused by the strength of the metal ion – crown ether interactions.

The ionic strength of the solution may contribute to the amount of cleaved DNA owing to the similar effects of the metal ions of the same charge. An experiment that probed the DNA photocleavage by 10 at the ionic strength of 50 mM and different molar ratios of Ca$^{2+}$ and Na$^+$ (Figure 4.27) was performed. The ionic strength, $I_c$ was calculated using eq 29. Ca$^{2+}$ was chosen as the representative metal ion.

$$I_c = \frac{1}{2} \sum c z^2$$  \hspace{1cm} (29)

In eq 29, $I_c$ is the ionic strength of the solution; $c$ is the molar concentration of the cation or anion; and $z$ is its formal charge.

**Figure 4. 26.** EtBr stained agarose gel (1%) showing the photocleavage ($\lambda_{irr} > 395$ nm, 12 min) of 100 $\mu$M pUC18 plasmid by 5 $\mu$M 10 (5 mM Tris, pH 7.5, 25 mM NaCl) in the presence of 25 mM metal ions. Lane 1: DNA only, dark; lane 2: DNA + 10, dark; lanes 3-7, DNA + 10, irradiated in air in the presence of 25 mM NaCl (lane 3); 25 mM KCl (lane 4); 25 mM CaCl$_2$ (lane 5); 25 mM CsCl (lane 6); 25 mM BaCl$_2$ (lane 7).
Figure 4.27. EtBr stained agarose gel (1%) showing the photocleavage ($\lambda_{irr} > 395$ nm, 12 min) of 100 μM pUC18 plasmid by 5 μM 10 (5 mM Tris, pH 7.5) in the presence of NaCl and/or CaCl$_2$ at $I_c = 50$ mM. Lane 1: DNA only, dark; lane 2-5: DNA + 10, irradiated in air in the presence of 50 mM NaCl (lane 2); 16.6 mM CaCl$_2$ (lane 3); 33.4 mM NaCl and 5.5 mM CaCl$_2$ (lane 4); 16.9 mM NaCl and 10.9 mM CaCl$_2$ (lane 5).

Figure 4.27 shows the DNA photocleavage by 5 μM 10 upon irradiation with visible light in air ($\lambda > 395$ nm) in the presence of various ions while keeping the ionic strength constant at $I_c = 50$ mM. Lane 2 shows DNA irradiated in air in the presence of 50 mM NaCl and 5 μM 10. The amount of nicked DNA in lane 2 after the subtraction of the nicked DNA present in the plasmid alone (lane 1) was set to 100 %. Surprisingly, the amount of photocleaved DNA in the presence of Ca$^{2+}$ ions (lanes 3-5) was independent of the [Ca$^{2+}$]:[Na$^+$] ratio (~60 %). It is worth noting that in other experiments under similar irradiation conditions the photocleavage of DNA by 5 μM 10 in the presence of 25 mM NaCl and 25 mM CaCl$_2$ ($I_c = 100$ mM) also resulted in 60 % nicked DNA relatively to the photocleavage in the presence of 50 mM NaCl ($I_c = 50$ mM). These results show that the ionic strength does not affect the photocleavage.
Owing to the presence of the negatively charged phosphate backbone, DNA is a polyanion, requiring the presence of cationic atmosphere (electrical double layer) close to its surface to balance its large negative charge. Therefore, keeping the $I_c$ constant is very important, since the thickness of the electrical double layer is inversely proportional to the square root of the $I_c$. Monovalent metal ions (e.g., Na$^+$) do not bind to specific sites on DNA, but rather stabilize DNA charge via coulombic interactions. The enhanced photocleavage in the presence of K$^+$ and Cs$^+$ is possibly due to the looser attraction by these ions to DNA compared to Na$^+$. Since K$^+$ and Cs$^+$ possess greater ionic radii, they have smaller charge density on their surface, and therefore bind to DNA less tightly than Na$^+$ and Li$^+$, thus shielding the negative charge of DNA less effectively. This phenomenon has been observed in the differences in DNA mobility through the gel as a function of cation.

In contrast, as was shown for multiply charged metal ions, they exhibit both coulombic attraction for the DNA at phosphate groups and binding to the bases. The specific binding of alkaline cations was confirmed by Fourier Transform IR, $^1$H NMR and crystallography. The divalent cations typically bind in the major groove of DNA between the N7 of purine bases and phosphates. Therefore, the reduced photocleavage in the presence of divalent metal ions could be due to stronger binding and better screening of the DNA charge by Ca$^{2+}$ and Ba$^{2+}$, shielding the deoxyribose from possible damage by diffusible intermediates. Also, a layer of cations around DNA can prevent dirhodium complexes from electrostatic binding to the DNA.
4.3.9. Electrochemistry of \([\text{Rh}_2(\mu-O_2\text{CCH}_3)(15\text{-crown-5-dppz})]^{2+}\)

Differential pulse voltammetry (DPV) (Figure 4.28) and cyclic voltammetry techniques (CV) were used to determine reduction potentials of 10 in degassed DMSO in the presence and absence of sodium salicylate (Figures 4.28 and 4.29), and the data are listed in Table 4.4. Four irreversible ligand-based reduction peaks of 10 were recorded in the absence of Na\(^+\). Upon addition of sodium salicylate, the waves at –976 and –620 mV were not disturbed, while those at –1204 and –1352 mV shifted by 52 and 18 mV, respectively to more positive potentials. In addition, new peak at –1711 mV was observed. Sodium salicylate itself was not reducible under similar experimental conditions.

The effect of complexation of crown ether systems by alkali ions on oxidation-reduction potentials was previously studied with several systems. Fullerene-based redox potentials of a fullerene-18-crown-6 conjugate shift positively from 40 to 90 mV upon addition of K\(^+\). The magnitude of the shift correlates with the distance between the K\(^+\) and fullerene. This observation is in agreement with the electrochemical behavior of Ni(II) complexes whose ligands were functionalized with 15-crown-5. The \(E_{1/2}(\text{Ni}^{2+/3+})\) potential did not change upon binding of Na\(^+\) to the crown ether owing to the large space between the Ni(II) center and crown ether. However, the ligand-based reduction potentials were easier to reduce by 11-125 mV. Similarly, the oxidation potentials of zinc(II) dithiolate complexes with crown ether ligands were not disturbed, while the ligand-based reduction potentials exhibited positive shifts by 80-120 mV. In general, the change in the reduction potential of the ligand coupled to the crown ether after the binding of the metal ion makes the ligand easier to reduce. The results presented...
here for the [Rh$_2$(μ-O$_2$CCH$_3$)$_2$(15-crown-5-dppz)$_2$]$^{2+}$ are consistent with those of systems previously reported.

**Figure 4.28.** Differential pulse voltogramms showing the reduction of 10 in dry DMSO (·······) in the absence of sodium salicylate and (——) in the presence of excess of sodium salicylate. Ferrocene (FeCp) is added as a reference ($E_{1/2}$ (FeCp$^{0}$) = 0.273 V vs NHE).$^{113}$
Figure 4.29. Cyclic voltamgrams showing the reduction of 10 in dry DMSO (⋯⋯) in the absence of sodium salicylate and (——) in the presence of excess of sodium salicylate. FeCp is added as a reference.

<table>
<thead>
<tr>
<th></th>
<th>$E_{1/2}$ of 10, mV</th>
<th>$E_{1/2}$ of (10 + Na$^+$), mV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-976</td>
<td>-976</td>
</tr>
<tr>
<td></td>
<td>-1204</td>
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<td></td>
<td>-1620</td>
<td>-1626</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>-1711</td>
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</table>

Table 4.4. Electrochemical data for 10 collected in degassed DMSO in the presence of FeCp in the presence and the absence of the sodium salicylate (vs NHE).
4.4. Concluding Remarks

The interaction of \(\text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(R_1,R_2-dppz)_2]^2+\) with DNA was investigated. The complexes do not intercalate into DNA duplex and cleave DNA upon irradiation with visible light (\(\lambda > 500\) nm, 20 min). The percent photocleavage decreases with the increase of the reduction potential of the R,R'-dppz ligand in the metal complex. A linear relationship between \(1/[\text{DNA}]_{\text{nicked}}\) vs the free energy \(|\Delta G^0|\) was established. It was shown that free axial positions are required for dirhodium complexes to be photoactive. Electrochemical measurements have shown that ligand-centered reduction potentials of \(\text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(15\text{-crown-5-dppz})_2](\text{O}_2\text{CCH}_3)_2\) shift to the positive direction upon addition of \(\text{Na}^+\). These results provide a basis for the design of new metal complexes as potential PDT agents.
Bibliography


(54) Turro, C. unpublished results


(86) Turro, C. Unpublished results.


CHAPTER 5

CYCLING THE DNA LIGHT SWITCH ON AND OFF

5.1. Background

\[
[Ru(bpy)_3]^{2+} \quad (bpy = 2,2'-bipyridine) \text{ is currently used as a benchmark in photoinduced electron and energy transfer due to its long-lived emissive excited state, photochemical stability, and ability to effect redox reactions from its excited state.}^{1,2} \text{ The ability of } *[Ru(bpy)_3]^{2+} \text{ to transfer an electron to various acceptors is utilized in numerous applications, including dye-sensitized solar cells,}^{3} \text{ oxidation of water,}^{4,5,6} \text{ organic light-emitting diodes, and organic light-emitting electrochemical cells.}^{7,8,9,10} \text{ Therefore, the photochemical and photophysical properties of } [Ru(bpy)_3]^{2+} \text{ have been studied in great detail.}^{11,12,13} \text{ The complex possesses an emissive } ^3\text{MLCT (metal-to-ligand charge transfer) excited state with } \Phi_{\text{em}} = 0.042 \text{ in water.}^{14}
\]

Interactions of Ru(II) complexes with DNA have also been investigated extensively and include variations in size, shape, and hydrophobicity of the ligation sphere.\textsuperscript{15,16,17} Electrostatic binding to the anionic DNA backbone is observed for \([Ru(NH_3)_6]^{2+}\) and \([Ru(bpy)_3]^{2+}\),\textsuperscript{18,19} while minor groove binding was reported for \([Ru(phen)_2(dpq)]^{2+}\) (phen = 1,10 phenathroline, dpq = dipyridoquinoxaline),\textsuperscript{20} and intercalation from the major groove by \([Ru(bpy)_2(dpdz)]^{2+}\) (dpdz = dipyrido[3,2-a:2',3'-c]phenazine) (Figure 5.1). These examples depict non-covalent DNA binding modes and
generally the binding affinity of the complexes to the duplex vary from one mode to another. DNA damage initialized by Ru(II) complexes typically occurs via the photoinduced generation of $^1\text{O}_2$ from the $^3\text{MLCT}$ state. $^1\text{O}_2$ attacks guanine via 1,4-cycloadDITION generating the unstable guanine endoperoxide, believed to be a precursor of 8-oxo-guanine. The latter is susceptible to base-sensitive cleavage as is the case upon addition of piperidine. The ability of $^1\text{O}_2$ to produce frank strand breaks in ds-DNA has been an issue of some debate. Multiple authors report the formation of nicked strands in plasmid DNA directly by $^1\text{O}_2$, which is supported by increased damage in D$_2$O owing to the longer lifetime of $^1\text{O}_2$ in this solvent. Interestingly, as was shown in PM2 DNA, the frequency of 8-oxo-guanine production is 17 times higher than the generation of single strand. This result is inconsistent with the direct cleavage of DNA by $^1\text{O}_2$. It is possible that endoperoxide may also be a common precursor for both 8-oxo-guanine and single strand breaks of DNA. However, some Ru(II) complexes bearing strongly oxidizing ligands such as hat (hat = 1,4,5,8,9,12-hexaazatriphenylene) or tap (tap = 1,4,5,8-tetraazaphenanthrene) were shown to photocleave DNA primarily by photoinduced guanine oxidation directly from their excited state instead of the generation of $^1\text{O}_2$, as determined by time-resolved laser spectroscopy. These complexes were shown to induce nicks in supercoiled DNA upon irradiation and to form covalent photoadducts with oxidized guanine bases.

$[\text{Ru(bpy)}_2(dppz)]^{2+}$ and $[\text{Ru(phen)}_2(dppz)]^{2+}$ are unique luminescent probes of DNA. They are non-emissive in water, but highly luminescent in CH$_3$CN, C$_2$H$_5$OH, and upon intercalation into the DNA duplex, thus making them “DNA light switch” complexes. Shielding of the nitrogen atoms on phenazine (phz) part of the dppz
ligand (Figure 5.1) from water upon DNA intercalation of the dppz ligand is believed to be the origin of the DNA light switch effect. Multiple time-resolved studies were undertaken with Ru(II) complexes possessing dppz ligands to elucidate the factors that control the changes in emission in various solvents and in DNA. The dramatic difference in the lifetime of the $^3$MLCT excited state of [Ru(phen)$_2$(dppz)]$^{2+}$ in water (260 ps), CH$_3$CN (660 ns), and bound to DNA (250 ns)$^{30}$ suggest either stabilization of the lowest $^3$MLCT state by hydrogen bonding with H$_2$O or the presence of multiple low-lying states, the energies of which depend on the solvent.$^{35}$ However, it was shown that the identity of the long-lived MLCT state in aprotic solvents and DNA environment is identical to that in H$_2$O.$^{34}$ Interestingly, the experiments performed with single strand oligonucleotides and [Ru(phen)$_2$(dppz)]$^{2+}$ showed that full intercalation of dppz ligand into DNA is not a prerequisite for turning “on” the emission.$^{34}$ These studies show that a combination of the shielding of the complex from water by a hydrophobic medium is enough to induce emission.

The temperature dependence of the emission of Ru(II) complexes possessing dppz ligands stems from the equilibrium between at least two low-lying $^3$MLCT excited states (Figure 5.2), both centered on the dppz ligand, one emissive and another dark.$^{36,37}$ The “bright” state in [Ru(L)$_2$(dppz)]$^{2+}$ (L = bpy, phen) and related Ru(II) polypyridyl complexes has been shown to possess a similar electronic distribution as the $^3$MLCT of [Ru(bpy)$_3$]$^{2+}$. 

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It was concluded that after relaxation to the $^3$MLCT excited state, the electron localizes mainly on the bpy-like part of the dppz ligand (Figure 5.2), giving rise to the “bright” state. In water, the non-emissive “dark” state is lower in energy than the “bright” state and is associated with the phz part of the dppz ligand. The “dark” state of $[\text{Ru(bpy)}_2(\text{dppz})]^2^+$ is characterized by lower enthalpy ($\Delta H \approx -51 \text{ kJ/mol}$) and entropy ($\Delta S \approx -150 \text{ J/mol/K}$) relative to the “bright” state. Therefore there is greater population of the “dark” state at low temperatures even in aprotic solutions, since it is enthalpically favored. When the temperature is increased to 254 K, the population of the “bright” state dominates owing to the entropic factor. As the temperatures is increased above 254 K, the complex remains emissive, however, the emission lifetime decreases due to the thermal population of the non-emissive metal-centered dd manifold located at higher energy.$^{38,39}$
Numerous Ru(II) and Os(II) complexes possessing dppz and related ligands have been investigated as DNA light switch complexes upon binding to duplex DNA.\textsuperscript{31,40,41} Since the initial reports of long-range charge transfer through DNA,\textsuperscript{42} a body of research has been amassed that supports the conclusion that duplex DNA is able to transport charge over long distances.\textsuperscript{43,44} Although the biological function of the long-range charge transfer remains unknown, it has been suggested that it may play an important role in the \textit{in vivo} detection of sites of oxidative damage on DNA,\textsuperscript{45} as well as in the suppression of DNA damage under conditions of oxidative stress.\textsuperscript{46} Various applications of the long distance charge transport by DNA have been explored, including electrochemical sensors of selected DNA sequences,\textsuperscript{47} hybridization,\textsuperscript{48} and base pair mismatches.\textsuperscript{49} Owing to the ability of DNA to selectively self-assemble into a wire-shaped structure, together with its capability to effect long-range charge transport and to
support chemical modifications, DNA may have potential applications as a template or as an element in DNA-based nanocircuits and molecular electronics.\textsuperscript{50,51} Recently, photoexcitation of a molecule intercalated between the bases of a short duplex oligonucleotide covalently bound to an electrode was shown to produce current over long distances, a property that may be useful in photoelectronics.\textsuperscript{52} In this system, however, the intercalator cannot be switched “off”, thus, irradiation always results in charge transport.\textsuperscript{52}

\begin{equation*}
[Ru(bpy)_2(tpphz)]^{2+} \text{ (tpphz = tetrapyrido[3,2-a:2',3'-c:3”,2”-h:2”,3”-j]phenazine)}
\end{equation*}
is an attractive target as a light switch owing to its high affinity for DNA, and a distal “phenanthroline-like” site allowing the coordination of a second metal (structure shown in Figure 5.3).\textsuperscript{53,54} The formation of a heterobimetallic complex bound to DNA with Cu\textsuperscript{2+}, [Ru(bpy)_2(tpphz)]^{2+}-DNA-Cu\textsuperscript{2+}, was previously reported,\textsuperscript{54a} where the Cu\textsuperscript{2+} ion was shown to quench the luminescence from intercalated [Ru(bpy)_2(tpphz)]^{2+}. However, detailed studies of the quenching mechanism were not conducted.\textsuperscript{54a} In addition, there have been no reports regarding the reversibility of quenching. In order for a light-switch complex to be useful for any potential application, its luminescence must be turned “on” and “off” in a controlled manner. For this purpose the DNA binding and luminescence switching studies of [Ru(bpy)_2(tpphz)]^{2+} and the control complex [Ru(bpy)_2(tapt)]^{2+} (tapt = 4,5,9,18-tetraazahehanthreno[9,10-b] triphenylene) are presented here. As shown in Figure 5.3, the tapt ligand does not possess distal nitrogen atoms for the binding of additional metal ions.
**Figure 5.3.** Schematic representation of the molecular structures of the [Ru(bpy)$_2$(tphz)]$^{2+}$ (left) and [Ru(bpy)$_2$(taptp)]$^{2+}$ (right).
5.2. Results and Discussions

5.2.1. Ground and Excited State Properties

The electronic absorption spectra of $[\text{Ru(bpy)}_2(tpphz)]^{2+}$ and $[\text{Ru(bpy)}_2(taptp)]^{2+}$ have been previously reported and are shown in Figure 5.4.\textsuperscript{55,56,57,58} The absorption spectra of the complexes display a bpy-centered $^1\pi\pi^*$ transition at 285 nm, $^1\pi\pi^*$ transitions of the tptap and tpphz ligands in the 350-400 nm region, and $^1\text{MLCT} (\text{Ru} \rightarrow \text{tptap or Ru} \rightarrow \text{tpphz})$ and $^1\text{MLCT} (\text{Ru} \rightarrow \text{bpy})$ at 400-500 nm. These spectral features are typical of Ru(II) complexes with bpy and related ligands, where in $[\text{Ru(bpy)}_2\text{L}]^{2+}$ polypyridyl complexes transitions in UV region are typically ligand-centered,\textsuperscript{59,60,61} while the visible region of the spectrum is characterized by the overlap of the $^1\text{MLCT} (\text{Ru} \rightarrow \text{bpy and Ru} \rightarrow \text{L})$ transitions.

![Figure 5.4. Electronic absorption spectra of $[\text{Ru(bpy)}_2(taptp)]^{2+}$ and $[\text{Ru(bpy)}_2(tpphz)]^{2+}$ in water.]}
The emission and excitation spectra of $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ and $[\text{Ru(bpy)}_2(\text{taptp})]^{2+}$ collected at 298 K and 77 K are shown in Figure 5.5. The emission maxima in H$_2$O, CH$_3$CN and CH$_3$OH / C$_2$H$_5$OH are listed in Table 5.1, along with luminescence quantum yields and lifetimes. The peak positions in the excitation spectra at room temperature coincide with those in the absorption spectrum and indicate that emission arises from photoexcited $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ and $[\text{Ru(bpy)}_2(\text{taptp})]^{2+}$.

Typically the emission maxima of Ru(II) polypyridyl complexes appear at a lower energy in H$_2$O compared to CH$_3$CN, owing to the greater polarity of the former which results in better stabilization of the $^3\text{MLCT}$ excited state.$^{62,63}$ The low temperature emission spectra collected in CH$_3$OH / C$_2$H$_5$OH glasses at 77 K elucidated vibronic structure with average $\Delta \nu \approx 1513$ cm$^{-1}$ and $\Delta \nu \approx 1043$ cm$^{-1}$ for $[\text{Ru(bpy)}_2(\text{taptp})]^{2+}$ and $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$, respectively. Such vibronic progressions are typical of Ru(II) MLCT excited states and correspond to vibrations associated with the polypyridyl ligands.$^{64}$ The emission maxima at 77 K are blue-shifted compared to those recorded at room temperature due to the inability of the frozen solvent to reorganize at 77 K. The excited state energy, $E_{00}$, can be estimated from the highest energy vibronic peak in the 77 K emission, which are observed at 581 nm for $[\text{Ru(bpy)}_2(\text{taptp})]^{2+}$ and 585 nm for $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ and correspond to 2.12 eV and 2.11 eV, respectively. For comparison, the $E_{00}$ values reported for $[\text{Ru(bpy)}_3]^{2+}$ and $[\text{Ru(bpy)}_2(\text{dppz})]^{2+}$ are equal to 2.01 and 2.24 eV, respectively.$^{21,65}$ Therefore, the $E_{00}$ values measured here for $[\text{Ru(bpy)}_2(\text{taptp})]^{2+}$ and $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ fall within the range of known values for related complexes.
Figure 5.5. Excitation ($\lambda_{\text{em}} = 630$ nm) spectra at (---) 300 K and (– – –) 77 K and emission spectra ($\lambda_{\text{exc}} = 450$ nm) at (----) 300 K and (-----) 77 K of 5 $\mu$M $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ (top) and $[\text{Ru(bpy)}_2(\text{tapt})]^{2+}$ (bottom) collected in CH$_3$OH/C$_2$H$_5$OH (4:1, v:v).
Table 5.1: Photophysical properties of 5 µM $[\text{Ru(bpy)}_2L]^{2+}$ (L = taptp, tpphz) collected in various solvents at 298 K and 77 K under N$_2$.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{em}, \text{nm}$</th>
<th>$\Phi_{em}$ (N$_2$, degassed CH$_3$CN)</th>
<th>$\tau$, ns</th>
<th>$\lambda_{em}, \text{nm}$</th>
<th>$\Phi_{em}$ (N$_2$, degassed CH$_3$CN)</th>
<th>$\tau$, ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$OH/C$_2$H$_5$OH</td>
<td>4738 (90%)</td>
<td>612</td>
<td>639.7</td>
<td>581</td>
<td>29 (10%)</td>
<td>4738 (90%)</td>
</tr>
<tr>
<td>CH$_3$CN</td>
<td>746 (81%)</td>
<td>627</td>
<td>9.0</td>
<td>1097</td>
<td>13 (19%)</td>
<td>746 (81%)</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>4075 (88%)</td>
<td>623</td>
<td>$1.7 \times 10^{-4}$</td>
<td>298 K</td>
<td>77 K</td>
<td>4075 (88%)</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>4075 (88%)</td>
<td>623</td>
<td>$1.7 \times 10^{-4}$</td>
<td>298 K</td>
<td>77 K</td>
<td>4075 (88%)</td>
</tr>
<tr>
<td>CH$_3$OH/C$_2$H$_5$OH</td>
<td>4738 (90%)</td>
<td>612</td>
<td>639.7</td>
<td>581</td>
<td>29 (10%)</td>
<td>4738 (90%)</td>
</tr>
<tr>
<td>CH$_3$CN</td>
<td>746 (81%)</td>
<td>627</td>
<td>9.0</td>
<td>1097</td>
<td>13 (19%)</td>
<td>746 (81%)</td>
</tr>
</tbody>
</table>

$\lambda_{exc} = 450$ nm; $\Phi_{em}$ measured relatively to $[\text{Ru(bpy)}_3]^{2+}$ ($\lambda_{em} = 610$ nm, $\Phi_{em} = 6.5 \times 10^{-2}$). $\lambda_{exc} = 532$ nm.
The emission maximum of $[\text{Ru}(\text{bpy})_2(\text{tpphz})]^{2+}$ is red-shifted by $\sim 10$ nm relatively to that of $[\text{Ru}(\text{bpy})_2(\text{taptp})]^{2+}$ in both CH$_3$CN and H$_2$O. As was shown previously by INDO/SCI calculations,$^{66}$ the HOMO-LUMO gap is stabilized upon replacement of the CH unit by nitrogen atoms in aromatic ligands. The shift in the $^3$MLCT emission to lower energy observed in $[\text{Ru}(\text{bpy})_2(\text{tpphz})]^{2+}$ relative to $[\text{Ru}(\text{bpy})_2(\text{tpphz})]^{2+}$ is consistent with these calculations. Both complexes are fairly emissive in CH$_3$CN and decay monoexponentially (Table 5.1). The emission maxima and lifetimes in CH$_3$CN are consistent with those previously reported in the literature,$^{56,57}$ at 620 nm for $[\text{Ru}(\text{bpy})_2(\text{taptp})]^{2+}$ ($\tau = 995$ ns) and 628 nm for $[\text{Ru}(\text{bpy})_2(\text{tpphz})]^{2+}$ ($\tau = 1097$ ns). These values are typical for the $^3$MLCT emission of $[\text{Ru}(\text{bpy})_2\text{L}]^{2+}$ polypyridyl complexes, ranging from 610 nm for $[\text{Ru}(\text{bpy})_2(\text{dpqC})]^{2+}$ ($\text{dpqC} = \text{dipyrido-6,7,8,9-tetrahydrophenazine}$) to 678 nm for $[\text{Ru}(\text{bpy})_2(\text{tap})]^{2+}$.21,60

Both complexes emit weakly in H$_2$O with the emission lifetimes 6-11 times shorter than those in CH$_3$CN. The shorter lifetimes in H$_2$O are believed to be due to the presence of hydrogen bonding from the solvent which stabilizes the “dark” state. The emission lifetimes of $[\text{Ru}(\text{bpy})_2(\text{taptp})]^{2+}$ (178 ns) and $[\text{Ru}(\text{bpy})_2(\text{tpphz})]^{2+}$ (16 ns) in H$_2$O are comparable with those of $[\text{Ru}(\text{bpy})_3]^{2+}$ (621 ns),$^{60}$ $[\text{Ru}(\text{bpy})_2(\text{dpq})]^{2+}$ (195 ns),$^{21}$ $[\text{Ru}(\text{bpy})_2(\text{tap})]^{2+}$ (145 ns),$^{60}$ and $[\text{Ru}(\text{bpy})_2(\text{hat})]^{2+}$ (102 ns),$^{67}$ but significantly greater than that of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (250 ps).$^{33}$
Table 5.2. Electrochemical data for [Ru(bpy)_2L]^2+ (L = taptp, tpphz, dppz, dpq, bpy), V vs NHE.

<table>
<thead>
<tr>
<th>L</th>
<th>E_{1/2}(Ru^{3+/2+})</th>
<th>E_{1/2}(L^{0/-})</th>
<th>E_{1/2}(bpy^{0/-})</th>
<th>E_{1/2}(bpy^{0/-})</th>
<th>E_{1/2}(Ru^{2+/+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>taptp</td>
<td>+1.57</td>
<td>-0.85</td>
<td>-1.14</td>
<td>-1.34</td>
<td>+1.27</td>
</tr>
<tr>
<td>tpphz</td>
<td>+1.58</td>
<td>-0.73</td>
<td>-1.17</td>
<td>-1.33</td>
<td>+1.38</td>
</tr>
<tr>
<td>dppz</td>
<td>+1.57</td>
<td>-0.73</td>
<td>-1.15</td>
<td>-1.39</td>
<td>+1.51</td>
</tr>
<tr>
<td>dpq</td>
<td>+1.54</td>
<td>-1.10</td>
<td>-1.25</td>
<td>-1.42</td>
<td>+1.08</td>
</tr>
<tr>
<td>bpy</td>
<td>+1.49</td>
<td>-1.12</td>
<td>-1.28</td>
<td>-1.51</td>
<td>+0.98</td>
</tr>
</tbody>
</table>

From reference 21.

The ground state oxidation and reduction potentials of various Ru(II) complexes in CH$_3$CN are presented in Table 5.2. The metal-based oxidation potentials measured for [Ru(bpy)$_2$L]$^{2+}$ (L = tpphz, taptp) are typical for [Ru(bpy)$_2$L]$^{2+}$ complexes.$^{21,56,68,69,70}$ The taptp and tpphz ligands are easier to reduce than the ancillary bpy ligands in [Ru(bpy)$_2$L]$^{2+}$ (L = tpphz, taptp) due to the larger $\pi$-system in the former which provide greater electron delocalization. Two additional reductions were also observed which are at potentials similar to those of other bpy-containing Ru(II) complexes and are assigned to bpy-centered reductions.$^{21,56}$

Since the E$_{00}$ values were obtained from the low-temperature emission and the E$_{1/2}(L^{0/-})$ (L = tpphz or taptp) were measured from electrochemistry, the excited state reduction potentials of [Ru(bpy)$_2$L]$^{2+}$ (L = tpphz or taptp), E$_{1/2}(Ru^{2+/+})$, can be calculated and are listed in Table 5.2,$^{65}$ allowing to estimate the value of the oxidation potential of the excited state.
5.2.2. Quenching of $[\text{Ru(bpy)}_2\text{L}]^{2+}$ (L = taptp, tpphz) Emission by Co$^{2+}$ and Zn$^{2+}$ in CH$_3$CN

Owing to the presence of the two distal nitrogen atoms on the tpphz ligand in $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$, the coordination of another metal ion to the tpphz ligand is possible. The absorption changes in $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ observed upon the addition of Co$^{2+}$ in CH$_3$CN are shown in Figure 5.6, which result in the blue shift of the $\pi\pi^*$ tpphz-based transition from 381 to 376 nm. Similar shifts in the absorption spectrum of $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ were observed upon titration of the complex with Zn$^{2+}$ and HClO$_4$ in CH$_3$CN due to the protonation of distal nitrogen atoms.$^{57}$

![Figure 5.6](image)

**Figure 5.6.** Absorption changes upon titration of 8 µM $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ by Co$^{2+}$ in CH$_3$CN.

The emission of $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ in CH$_3$CN can be quenched by the addition of Co$^{2+}$ and Zn$^{2+}$. The Stern-Volmer plot for the quenching of the emission intensity and
the lifetime of [Ru(bpy)₂(tpphz)]²⁺ by Co²⁺ in CH₃CN is depicted in Figure 5.8. Figure 5.8 shows that while the emission intensity decreases, the emission lifetime remains unchanged, indicative of static quenching with the formation of non-emissive ground state adduct.⁷¹,⁷² It is apparent from Figure 5.8, the quenching curve I₀/I exhibits an upward curvature starting at ~3 µM Co²⁺. At [Co²⁺] > 3 µM, the slope of the line changes. A dependence of the slopes and onset of curvature on the Stern-Volmer plot on [Cl⁻] was observed, indicative of equilibrium present in solution that depends on this ion.

The absorption spectrum of [Ru(bpy)₂(taptp)]²⁺ does not changes upon addition of Zn²⁺ or Co²⁺, ruling out adduct formation as was expected due to the absence of the distal nitrogen atoms. However, the emission intensity and the lifetime are quenched upon the addition of Co²⁺. As shown in Figure 5.9, the decrease in emission intensity of the complex is similar to that of the lifetime upon the addition of Co²⁺ and the I₀ / I much smaller than in [Ru(bpy)₂(tpphz)]²⁺ complex. These results point toward a dynamic mechanism of quenching, associated with energy loss due to collisions between [*Ru(bpy)₂(taptp)]²⁺ and Co²⁺, resulting in energy transfer.⁷¹ No quenching was observed upon addition of Zn²⁺.

It is worth noting that upon addition of Zn²⁺ or Co²⁺ to CH₃CN solutions of [Ru(bpy)₂(taptp)]²⁺ and [Ru(bpy)₂(tpphz)]²⁺ the Ru- and taptp-based oxidation and reduction potentials were not affected, while the tpphz-based reduction potential shifted by 0.13 V to a more positive potential, consistent with metal ion coordination to the tpphz ligand. Metal coordination makes the tpphz ligand easier to reduce, thus lowering the energy of the MLCT state.
Figure 5.7. Stern-Volmer plot of the quenching of the (×) emission lifetimes, \( \tau \), and the (●) intensity, I, of 8 \( \mu \)M [Ru(bpy)\(_2\)(tpphz)]\(^{2+}\) by Co\(^{2+}\) in degassed CH\(_3\)CN.

The quenching of [Ru(bpy)\(_2\)(tpphz)]\(^{2+}\) by Co\(^{2+}\) in CH\(_3\)CN shown in Figure 5.8 cannot be due to electron transfer, since the photoreduction of [Ru(bpy)\(_2\)(tpphz)]\(^{2+}\) by Co\(^{2+}\) or Zn\(^{2+}\) is not favorable since \( E_{1/2}(M^{3+/2+}) > 1.7 \) V vs NHE (M = Co, Zn), while the oxidation potential \( E(\text{Ru}^{2+/3+}) \) of [Ru(bpy)\(_2\)(tpphz)]\(^{2+}\) complex is +1.38 V vs NHE.\(^{73}\) Energy transfer from *[Ru(bpy)\(_2\)(tpphz)]\(^{2+}\) to low-lying empty LF (ligand-field) states of the Co\(^{2+}\) ion is a possible quenching mechanism. However, LF of the Zn\(^{2+}\) that possess d\(^{10}\) electron configuration, are not available. Therefore, coordination of the Zn\(^{2+}\) or Co\(^{2+}\) coordination may also influence electronic structure of the tpphz ligand making the “dark” state more populated than the “bright” state.

The bis-homonuclear complex [(bpy)\(_2\)Ru(tpphz)Ru(bpy)\(_2\)]\(^{4+}\) was previously synthesized and its photophysical properties were compared to those of the mononuclear
complex [Ru(bpy)$_2$(tpphz)]$^{2+}$ The emission lifetime (90 ns) and quantum yield ($\Phi_{em} = 8.4 \times 10^{-4}$) of [(bpy)$_2$Ru(tpphz)Ru(bpy)$_2$]$^{4+}$ measured in CH$_3$CN at room temperature were significantly lower than those of its mononuclear analogue, [Ru(bpy)$_2$(tpphz)]$^{2+}$ (1090 ns, $\Phi_{em} = 4.5 \times 10^{-2}$). Moreover, the bis-heteronuclear complex [(bpy)$_2$Ru(tpphz)Os(bpy)$_2$]$^{4+}$ is not luminescent in H$_2$O or CH$_3$CN and is weakly emissive in CH$_2$Cl$_2$ with $\tau = 1.5$ ns.

![Stern-Volmer plots of the quenching of the (×) emission lifetimes, $\tau$, and the (●) intensity, I, of 12 µM [Ru(bpy)$_2$(taptp)]$^{2+}$ by Co$^{2+}$ in degassed CH$_3$CN.](image)

**Figure 5.8.** Stern-Volmer plots of the quenching of the (×) emission lifetimes, $\tau$, and the (●) intensity, I, of 12 µM [Ru(bpy)$_2$(taptp)]$^{2+}$ by Co$^{2+}$ in degassed CH$_3$CN.

5.2.3. DNA Light-Switch

Both [Ru(bpy)$_2$(taptp)]$^{2+}$ and [Ru(bpy)$_2$(tpphz)]$^{2+}$ are weakly emissive in H$_2$O and a significant increase in their luminescence is observed upon the addition of DNA. This behavior is commonly known as the DNA light-switch, and in some cases it is
consistent with the intercalation of the complexes.\textsuperscript{53,57,77} The emission intensity of $[\text{Ru(bpy)}_2(\text{tpphz})]^2^+$ increases 59 times upon DNA binding, and the emission maximum shifts from 634 nm ($\tau = 16$ ns) to 628 nm ($\tau = 640$ ns). Upon intercalation into DNA, a 4-fold increase in the emission intensity is observed for $[\text{Ru(bpy)}_2(\text{taptp})]^2^+$ with a shift of the emission maximum from 623 nm ($\tau = 178$ ns) to 609 nm with $\tau_1 = 31$ ns (40%), $\tau_2 = 569$ ns (60%). Biexponential emission decays were previously reported for $[\text{Ru(phen)}_2(\text{dppz})]^2^+$ and related complexes in the presence of DNA and has been attributed to multiple binding modes.\textsuperscript{78,79} Therefore, the biexponential decay for $[\text{Ru(bpy)}_2(\text{taptp})]^2^+$ and monoexponential decay for $[\text{Ru(bpy)}_2(\text{tpphz})]^2^+$ in the presence of DNA is not unusual.\textsuperscript{53,79}

5.2.4. DNA Binding

Various methods are available to investigate the DNA binding modes of molecules, including the changes in the relative viscosity of DNA solutions in the presence of the agent and shifts in the DNA melting temperature upon DNA binding. In addition, the DNA binding constant, $K_b$, of a metal complex can often be measured using the changes in the absorption and emission of the complex as a function of DNA concentration, or through equilibrium dialysis. Figure 5.9 shows the changes in the relative viscosity of a solution of 2.4 mM DNA (5 mM Tris, pH 7.5, 50 mM NaCl) upon addition of increasing concentrations of $[\text{Ru(bpy)}_2(\text{taptp})]^2^+$ and $[\text{Ru(bpy)}_2(\text{tpphz})]^2^+$. Both metal complexes increase the relative viscosity of DNA, which clearly shows that both complexes intercalate between the DNA bases (Figure 5.10).\textsuperscript{80,81,82} A similar trend is observed upon addition of EtBr, a model intercalator, while the Hoechst 33258, a
minor groove binder, does not result in any changes to the relative viscosity of the DNA solutions (Figure 5.9).

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**Figure 5.9.** Relative viscosity plot of 2.4 mM herring sperm DNA upon addition of (o) EtBr, (●) [Ru(bpy)2(taptp)]2+, (×) [Ru(bpy)2(tpphz)]2+, and (□) Hoechst 33258 in 5 mM Tris, pH 7.5, 50 mM NaCl.

Equilibrium dialysis was used to measure the DNA binding constant of the [Ru(bpy)2L]2+ (L = taptp, tpphz) complexes. A DNA solution (100 μM CT DNA, 2 ml) was dialyzed against a 15 μM solution (200 mL) of each complex (5 mM Tris, pH 7.5, 50 mM NaCl) and resulted in DNA binding constants of $4.2 \times 10^5$ M$^{-1}$ and $3.5 \times 10^5$ M$^{-1}$ for [Ru(bpy)2(taptp)]2+ and [Ru(bpy)2(tpphz)]2+, respectively. These values are typical for intercalators. For example, the intercalator [Ru(bpy)2(phi)]2+ (phi = 9,10 phenanthrenequinonediimine), with $K_b = 1.6 \times 10^5$, exhibits a similar binding constant.
to [Ru(bpy)$_2$(taptp)]$^{2+}$ and [Ru(bpy)$_2$(tphpz)]$^{2+}$. Similarly, the DNA binding constant of EtBr was measured to be $1.7 \times 10^5$ M$^{-1}$ under the same dialysis conditions.$^{83,84}$

The changes in emission of a probe as a function of DNA concentration have been previously used for the determination of DNA binding constants of complexes.$^{31c}$ The increase in emission intensity of [Ru(bpy)$_2$(tphpz)]$^{2+}$ and [Ru(bpy)$_2$(taptp)]$^{2+}$ upon addition of DNA was used to obtain the value of $K_b$ for each complex by fit to the eq 3. Fits of the changes of the emission intensity of each complex upon addition of DNA to eq 3 result in $K_b = 8.0 \times 10^6$ M$^{-1}$ ($s = 2.22$) and $K_b = 3.6 \times 10^5$ M$^{-1}$ ($s = 0.69$) for [Ru(bpy)$_2$(tphpz)]$^{2+}$ and [Ru(bpy)$_2$(taptp)]$^{2+}$, respectively. A representative plot for [Ru(bpy)$_2$(tphpz)]$^{2+}$ is shown in Figure 5.10. It should be noted that for [Ru(bpy)$_2$(tphpz)]$^{2+}$, the value of $K_b$ obtained from the emission titration is 22 times greater than that calculated from equilibrium dialysis. One possible explanation is the enhancement of aggregation through $\pi$-stacking interactions by [Ru(bpy)$_2$(tphpz)]$^{2+}$ on the DNA surface, which would result on emission enhancement by complexes that are not intercalated. Surface aggregation of metal complexes has been previously observed for cationic complexes possessing hydrophobic ligands.$^{85}$ Extensive $\pi$-stacking dimerization of [Ru(bpy)$_2$(tphpz)]$^{2+}$ and related complexes in H$_2$O and D$_2$O has been observed by $^1$H NMR by us and others.$^{86,87}$ [Ru(bpy)$_2$(taptp)]$^{2+}$ is also expected to exhibit extensive aggregation in solution, however, the $K_b$ values obtained from equilibrium dialysis and emission titrations vary reasonably within an experimental error.
Figure 5.10. Plot of \((I - I_0) / (I - I_f)\) vs [DNA] for 5 µM \([\text{Ru(bpy)}_2(\text{tpphz})]^2^+\) measured at 620 nm \((\lambda_{\text{exc}} = 450 \text{ nm})\) in 5 mM Tris, pH = 7.5, 50 mM NaCl.

Deviations from Beer’s law were observed for \([\text{Ru(bpy)}_2L]^2^+\) \((L = \text{taptp, tpphz})\) complexes in water, especially in the region assigned to the \(\pi^*\) transition associated with the taptp and tpphz ligands. Strong hypochromic (38%) and bathochromic (380 to 387 nm) shifts were observed upon addition of up to 150 µM DNA to a 7.5 µM solution of \([\text{Ru(bpy)}_2(\text{tpphz})]^2^+\) (5mM Tris, pH 7.5, 50 mM NaCl), indicating strong interactions between the complex and the DNA duplex (Figure 5.12). The DNA binding constant, \(K_b\), calculated from fits of the absorption changes of \([\text{Ru(bpy)}_2(\text{tpphz})]^2^+\) to eq 1 (Figure 5.11, inset) was found to be \(5.0 \times 10^7 \text{ M}^{-1}\) \((s = 0.77)\) which is greater than that obtained by equilibrium dialysis by approximately two orders of magnitude. Owing to the self-aggregation of the complexes in water and the enhancement of surface aggregation in the presence of DNA, reliable \(K_b\) values could not be obtained using this method.
Figure 5.11. Absorption changes of 7.5 µM [Ru(bpy)$_2$(tpphz)]$^{2+}$ upon addition of 0, 25, 50, 75, 100, 125, and 150 µM DNA (5 mM Tris, pH 7.5). Inset shows the fit of the absorption changes at 380 nm and fit to eq 1.

Less pronounced absorption shifts were observed for [Ru(bpy)$_2$(taptp)]$^{2+}$ upon addition of DNA. The intensity of the ππ$^*$ transition associated with the taptp ligand in 10 µM Ru(bpy)$_2$(taptp)]$^{2+}$ decreases upon addition of up to 18 µM DNA, and then increases upon addition of up to 130 µM DNA, a result contrary to the hypochromic shifts observed for [Ru(bpy)$_2$(tpphz)]$^{2+}$. Since self-aggregation of the complex in water or buffer results in negative deviations from Beer’s law, the increase of the absorption upon addition of DNA is likely due to the dissociation of dimers or higher order aggregates of [Ru(bpy)$_2$(taptp)]$^{2+}$ when DNA is added. Further addition of DNA into
solution results in decrease of absorbance consistent with DNA intercalation or enhanced surface aggregation. Owing to this anomalous behavior, fits to eq 1 are not possible for this complex in order to obtain $K_b$. Similar behavior was observed for $[\text{Ru(DAP)}_3]^{2+}$ ($\text{DAP} = 1,12$-diazaperylene).88

5.2.5. DNA Photocleavage

Irradiation of pUC18 plasmid in the presence of $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ and $[\text{Ru(bpy)}_2(\text{tapt})]^{2+}$ with visible light ($\lambda_{\text{irr}} > 395$ nm) results in single strand breaks of DNA producing the nicked form (Figure 5.12). Lane 1, the control lane, with only plasmid in a dark, shows the undamaged supercoiled pUC18 with a small amount of nicked DNA. Neither complex cleaves DNA in the dark (Lanes 2 and 5) or upon irradiation in the absence of $O_2$ (Lanes 4 and 7). However, nicked DNA is formed upon irradiation in air (Lanes 3 and 6). A small shift in the DNA migration of the supercoiled and relaxed forms of DNA is observed with intercalated Ru(II) complexes (Lanes 2-7) relatively to the control (Lane 1). The retardation of DNA mobility may be due to the high molecular weight ($> 1000$ a.u.) of the DNA with the intercalated Ru compounds, together with a lower overall negative charge on DNA molecule.

The DNA photocleavage of $[\text{Ru(bpy)}_2(\text{tapt})]^{2+}$ shown in Figure 5.12 was inhibited by NaN$_3$ (Lane 3) but was not affected by HCOONa (Lane 4) or mannitol (Lane 5), both of which are well known OH$^-$ scavengers, ruling out the participation of OH$^-$ in the photocleavage.89,90 The inhibition of DNA damage by NaN$_3$ is likely due to its effective scavenging of $^1O_2$.91,92,93 In addition, the emission lifetimes of $[\text{Ru(bpy)}_2(\text{tpphz})]^{2+}$ and $[\text{Ru(bpy)}_2(\text{tapt})]^{2+}$ measured in air-saturated solutions are
shorter (10 and 158 ns, respectively) than in degassed (16 and 178 ns, respectively) due to the quenching of the reactive \(^3\)MLCT excited state by O\(_2\).\(^{94}\) Assuming that the concentration of O\(_2\) in air-saturated solution is 0.27 mM,\(^{73}\) the quenching constants, \(k_q\) were calculated to be \(1.4 \times 10^{11}\) and \(2.6 \times 10^9\) M\(^{-1}\)s\(^{-1}\) for \([\text{Ru(bpy)}_2(\text{tpphz})]^ {2+}\) and \([\text{Ru(bpy)}_2(\text{taptp})]^ {2+}\), respectively. As previously shown, \(^1\)O\(_2\) oxidizes guanine, G (\(E_{1/2}(G^{+0}) = + 1.29\) V vs. NHE) at 5'-G or 5'-GG-3' sites.\(^{95,96}\)

The oxygen-dependent photocleavage by \([\text{Ru(bpy)}_2(\text{tpphz})]^ {2+}\) and \([\text{Ru(bpy)}_2(\text{taptp})]^ {2+}\) is consistent with Ru(II) complexes previously reported, since the generation of \(^1\)O\(_2\) is typical for Ru(II) complexes.\(^{77}\) Numerous publications have confirmed the possible formation of \(^1\)O\(_2\) and O\(_2^-\) by energy or charge transfer, respectively, from the \(^3\)MLCT excited state.\(^{94}\) Both reactions are thermodynamically feasible for \([\text{Ru(bpy)}_2(\text{tpphz})]^ {2+}\) and \([\text{Ru(bpy)}_2(\text{taptp})]^ {2+}\). Since the energy gap between ground state and the singlet excited state of O\(_2\) is \(\sim 0.98\) eV, energy transfer from \(^*\)[RuL\(^{2+}\)] to O\(_2\) is favorable. With \(E_{1/2}(\text{RuL}^{3+/2+*})\) of -0.53 and -0.55 V vs NHE for \([\text{Ru(bpy)}_2L]^ {2+}\) (L = taptp, tpphz), respectively, and \(E_{1/2}(O_2^{0/-})\) \(\sim -0.16\) V vs NHE, electron transfer to generate O\(_2^-\) is also possible.\(^{95}\) However, the DNA photocleavage of the complexes is not inhibited by O\(_2^-\) scavengers. It is possible that following the formation of superoxide, O\(_2^-\) does not escape the solvent cage, therefore increasing the back reaction rate to regenerate the starting materials. Alternatively, the rate constant for energy transfer to O\(_2\) may be much faster than that for electron transfer.
Figure 5.12. EtBr stained agarose gel (1%) showing the photocleavage ($\lambda_{irr} > 395$ nm, 15 min) of 100 $\mu$M pUC18 plasmid with 10 $\mu$M Ru(II) complexes (5 mM Tris, pH 7.5, 50 mM NaCl). Lane 1, DNA only, dark; lanes 2-4, plasmid + [Ru(bpy)$_2$(taptp)]$^{2+}$: dark in air (lane 2), irradiated in air (lane 3), irradiated after six freeze-pump-thaw cycles (lane 4); lane 5-7, plasmid + [Ru(bpy)$_2$(tphh)]$^{2+}$: dark in air (lane 5), irradiated in air (lane 6), irradiated after six freeze-pump-thaw cycles (lane 7).

Figure 5.13. EtBr stained agarose gel (1%) showing the photocleavage ($\lambda_{irr} > 395$ nm, 15 min) of 100 $\mu$M pUC18 plasmid by 10 $\mu$M [Ru(bpy)$_2$(tauptp)]$^{2+}$ (5 mM Tris, pH 7.5, 50 mM NaCl). Lane 1, DNA only, dark; lane 2-5, DNA + [Ru(bpy)$_2$(tauptp)]$^{2+}$: irradiated in air (lane 2), irradiated with 2 mM NaN$_3$ (lane 3), irradiated with 2 mM HCOONa (lane 4), irradiated with 2 mM mannitol (lane 5).
5.2.6. Quenching of DNA Light-Switch Emission by Co$^{2+}$ and Zn$^{2+}$

Both [Ru(bpy)$_2$(taptp)]$^{2+}$ and [Ru(bpy)$_2$(tpphz)]$^{2+}$ complexes exhibit DNA light switch behavior and may be used as emissive intercalative probes of DNA. The presence of terminal nitrogen atoms on the tpphz ligand permit the binding of other transition metal ions, that may quench the $^3\text{MLCT}$ emission from intercalated [Ru(bpy)$_2$(tpphz)]$^{2+}$. Static luminescence quenching of [Ru(bpy)$_2$(tpphz)]$^{2+}$ and [Ru(bpy)$_2$(ppz)]$^{2+}$ (ppz = 4',7'-phenanthrolino-5',6':5,6-pyrazine) by Cu$^{2+}$ was observed when complexes were intercalated between the DNA bases, since the lifetime of the emission was independent of [Cu$^{2+}$]. Titration of [Ru(bpy)$_2$(tpphz)]$^{2+}$ intercalated into a DNA duplex by Co$^{2+}$ and Zn$^{2+}$ ions resulted in the blue shift of the $\pi\pi^*$ transition (Ru $\rightarrow$ tpphz) from 387 nm to 383 nm, indicative of coordination of metal ions to the tpphz ligand.

Figure 5.14 shows the changes in the emission intensity of DNA-intercalated [Ru(bpy)$_2$(tpphz)]$^{2+}$ upon addition of Co$^{2+}$, which results in emission quenching at 1:1 [Co$^{2+}$]:[Ru$^{2+}$]. It should be noted that the mechanism of quenching of intercalated [Ru(bpy)$_2$(tpphz)]$^{2+}$ is static with no change in lifetime in the presence of Co$^{2+}$ or Zn$^{2+}$. This results show that Co$^{2+}$ (or Zn$^{2+}$) and [Ru(bpy)$_2$(tpphz)]$^{2+}$ form a non-emissive or weakly emissive ground-state adduct and that the emission observed in the presence of Co$^{2+}$ (or Zn$^{2+}$) stems from unbound [Ru(bpy)$_2$(tpphz)]$^{2+}$. The final emission intensity is $\sim 5\%$ of the initial value ($\tau_0 = 15\text{ ns}$), indicating that the Ru-tpphz-Co$^{2+}$ adduct is weakly emissive. In contrast, the emission intensity from intercalated [Ru(bpy)$_2$(taptp)]$^{2+}$ is not affected by the presence of similar concentrations of Co$^{2+}$ or Zn$^{2+}$ (Figure 5.14).
Interestingly, upon addition of Co$^{2+}$ or Zn$^{2+}$ the emission intensity of DNA-bound [Ru(bpy)$_2$(tpphz)]$^{2+}$ decreases in a step-like manner, with the initiation of quenching at 1:1 ratio of [M$^{2+}$] to [Ru$^{2+}$] (M = Co, Zn) and the quenching is complete at 2:1 ratio. In contrast, upon addition of Cu$^{2+}$ the emission intensity of the complex decreases linearly and is completely quenched at 1:1 ratio of [Cu$^{2+}$] and [Ru$^{2+}$].$^{54}$ Such a delay in quenching is explained by the presence of Cl$^{-}$ anions in the DNA solution (5 mM Tris, pH 7.5, 50 mM NaCl) such that chloride dissociation from the Co$^{2+}$ and Zn$^{2+}$ is required for the metal ions to bind with the tpphz nitrogens. Indeed, at low [NaCl] (< 20 mM) the emission intensity decrease follows linear behavior upon the addition of Co$^{2+}$. Quenching experiments in the presence of various concentrations of NaCl are consistent with this interpretation.
Relative viscosity measurements show that the emission quenching is not due to the displacement of [Ru(bpy)$_2$(tpphz)]$^{2+}$ by Co$^{2+}$ (and Zn$^{2+}$) from the DNA duplex. [Ru(bpy)$_2$(tpphz)]$^{2+}$ remains intercalated since the relative viscosity of DNA solutions remain unchanged at 1:2, 1:1 and 2:1 [Ru]:[Co$^{2+}$] ratios relative to the control, when no Co$^{2+}$ is added to intercalated [Ru(bpy)$_2$(tpphz)]$^{2+}$ (Figure 5.15).

The intercalation of metal complexes can take place from the minor or major grooves.$^{53,79}$ The direct competition experiments with the minor groove binder distamycin and the major groove binder $\Delta$-$\alpha$-[Rh[(R,R)-Me$_2$trien]phi]$^{3+}$ ((R,R)-Me$_2$trien = 2R, 9R-diamino-4,7-diazadecane, phi = 9,10-phenanthrenequinone diimine) confirmed that the major groove is the preferable binding site for [Ru(bpy)$_2$(dppz)]$^{2+}$. $^{97}$ If [Ru(bpy)$_2$(tpphz)]$^{2+}$ binds from the major groove, then the Co$^{2+}$, Zn$^{2+}$ or Cu$^{2+}$ ions must bind to the tpphz ligand from the minor groove as shown in Figure 5.16.

**Figure 5.15.** Relative viscosity changes of 1 mM herring sperm DNA (5mM Tris, 50 mM NaCl, pH 7.5) as a function of added [Ru(bpy)$_2$(tpphz)]$^{2+}$, keeping [Ru]:[DNA] as: (●) no Co$^{2+}$; (♦) 2:1; (□) 1:1; (+) 1:2.
5.2.7. Cycling the Light Switch ON and OFF

The quenched emission from intercalated [Ru(bpy)$_2$(tpphz)]$^{2+}$-M$^{2+}$ can be completely recovered upon addition of EDTA (ethylenediaminetetraacetic acid), a chelating agent (Figure 5.17). Starting with 12 µM [Ru(bpy)$_2$(tpphz)]$^{2+}$ and 25 µM Co$^{2+}$ in the presence of 120 µM DNA (DNA light-switch is OFF), the emission can be recovered completely through the addition of 18 µM EDTA. The concentrations of EDTA and Co$^{2+}$ required to turn the DNA light-switch ON and OFF are not equimolar due to difference in binding constants of Co$^{2+}$ to EDTA and the distal tpphz nitrogen atoms. The binding constants for Co$^{2+}$ with bpy and EDTA ligands $2.5 \times 10^6$ and $2.8 \times 10^{16}$ M$^{-1}$, respectively.
Figure 5.17 shows the changes in the relative emission intensity upon successive additions of Co\(^{2+}\) and EDTA to the same solution, thus flipping emission OFF and ON, respectively. It is important to emphasize that emission intensity is regenerated completely. The quenching and recovery of emission is instantaneous in this case.

**Figure 5.17.** Changes in the relative emission intensity of a solution containing 12 µM [Ru(bpy)_2(tpphz)]^{2+} and 120 µM DNA (5 mM Tris, pH = 7.5, 50 mM NaCl) upon successive additions of Co\(^{2+}\) and EDTA (\(\lambda_{\text{exc}} = 450\) nm, \(\lambda_{\text{em}} = 628\) nm).
5.3. Concluding Remarks

The DNA intercalation by [Ru(bpy)$_2$(taupt)$_2$]$^{2+}$ and [Ru(bpy)$_2$(tppz)$_2$]$^{2+}$ was established by changes in the relative viscosity and was supported by changes in the absorption of the probe increase in emission intensity. The DNA binding constants by the two complexes were determined using equilibrium dialysis. Both complexes show typical “DNA-light switch” behavior with a large increase in the emission intensity in buffer upon intercalation into the DNA duplex. Both complexes produce a nicked plasmid DNA upon irradiation with visible light ($\lambda_{irr} > 395$ nm) via the production of $^{1}$O$_2$ by energy transfer.

The emission of intercalated [Ru(bpy)$_2$(tppz)$_2$]$^{2+}$ can be statically quenched in the presence of Co$^{2+}$ and Zn$^{2+}$ both in CH$_3$CN and when the complexes are intercalated between the DNA bases. The statically quenched emission can be regenerated through the addition of EDTA. Several “on-off” cycles can be accomplished upon successive addition of Co$^{2+}$ and EDTA to solution which contains [Ru(bpy)$_2$(tppz)$_2$]$^{2+}$ intercalated into DNA.

To our knowledge, this work presents the first example of a reversible DNA light-switch.
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Mononuclear platinum complexes Pt(NH$_3$)Cl$_2$, Pt(bpy)Cl$_2$, and Pt(dppz)Cl$_2$ were studied for their DNA binding and photocleavage properties. It was found that Pt(NH$_3$)Cl$_2$ and Pt(bpy)Cl$_2$ bind covalently to DNA, while Pt(dppz)Cl$_2$ prefers intercalative binding mode as was shown by gel mobility assays and changes in relative viscosity. Pt(dppz)Cl$_2$ photocleaves DNA upon irradiation with $\lambda > 395$ nm (15 min) equally under both aerobic and anaerobic conditions, while Pt(NH$_3$)Cl$_2$ and Pt(bpy)Cl$_2$ are do not effect the DNA photocleavage. Evidently, the presence of the photoactive dppz ligand is responsible for such a dramatic difference in photochemical properties of these complexes. However, for potential use in PDT, the absorption maximum of Pt(dppz)Cl$_2$ needs to be shifted to lower energies and its solubility in water needsto be improved.

The photocleavage of ds-DNA by a series of dinuclear [Rh$_2$(µ-O$_2$CCH$_3$)(R$_1$R$_2$-dppz)$_2$](O$_2$CCH$_3$)$_2$ complexes (R$_1$ = R$_2$ = NO$_2$, Cl, H, CH$_3$, OCH$_3$, 15-crown-5, 18-crown-6 or R$_1$ = H, R$_2$ = NO$_2$, Cl, CH$_3$, OCH$_3$) was studied. It results in the production of the nicked form of DNA ($\lambda > 495$ nm, 20 min), and proceeds through an oxygen-independent pathway. The percent of nicked DNA decreases with the increase of the reduction potential of the R,R’-dppz ligand in the metal complex. A linear relationship between $1/[$DNA$_{\text{nicked}}$] vs the free energy $|\Delta G^0|$ was established. It was shown that the
[Rh₂(µ-O₂CCH₃)(R₁,R₂-dppz)₂](O₂CCH₃)₂ complexes do not intercalate into ds-DNA (K₀ = 4 × 10³).

The DNA light switch complexes [Ru(bpy)₂(tpphz)]Cl₂ and [Ru(bpy)₂(taptp)]Cl₂ are weakly luminescent in water and become fairly emissive upon intercalation into ds-DNA. The emission of intercalated [Ru(bpy)₂(tpphz)]²⁺ can be quenched by the addition of transition metal ions, such as Co²⁺ and Zn²⁺. Due to the presence of two distal nitrogens on the tpphz ligand the formation of the ground-state Ru(tpphz)-DNA-M²⁺ (M = Co²⁺, Zn²⁺) adduct is possible. Upon addition of a chelating agent, EDTA, the emission of intercalated [Ru(bpy)₂(tpphz)]²⁺ can be completely regenerated. Several cycles of the quenching and regeneration of the emission intensity can be accomplished upon successive addition of Co²⁺ and EDTA to the same solution of [Ru(bpy)₂(tpphz)]²⁺ and DNA. As expected, the emission intensity of intercalated [Ru(bpy)₂(taptp)]²⁺ was not affected by the addition of Co²⁺ or Zn²⁺. These results show that the DNA light switch can be controlled chemically by external agents.


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