Influence of Protonation State on the Excited State Dynamics of a Photobiologically Active Ru(II) Dyad

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Supporting Information

ABSTRACT: The influence of ligand protonation on the photophysics of a ruthenium (Ru) dyad bearing the 2-(1-pyrenyl)-1*H*-imidazo[4,5-*f*][1,10]-phenanthroline (ippy) ligand was investigated by time-resolved transient absorption spectroscopy. It was found that changes in the protonation state of the imidazole group led to changes in the electronic configuration of the lowest lying excited state. Formation of the fully deprotonated imidazole anion resulted in excited state signatures that were consistent with a low-lying intraligand (IL) triplet state. This assignment was supported by time-dependent density functional theory (TDDFT) calculations. IL triplet states have been suggested to be potent mediators of photodynamic effects. Thus, these results are of interest in the design of Ru metal complexes as photosensitizers (PSs) for photodynamic therapy (PDT).



1. INTRODUCTION

Due to their rich photophysical and electrochemical properties, ruthenium(II) polypyridyl complexes (RPCs) have long been considered as photosensitizers (PSs) for use in a wide variety of applications spanning molecular devices,^{1,2} dye-sensitized solar cells,^{3–7} photocatalysts,^{8–11} and luminescence sensing applica-tions.^{12–17} More recently, there has been a growing interest in RPCs as metallopharmaceuticals, including biomedical diagnostics and agents for photodynamic therapy (PDT).¹⁸⁻²¹ This general interest in RPCs and Ru-based coordination complexes derives, in part, from their modular architectures, which can be modified by rational design to provide access to a wide variety of triplet excited-state configurations with characteristic reactivities: metal-to-ligand charge transfer (MLCT), metalcentered (MC), ligand-centered (LC) or intraligand (IL), intraligand charge transfer (ILCT), ligand-to-ligand charge transfer (LLCT), metal-to-metal charge transfer (MMCT), and others.²

Some of these triplet excited states, alone or working in concert, can participate in type II energy- and type I electron-transfer processes with molecular oxygen to form reactive oxygen species (ROS) such as singlet oxygen $(^{1}O_{2})$ and superoxide $(O_{2}^{\bullet-})$. Others undergo redox chemistry directly with biological targets via type III processes, or form covalent

adducts with biomolecules such as DNA through ligand exchange reactions (type IV).²³ Collectively, these excitedstate pathways constitute a potent and selective mechanism for triggering cell death when the accumulated PS is exposed to light of an appropriate wavelength. This photocytoxicity induced by otherwise nontoxic prodrugs forms the basis of the anticancer modality known as PDT, which has traditionally employed organic PSs that generate ${}^{1}O_{2}$ from the formally forbidden triplet $\pi\pi^{*}$ excited state of the PS (porphyrins, chlorins, bacteriochlorins, and phthalocyanines, etc.).²⁴

There is much interest in exploiting RPCs and other Ru coordination complexes for PDT because ${}^{1}O_{2}$ sensitization can be much more efficient in these systems, owing to the near unity quantum yields for triplet-state formation as a result of the larger spin orbit coupling (SOC) induced by the heavy Ru metal center. In addition, some RPCs derived from π -expansive ligands possess low-energy, long-lived ³IL states that are extremely sensitive to trace oxygen (and are thus capable of generating ${}^{1}O_{2}$ at low oxygen tension).²⁵ Other RPCs have been cleverly designed to exploit steric strain in the

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coordination sphere to access dissociative ³MC states that covalently modify DNA (or other biomolecules) and do not require oxygen at all.^{21,26–30} The ability of certain RPCs to exert their phototoxic effects in hypoxic environments is a key advantage over the organic PSs because some of the most aggressive and drug-resistant malignancies are hypoxic,^{31,32} and this point in particular has generated ongoing interest in developing Ru-based PSs for light-activated cancer therapy.

We have previously reported several classes of Ru-based metal-organic dyads, hybrid systems composed of a Ru scaffold tethered or contiguously fused to a π -expansive organic chromophore, that generate potent PDT effects.³³⁻³⁷ The purpose of the π -expansive organic unit is to contribute a lowenergy, long-lived ³IL state in energetic proximity to the ³MLCT (≤ 2.1 eV). This construct exploits the efficient tripletstate formation known for Ru complexes and the long intrinsic triplet-state lifetimes of organic compounds. The rationale is that the resulting Ru dyads should be extremely sensitive to triplet-state quenchers. Work pioneered by Ford and Rodgers showed that ³MLCT-³IL equilibration can lengthen ³MLCT lifetimes drastically compared to the lifetimes of the parent $[Ru(bpy)_3]^{2+}$ and $[Ru(tpy)_2]^{2+}$ complexes (bpy = 2,2'bipyridine, tpy = 2,2',2''-terpyridine).³⁸⁻⁴⁰ Complementary studies revealed that lowest lying "pure" ³IL states that do not thermally equilibrate with ${}^{3}MLCT$ states give rise to even longer intrinsic lifetimes (up to 150 μ s). ${}^{41-43}$ We and others have demonstrated that these long-lived ³IL states are highly photosensitizing toward cancer cells, making the Ru dyads (in general) excellent PSs for PDT.^{44–46} Moreover, it appears that these ³IL states can be directly populated with red light, where molar extinction coefficients are exceedingly small due to the forbidden nature of the singlet-triplet absorption transition. The result is that nominally blue-green absorbing PSs yield potent PDT effects even with red light activation.²

As part of our exploration of ³IL states in a variety of Ru dyad constructs, we have shown that complexes of 2-(1-pyrenyl)-1Himidazo[4,5-*f*][1,10]phenanthroline (ippy) exhibit potent PDT effects against cancer cells, bacteria, and bacterial biofilms.³⁴ Supported by Stern–Volmer quenching constants (K_{SV}) as large as 0.2393 Torr^{-1,48} [Ru(bpy)₂(ippy)]²⁺ and its derivatives are extremely sensitive to trace oxygen owing to ³MLCT-³IL excited-state equilibration that prolongs the intrinsic ³MLCTstate lifetime. This sensitivity may be responsible for the potent in vitro PDT effects elicited by the Ru ippy dyads. The ippy ligand is crucial to this photobiological activity because it contributes a pyrene-centered ³IL transition in energetic proximity to the lowest lying ³MLCT (~2.1 eV), and we have shown previously that access to this state is strongly influenced by the orientation of the pyrenyl unit relative to the imidazo[4,5-*f*][1,10]phenanthroline (ip) coordinating ligand.³⁶ It stands to reason that the protonation state of the imidazole functionality of the ip ligand will influence the ip-py dihedral angle and that such pH-dependent conformational changes will control access to ³IL states.

The ip moiety of the ippy ligand is pH responsive in both the ground and excited states of these dyads,^{49–51} making it possible to exploit the ionization state of the imidazole group for pH-dependent DNA binding and damage as a means of providing selectivity for diseased cells.^{52,53} Moreover, the ionization state of the imidazole group should also have a profound influence on the excited-state dynamics (and hence photobiological acitivity) over a physiologically relevant pH range and may offer an added layer of selectivity for diseased

cells with pH abnormalities due to altered metabolic activity. In the present study, we explore the influence of the protonation state of the ionizable imidazole group on the contribution of ³IL states to the excited-state dynamics in a Ru ippy dyad.

2. EXPERIMENTAL PROCEDURES

2.1. Materials. The synthesis of RuH was described previously.³⁴ RuH was dissolved in distilled water for spectroscopic measurements (pH 6.9). HCl (10 M) or NaOH (10 M) was added to adjust the protonation state of RuH to RuH₂ or Ru, respectively. Final pH values for the HCl and NaOH solutions of RuH and Ru were 0.88 and 13.7, respectively. The freeze-pump-thaw method was used to remove oxygen from samples for nanosecond transient absorption measurements.

2.2. Instrumentation. Steady-state UV/vis absorption spectra were recorded in a 1 cm quartz cell on a JASCO V-670 spectrophotometer. Steady-state emission spectra were recorded with a JASCO FP-6200 spectrofluorometer.

The setup for femtosecond transient absorption is described elsewhere.^{54,55} Samples of RuH, RuH₂, or Ru were excited at 403 nm, and their photoinduced dynamics were probed by a white-light supercontinuum. The photophysics of the deprotonated species Ru was also interrogated with excitation at 483 and 513 nm. Sample concentrations were adjusted to yield an optical density of approximately 0.5 at the respective excitation wavelength in a 1 mm quartz cell. For kinetic analyses, the transient absorption data were chirp-corrected and subsequently analyzed by global fitting using a sum of exponentials.

Nanosecond transient absorption measurements of RuH were performed in water (pH = 7) before and after addition of HCl (pH < 7) or NaOH (pH > 7) in a sealed quartz cell with an optical path length of 1 cm (OD = 0.5 at 410 nm). The setup (commercially available from *Pascher Instruments AB*) was used as described in literature.³⁴ The solutions were excited with 10 ps pulses, centered at 410 nm, while the probewavelength was scanned between 380 and 800 nm in steps of 10 nm. Quantitative data analysis was based on a global fit of the data.

During the time-resolved spectroscopic experiments, sample integrity was ensured by recording absorption spectra of the samples before and after each spectroscopic experiment. No spectral changes indicative of sample degradation were observed.

2.3. Computational Methods. Structural and electronic data for the Ru dyad with its imidazole group in the fully deprotonated (Ru), protonated (RuH), and fully protonated (RuH₂) forms were obtained from quantum chemical calculations performed using Gaussian 09. For the complex with its imidazole group in the neutral form RuH, two isomers were investigated that differ by which nitrogen atom of the imidazole ring is protonated. The geometries, vibrational frequencies, and normal coordinates of the electronic singlet ground state were calculated by DFT using the B3LYP exchange-correlation functional, a functional based on B3LYP and denoted as B3LYP35, combining 35% of exactexchange, 58.5% of nonlocal B88 exchange, and the LYP correlation as well as the long-range corrected CAM-B3LYP functional and employing the 6-31G(d) double- ζ basis set for all main group elements. For the Ru atom, the 28-electron relativistic core potential MWB was applied with its basis set describing the electrons (4s, 4p, 4d, and 5s) explicitly and the inner shells by means of a core potential. To correct for the lack



Figure 1. (A) Structures and abbreviations of the three protonation states probed in this study. (B) Absorption (solid) and emission (dashed) spectra ($\lambda_{ex} = 450$ nm, OD = 0.032, 1 cm path length) for RuH (black), RuH₂, (red), and Ru (blue). Note: the tiny shoulder at 675 nm is a spectrometer artifact.



Figure 2. Experimental (dashed) and calculated (solid) absorption spectra of RuH (A), RuH₂, (B) and Ru (C), whereby the gray and black solid graphs represent the unshifted or shifted calculated spectra. In (A) and (B) MLCT transitions (S_4 , S_7/S_4 , S_9) are shifted by 5000 cm⁻¹ and LC transition (S_9/S_2 , S_6) are shifted by 2500 cm⁻¹. In (C) MLCT transitions (S_7) are shifted by 6500 cm⁻¹ and LC transition (S_1 , S_6) are shifted by 2000 cm⁻¹. The calculated oscillator strengths are represented by black and gray bars. A Lorentzian function with a full-width at half-maximum (fwhm) of 2500 cm⁻¹ was employed to broaden the transitions in the simulated spectrum. Only MLCT states were considered for the shifted calculated spectra.

of anharmonicity and the approximated treatment of electron correlation, the harmonic vibrational frequencies were scaled by a factor of 0.97 (B3LYP) and 0.95 (B3LYP35 and CAM-B3LYP). The vertical excitation energies, oscillator strengths, and analytic Cartesian energy derivatives of the excited states were obtained by TDDFT within the adiabatic approximation and by utilizing the same functionals (B3YLP, B3LYP35 and CAM-B3YP), basis set, and pseudopotential.

The simulated absorption spectra have been determined on the basis of the 100 lowest excited singlet states. Effects of solvent interactions (water: $\varepsilon = 78.355$) on the equilibrium structure, vibrational frequencies, excitation energies, transition

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Table 1. Calculated Vertical Excitation Energies (E^{e}), Oscillator Strengths (f), and Singly-Excited Configurations of the Main Excited States and Experimental Absorption Maxima^{*a*}

state	contributing transition	weight/%	$E^{\rm e}_{\rm Cal} (E^{\rm e}_{\rm Shift})/{\rm eV}$	$\lambda_{\mathrm{Cal}}~(\lambda_{\mathrm{Shift}})/\mathrm{nm}$	f	$\lambda_{\rm Exp}/{ m nm}$
		R	μH			
S_4	d_{xz} (196) $\rightarrow \pi^*_{bpy,phen}(199)$ (MLCT)	33	3.50 (2.88)	354 (431)	0.8517	460
	$\pi_{\text{pyr}} (198) \rightarrow \pi^*_{\text{bpy,phen}}(199) (\text{ILCT})$	16				
	$\pi_{\text{pyr}} (198) \rightarrow \pi^*_{\text{bpy,phen}}(200) (\text{ILCT})$	12				
	d_{xz} (196) $\rightarrow \pi^*_{bpy,phen}(200)$ (MLCT)	9				
	$\pi_{\text{pyr}} (198) \rightarrow \pi^*_{\text{phen,im,pyr}} (202) (\text{ILCT})$	8				
S ₇	d_{xy} (195) $\rightarrow \pi^*_{bpy,phen}(200)$ (MLCT)	64	3.59 (2.97)	345 (417)	0.0961	425
	$d_{xz} (196) \to \pi^*_{bpy}(201) (MLCT)$	12				
S ₉	$\pi_{\text{pyr}} (198) \rightarrow \pi^*_{\text{phen,im,pyr}}(202) (\text{ILCT})$	54	3.67 (3.38)	338 (367)	0.4699	363
	d_{xz} (196) $\to \pi^*_{bpy,phen}(199)$ (MLCT)	14				
		Ru	ıH ₂			
S ₂	$\pi_{\rm pyr} (198) \to \pi^*_{\rm phen} (199) (ILCT)$	31	3.39 (3.08)	366 (403)	0.8659	368
	d_{xz} (196) $\to \pi^*_{phen}$ (199) (MLCT)	23				
	d_{xz} (196) $\rightarrow \pi^*_{phen,im,pyr}$ (200) (MLCT)	14				
	$\pi_{\text{pyr}} (198) \rightarrow \pi^*_{\text{phen,im,pyr}} (200) (\text{ILCT})$	12				
S_4	$d_{yz} (197) \to \pi^*_{bpy}(203) (MLCT)$	63	3.44 (2.82)	361 (440)	0.2263	(460)
	$\pi_{\rm pyr} (198) \to \pi^*_{\rm phen} (199) (ILCT)$	10				
	d_{yz} (197) $\to \pi^*_{phen,im}(201)$ (MLCT)	9				
S ₆	d_{xz} (196) $\to \pi^*_{phen}$ (199) (MLCT)	32	3.52 (3.20)	353 (387)	0.1137	(-)
	$\pi_{\text{pyr}} (198) \rightarrow \pi^*_{\text{phen,im,pyr}} (200) (\text{ILCT})$	23				
	d_{xz} (196) $\rightarrow \pi^*_{phen,im,pyr}$ (200) (MLCT)	14				
	$\pi_{\rm pyr} (198) \to \pi^*_{\rm phen} (199) (ILCT)$	12				
	$d_{yz} (197) \to \pi^*_{bpy}(203) (MLCT)$	9				
S ₉	$d_{xy} (195) \to \pi^*_{bpy}(202) (MLCT)$	73	3.68 (3.06)	337 (405)	0.1286	387
		F	Ru			
S_1	$\pi_{\rm pyr} (198) \to \pi^*_{\rm phen}(201) (ILCT)$	74	3.10 (2.98)	400 (416)	0.5083	(427)
	$\pi_{\rm pyr} (197) \to \pi^*_{\rm phen}(201) (ILCT)$	14				
S ₆	$\pi_{\rm pyr} (198) \to \pi^*_{\rm pyr} (202) (IL)$	88	3.48 (3.35)	356 (370)	0.9328	382
S ₇	$d_{xy} (195) \rightarrow \pi^*_{by}(199) (MLCT)$	78	3.50 (2.70)	354 (460)	0.1388	(466)
The corres	ponding orbitals are depicted in the Suppor	ting Information				

dipole moments, and excited-state gradients were taken into account by the integral equation formalism of the polarizable continuum model. For calculating excitation energies and excited-state gradients, where only fast reorganization of the solvent is important, the nonequilibrium procedure of solvation was used.

The method used herein to shift excited states energies is based on calculated resonance Raman intensities and does not produce a precise match between calculated and observed absorption spectra. This approach assumes that the disagreement between calculated and observed Raman spectra is mainly due to errors in the excited-state energies. However, it should be noted that also errors in excited-state gradients and force constants may be responsible for the disagreement. Collection of Raman excitation profiles is one way to confirm whether our energy-shift approach is correct but was not carried out as part of this study.

3. RESULTS AND DISCUSSION

3.1. Steady-State Absorption Spectra. UV/vis absorption was used to monitor the individual protonation states of the imidazole-containing Ru dyad (Figure 1A). Photometric titration with acid or base indicated that three protonation states of RuH were individually accessible (Figure 1B).³⁶ An aqueous solution of the neutral imidazole form RuH displayed two intense absorption bands at 363 and 460 nm and a shoulder near 425 nm. Addition of HCl produced a new shoulder at 390 nm concomitant with a decrease in the visible

absorption bands and a bathochromic shift in the 460 nm absorption maximum by 5 nm (0.05 eV). By contrast, addition of NaOH caused a global decrease in the absorption signature of RuH, with the disappearance of the two distinct bands characterizing the neutral species to yield a single broad band near 380 nm. These changes are similar to what was observed for the analogous Ru metal complex lacking the pyrenyl unit, where pK_{a1} and pK_{a2} were determined to be 1.97 and 10.48,⁵¹ respectively. Despite notable differences between the absorption spectra of the individual protonation states, emission was centered at 600 nm irrespective of the pH but did increase in intensity with decreasing pH.

TDDFT was used to identify the electronic states that contribute to the absorption characteristics observed for the individual protonation states. On the basis of the calculations of resonance Raman intensities and their comparison to experimental data, a shift of the calculations is necessary (for more information see the Supporting Information). In previous studies on the photophysical properties of RPCs with 4Himidazole ligands, very similar bathochromic shifts of the calculated excitation energies (CAM-B3LYP/6-31G(d)) have been obtained with respect to the absorption spectra.^{56,57} The experimental and calculated absorption spectra are compared in Figure 2. Furthermore, Table 1 summarizes the properties of the excited states that contribute most to the absorption in the visible spectral range (molecular orbitals involved in these excitations, Supporting Information). The lowest energy absorption band observed for RuH, was determined to be

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Figure 3. Transient absorption spectra (λ_{pump} = 403 nm) collected at different delay times for RuH (A) and RuH₂ (B). Transient absorption traces collected at selected wavelengths for RuH (C) and RuH₂ (D). Decay-associated spectra and time constants for RuH (E) and RuH₂ (F). All spectra are normalized to the maximum signal.

due to the MLCT state (S_4 , 460 nm in the calculated spectrum), with electron density transferred from the Ru metal center to both the bpy and ippy ligands. A weaker MLCT state (S_7) with a shoulder at 425 nm in the experimental spectrum was also apparent. The higher energy excitation (S_9) corresponded to an ILCT state from the pyrene to the ip portion of the ippy ligand.

Calculations predicted the first absorption band (S_4) to be shifted by 9 nm (0.06 eV) to 440 nm upon protonation of RuH to form RuH₂, alongside a decrease in oscillator strength from 0.85 for RuH to 0.23 for RuH₂. This prediction agrees with the experimental spectra that show a decrease in the absorption bands by 23% with protonation. The intense absorption band (S_2) corresponded to the experimental absorption band at 368 nm and contains contributions from both ILCT and MLCT states. The weak shoulder experimentally observed at 387 nm was associated with an MLCT state (S_9) . It appeared that in RuH₂ the IL character of the visible absorption was increased compared to that in RuH. This observation was supported by the formation of an intense transition from the electronic ground state to the excited state S_2 .

Deprotonation of RuH to form Ru led to important differences in the electronic structures of the excited states: the influence of IL and ILCT states is magnified compared to the case for both RuH and RuH₂. The two intense bands for Ru at 370 (S_6) and 416 nm (S_1) corresponded to pure ILCT and IL states, respectively. The MLCT character was related to the red-shifted state S_7 . Notably, the nature of the energetically low-lying absorption band changed from being dominated by

MLCT states in the case of RuH and RuH_2 to mainly IL states for Ru. The IL states, which dominate the electronic properties of Ru in the visible spectral range, were mainly associated with the pyrene moiety.

3.2. Femtosecond Transient Absorption Spectrosco-py. After having discussed the ground-state absorption properties of the complex in its individual protonation states, we now shift the focus to the pH-dependent photoinduced processes in RuH.

Femtosecond Transient Absorption of RuH/RuH₂ in Water upon Excitation at 403 nm. We have previously reported the time-dependent transient absorption kinetics for RuH in water. Upon pumping at 403 nm, the signals of RuH₂ evolve similarly and resemble the spectroscopic features of RuH in water (Figure 3). The spectra were characterized by ground-state bleaching (GSB) at early delay times and probe wavelengths below 500 nm, which disappeared within 20 and 100 ps after photoexcitation for RuH and RuH₂, respectively. Aside from the GSB, the pump-probe signal was dominated by excitedstate absorption (ESA) with two discernible maxima at 515 and 555 nm, which evolved with increasing delay time. The spectral shape of the pump-probe signal for RuH remained rather constant as a function of delay time, whereas that for RuH₂ displayed a shift in the global maximum of the differential absorption signal from 555 (at 1 ps) to 515 nm (at 1000 ps).

Insight into the characteristic time-scales governing the excited-state evolution were obtained by analyzing the pumpprobe kinetics (Figure 3C,D). The kinetics recorded for RuH at all probe wavelengths reflected the formation of a long-lived ESA within about 200 ps, which did not change within the experimental time window (1.8 ns). However, transient data for RuH_2 show a discernible probe wavelength dependence: at 650 nm an ESA decay at long delay times was observed, whereas a constant transient absorption signal was observed at 520 nm. These qualitative differences in the transient absorption profiles of RuH and RuH₂ indicate that protonation of RuH substantially alters the photophysical dynamics in this system.

Two- and three-exponential fit functions were required to quantitatively describe the transient absorption kinetics of RuH and RuH₂, respectively (Table 2). The assignment of the

Table 2. Photophysical Parameters Determined for RuH, RuH_{21} and Ru in Water^a

compound	τ_1/ps	τ_2/ps	$ au_3/\mathrm{ps}$	$ au_4/\mu { m s}$	$ au_{ m em1}/\mu{ m s}$	$ au_{ m em2}/\mu m s$
RuH	2.2	33		52	0.6	26
RuH ₂	1.6	18	195	52	0.9	24
Ru	0.5	8	830	51	0.6	

^{*a*}Transient absorption time constants were obtained by global fit of the femtosecond time-resolved absorption data ($\lambda_{pump} = 403 \text{ nm}$) with two or three exponential functions (τ_{1-3}) and an infinite component, or with one exponential function (τ_4) for nanosecond time-resolved measurements ($\lambda_{pump} = 410 \text{ nm}$). Emission lifetimes ($\tau_{em1,2}$) were obtained at a probe wavelength of 600 nm.

observed components to molecular processes for RuH was reported earlier.⁵⁸ The decay-associated spectra obtained from the multiexponential analysis of the pump-probe data (Figure 3E,F) suggested that τ_1 and τ_2 describe identical photophysical processes in either protonation state. The shorter time-constant τ_1 present in both RuH (2.2 ps)³⁶ and RuH₂ (1.6 ps) was assigned to vibrational relaxation within the ³MLCT manifold. The formation of the ³MLCT state by rapid intersystem crossing from the photoexcited ¹MLCT state^{59,60} was not resolved in our experiments due to the limited temporal resolution. The slightly longer time constant τ_2 , 33 (RuH)³⁶ and 18 ps (RuH₂), was attributed to rotation around the ip–py bond. This geometrical change in the electronically excited state enables electronic coupling between the ip and py units, and, consequently, facilitates the population of an IL triplet state via energy transfer.^{54,61}

For RuH₂ a third excited-state process ($\tau_3 = 195 \text{ ps}$) was needed to accurately describe the subnanosecond photophysics. This much longer kinetic component displayed increasing/ decreasing differential absorption at wavelengths shorter/longer than 575 nm. Similar transient absorption features were observed for Ru and will be assigned in detail on the basis of DFT calculations later in this paper. On the basis of the observations for Ru, the positive or negative Δ OD signals of RuH₂ below or above 575 nm were assigned to MLCT or IL states, respectively. Hence, τ_3 is assigned to a py \rightarrow ip energy transfer.

For RuH, a similar positve signal is reflected in the infinite component as a weak shoulder around 620 nm (Figure 3E) and is also assigned to the py \rightarrow ip energy transfer. Although the decay of this state in RuH exceeds the temporal window accessible in this spectroscopic experiment (hence the spectroscopic signature appears in the infinite component of the pump-probe data anaylsis), it decays rather rapidly in RuH₂ and constitutes the component associated with $\tau_3 = 195$ ps (vide supra).

Femtosecond Transient Absorption of Ru in Water upon Excitation at 403/483/513 nm. Deprotonation of RuH to form Ru yielded excited-state processes that were significantly different from both RuH and RuH₂ (Figure 4). Excitation at 403 nm produced a single broad and relatively unstructured ESA band with a maximum at 610 nm. The intensity of this band increased during the first 50 ps and then decayed on the subnanosecond time scale (Figure 4A,B). These kinetics were rationalized by considering the results of the TDDFT



Figure 4. Transient absorption spectra collected at 1700 ps for Ru upon pumping at 403 (A), 483 (B), or 513 nm (C). Spectra are normalized to the maximum value of the data. Decay-associated spectra of Ru after excitation at 403 nm (D). Scattering of the pump beam in spectra B and C has been omitted for clarity.

calculations (Figure 2C). The calculated ground-state absorption for Ru at 403 nm was ascribed to S_1 (ILCT) and S_6 (IL) transitions. The calculated absorption spectra for RuH and RuH₂, in contrast, displayed MLCT character at 403 nm. Hence, we assume that the origin of the photoinduced excitedstate dynamics of Ru is of a very different nature compared to the dynamics of its pronated counterparts. To quantitatively assess the experimental data upon excitation at 403 nm, a triexponential global fit was applied that yielded time constants $\tau_1 = 0.5$, $\tau_2 = 8$, and $\tau_3 = 830$ ps (Figure 4D). The infinite component, reflecting absorption by a very long-lived excited state, was characterized by a maximum at 610 nm. This differed from RuH and RuH₂, whereby the infinite component showed a characteristic absorption maximum below 575 nm. Absorption at 610 nm is characteristic of ³IL states, which for Ru is directly populated upon 403 nm excitation.^{62,63}

To verify this hypothesis, femtosecond transient absorption data for Ru were also recorded with excitation at 483 and 513 nm (see Supporting Information for transient spectra and kinetic traces), which, according to the TDDFT results, populates an MLCT state (S_7) (Figure 2C). Notably, the maximum of the differential absorption spectrum recorded at long delay times was shifted from 610 nm (λ_{ex} = 403 nm) to 560 nm (λ_{ex} = 483 or 513 nm) (Figure 4B,C and the Supporting Information). These pump wavelength-dependent differential absorption spectral changes agreed with the quantum mechanical calculations and support the notion that ³MLCT states can be accessed in Ru with longer pump wavelengths. Therefore, the infinite component that was observed with 403 nm excitation has been assigned to a ³IL state that does not decay within the temporal window of our experiments.

The following discussion of Ru is restricted to the transient absorption data collected with excitation at 403 nm, which populates IL and ILCT states. To describe the data quantitatively, a three-exponential fit function was necessary. As for the other protonation states, the short time constant (τ_1 = 0.5 ps) has been attributed to vibrational relaxation of the initially formed excited states (Figure 4F). The spectral contributions associated with τ_1 show two distinct maxima at 570 and 621 nm, which reflect the expected shorter wavelength ³MLCT and longer wavelength ³IL states, respectively. The 8 ps component was ascribed to the rotation about the ip-py coannular bond. This rotation was accelerated up to 4-fold for Ru (RuH, 33 ps; RuH₂, 18 ps) and could be due to the smaller dihedral angle of 11.5° estimated for Ru in the ground state in comparison to that for the two other protonation states (RuH, 35.0/39.5°; RuH₂, 40.6°). Compared to the protonated species, an energy transfer from the py \rightarrow ip is not observed, which indicates that the energy gap in case of Ru is increased. The 830 ps component reflects ground-state recovery from the nonthermalized ³MLCT state. The ultrafast dynamics for all three protonation states are illustrated in the Jablonski diagrams in Figure 5 as well as the two geometries of the ³IL state.

3.3. Nanosecond Transient Absorption and Emission. Previously, the slow ground-state recovery was detailed for the monoprotonated species RuH⁵⁸ and serves as a reference for analyzing the slower kinetics for Ru and RuH₂ (time constants are summarized in Table 2). The transient spectra captured at 12.5 μ s after photoexcitation reflect the essential features of the infinite component documented in the femtosecond measurements (Figure 6, for temporal evolution of RuH₂ and Ru see the Supporting Information). The maxima observed both for





Figure 6. Nanosecond transient absorption spectra collected at 12.5 μ s for RuH (black), RuH₂ (red), and Ru (blue) upon pumping at 410 nm. Spectra are normalized to the maximum value of the data.

RuH and RuH₂ were centered at 500-510 nm and indicate that the nature of the long-lived states is similar in both compounds. In contrast, the deprotonated species Ru produced a broad band with a maximum at 600 nm. This spectral shift of the long-lived state with respect to RuH and RuH₂ is characteristic of a more pronounced contribution of the ³IL state to the signal.

From emission measurements, two radiative lifetimes were calculated for the monoprotonated species RuH, i.e., 0.6 and 26 μ s.³⁶ These emission lifetimes correspond to radiative decay from the ³MLCT state (0.6 μ s) and delayed emission (26 μ s). Delayed emission, and hence the repopulation of the ³MLCT state from the ³IL state, is enabled due to the small ³MLCT–³IL energy gap.⁴¹ The long-lived component observed in the transient absorption data (52 μ s) was assigned to nonradiative decay from the ³IL state.³⁶ The presence of two distinct yet similar microsecond time constants (26 and 52 μ s) might be explained by two energetically close lying ³IL states. However, the nature of these close lying ³IL states remains to be detailed.

The photophysics measured for RuH_2 were similar to those observed for RuH, with emission lifetimes of 0.9 and 24 μ s, which were attributed to spontaneous and delayed ³MLCT emission, respectively. Similarly, nonradiative decay from the ³IL to the ground state was observed with a time constant of 52 μ s. For RuH₂ the transient absorption kinetics were biexponential (22 and 52 μ s), where the 22 μ s component was attributed to the delayed emission.

Only short-lived emission (0.6 μ s) from the ³MLCT state of Ru was observed alongside nonradiative decay of the ³IL with the characteristic time constant of 51 μ s. The absence of a delayed emission suggests that the energy gap between the ³MLCT and ³IL states is increased compared to those for RuH and RuH₂ and excited-state equilibration is not possible.

4. CONCLUSIONS

To our knowledge this is the first systematic study of the influence of ionization state on the excited-state dynamics of a Ru dyad used for PDT applications. The protonated (positively charged), neutral, and deprotonated (negatively charged) forms of Ru ippy were generated in situ by adjusting the pH with strong acid or base. Changes in the protonation state of the imidazole group were found to influence the electronic nature of the lowest lying excited state.

For the neutral and protonated species RuH and RuH₂, the picosecond dynamics reveal a vibrational relaxation of the ³MLCT centered on the ippy ligand and subsequent torsional motion of the pyrene system. An additional sub-100 ps process was observed for RuH2, which is associated with an energy transfer from ³IL to the ³MLCT state. In the case of the deprotonated species Ru, substantially different photoinduced dynamics was apparent, supported by simultaneous population of both IL and MLCT states. After vibrational relaxation and fast planarization of the ³IL state, energy transfer from the ³MLCT to the ³IL state was observed. Transient absorption and emission lifetime experiments on the microsecond time scale indicated ground-state recovery from ³IL states of all protonation states. Two emission lifetimes were identified for RuH and RuH₂, which represent spontaneous and delayed emission of the ³MLCT state. This suggests an equilibrium between the ³MLCT and ³IL states. In the case of Ru, only the spontaneous emission was detected, which reflects a lower energy ³LL state.

It is intriguing to consider that highly photosensitizing ³IL states can be selected for by simply changing the protonation state of an ionizable functional group like imidazole. Although the charge associated with the various protonation states of an ionizable molecule is known to influence its cellular uptake, localization, and affinity for certain biological targets, PSs have not been well-studied in terms of how charge might also influence the photophysical dynamics that give rise to potent PDT effects. Given that some Ru complexes exhibit pH-dependent DNA binding that leads to selectivity for diseased cells, pH-dependent population of highly photosensitizing ³IL states could prove to be a convenient means for improving this selectivity even further. Studies are currently underway to better understand the factors that influence this relationship and develop systems that select for ³IL states at reduced pH.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.6b05957.

Moleular orbitals from quantum chemical calculations, resonance Raman spectra, and femtosecond and nanosecond transient absorption spectra (PDF)

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Notes

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