Mimicking Electron Transfer Reactions in Photosystem II: Synthesis and Photochemical Characterization of a Ruthenium(II) Tris(bipyridyl) Complex with a Covalently Linked Tyrosine

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Abstract: In the natural photosynthetic reaction center photosystem II, absorption of a photon leads to photooxidation of the primary electron donor P_{680}, which subsequently retrieves electrons from a tyrosyl residue, functioning as an interface to the oxygen-evolving manganese complex. In a first step toward mimicking these reactions, we have made a Ru(II)−polypyridine complex with an attached tyrosyl moiety. The photoexcited ruthenium complex played the role of P_{680} and was first oxidized by external acceptors. Combined transient absorbance and EPR studies provided evidence that the Ru(III) formed was reduced by intramolecular electron transfer from the attached tyrosine, with a rate constant of 5×10^4 s^{-1}. Thus we show that a tyrosine radical could be formed by light-induced electron transfer reactions, and we indicate future directions for developing a closer analogy with the photosystem II reactions.

Introduction

In oxygenic photosynthesis, light energy drives the electron transfer from water to carbon dioxide. By oxidizing water, the biosphere is provided with an infinite electron source. Water oxidation is performed by photosystem II (PSII), which is a large membrane-bound protein complex.[1−4] The central core proteins D1 and D2 carry the different cofactors involved in the photochemistry, including a redox-active tyrosyl residue, tyrosine Z (TyrZ).[1−5] Also associated with the core of PSII is a tetranuclear manganese complex which catalyzes the oxidation of water to molecular oxygen.[2,3]

When the primary electron donor chlorophylls, P_{680}, are excited by a light quantum, an electron is transferred to the electron acceptors, a pheophytin and two quinones, Q_A and Q_B.[2,5] The oxidized P_{680} has a redox potential of +1.12 V (vs NHE) and recaptures within nanoseconds an electron from TyrZ, which then forms a neutral radical.[1,2] TyrZ is re-reduced when electrons are extracted from the Mn complex, within 30−1.2 ms depending on the oxidation state of the Mn complex.[2−8] Fourconsecutive electron abstractions lead to the oxidation of two water molecules and the release of one oxygen molecule, during which the Mn complex stores the accumulated oxidizing equivalents.

The mechanism for water oxidation has earlier been thought to involve simple electron transfer from the Mn complex to the oxidized TyrZ, whereas the Mn cluster alone would bind and oxidize water. However, results from recent investigations are difficult to reconcile with TyrZ being a pure electron transfer intermediate.[9,10] Also, spectroscopic data indicated a shorter distance (ca. 4.5 Å) between TyrZ and the Mn complex than previously believed.[11] It was therefore proposed that the tyrosyl radical abstracts hydrogen atoms from water coordinated to the manganese cluster,[12] in analogy with known metal−radical enzyme mechanisms.[13] Quantum chemical calculations recently provided strong support for the feasibility of this mechanism.[14] The results from these calculations show that the O−H-bond strength in water coordinated to manganese complexes is lowered by about 30 kcal/mol, allowing direct hydrogen abstraction by a tyrosyl radical. The proposed new model suggests a conceptual change in the view of the water-oxidizing complex, from being a metal redox center to a metallo-radical one.

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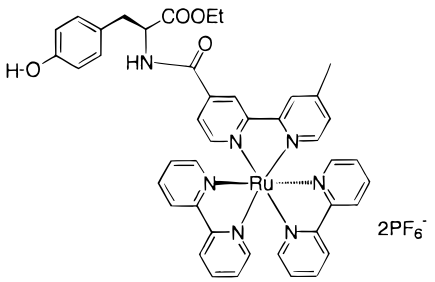

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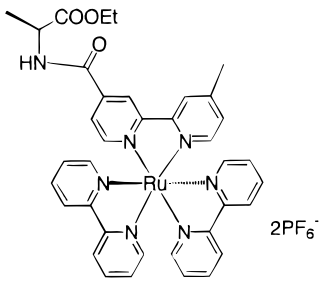

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Figure 1. Model compound **1** and reference compound **2**, synthesized by linking L-tyrosine ethyl ester and L-alanine ester, respectively, to $Ru^{II}(bpy)_2$(4-Me-4′-COCl-2,2′-bpy) via an amide bridge.

The aim of the present work is to construct a model system for the water-oxidizing complex in PSII (Figure 1). In the first step, we wanted to construct a system where a chromophore is photooxidized with the aid of an electron acceptor and then re-reduced by electron transfer from a tyrosyl residue, to mimic part of the electron transfer reactions in PSII. By this process, the tyrosyl residue should be capable of forming a neutral radical (Figure 2), similar to TyrZ in PSII. The results from this project are presented in this paper. In later stages of development, a secondary electron donor based on manganese will be introduced, which can act in conjunction with the tyrosyl radical in a fashion analogous to the reactions in PSII.

The photophysical and electrochemical properties of ruthenium(II) tris(2,2′-bipyridine) (bpy) complexes are well understood.[15−17] The redox potential of a $Ru^{II}(bpy)_3/Ru^{III}(bpy)_3$ pair is typically +1.26 V vs NHE;[19,20] thus we have chosen $Ru^{II}(bpy)_2$(4,4′-Me_2-bpy) to play the role of P_{680}. The redox potential of free tyrosine is +0.93 V at pH 7;[18] hence the reduction of the oxidized ruthenium complex by the tyrosyl residue will be thermodynamically feasible. The Ru(II) complex was covalently linked to an L-tyrosine to form compound **1** (Figure 1). This was subjected to laser flash photolysis in the presence of electron acceptors. Time-resolved emission, transient absorption spectroscopy and EPR spectroscopy were used to follow the photoinduced oxidation of the tyrosyl moiety and re-reduction of the Ru(II) complex. The results are very promising for the further development of systems that can mimic the water-oxidizing complex in PSII.

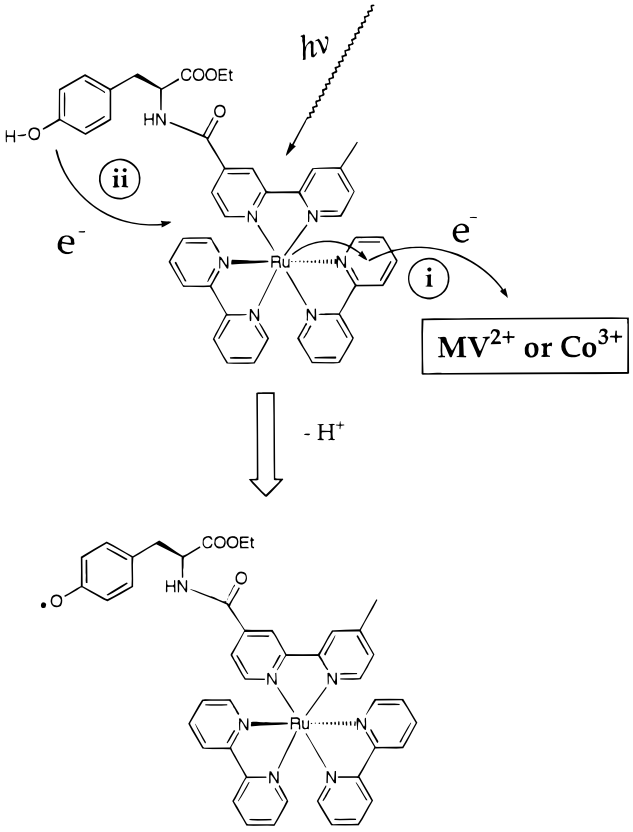

Figure 2. Reaction scheme proposed for the electron transfer events following excitation of **1** in the presence of an electron acceptor. The excited state quenching (i) produces Ru(III) and a reduced acceptor. In step (ii) an electron is transferred from the tyrosyl moiety to Ru(III), restoring the photosensitizer.

Experimental Section

Synthesis. [$Ru^{II}(bpy)_2$(4-Me-4′-(CONH-L-tyrosine ethyl ester)-2,2′-bpy)]$(PF_6)_2$ (1). [$Ru^{II}(bpy)_2$(4-Me-4′-COOH-2,2′-bpy)](PF_6)[21] (400 mg, 0.43 mmol) was dissolved in 30 mL of thionyl chloride, and the solution was heated to reflux under argon for 2 h. Evaporation of the excess thionyl chloride in vacuum gave a dark red oil [$Ru^{II}(bpy)_2$(4-Me-4′-COCl-2,2′-bpy)]Cl_2, which was immediately used for the next step of the reaction.

L-Tyrosine ethyl ester (160 mg, 0.65 mmol) was suspended in acetonitrile (15 mL, 99.9%), and the solid in the suspension was dissolved after triethylamine (0.4 mL, 2.5 mmol) was added. To this clear solution was added dropwise within 5 min the above [$Ru^{II}(bpy)_2$(4-Me-4′-COCl-2,2′-bpy)]Cl_2 complex in acetonitrile (5 mL 99.9%), while white smoke was formed above the solution in the reaction flask. The solution was heated to reflux under argon for 2.5 h and then cooled to room temperature. White crystals (NEt_3·HCl) were formed and filtered off, and the filtrate was concentrated to about 5 mL. The crude product was purified by repetitive column chromatography on neutral aluminum oxide with gradient eluents, dichloromethane and dichloromethane/methanol (94:6, v/v). The desired fractions (controlled by TLC and ^{1}H NMR) were combined, and the solvents were evaporated to dryness to give a red solid. The red solid was dissolved in water, and to this water solution was added a concentrated ammonium hexafluorophosphate aqueous solution to form a red precipitate. Filtration and washing with water and then with ether gave a red solid in 70% yield, after drying in vacuum at room temperature. The product purity was controlled with electrospray ionization mass spectroscopy (ESI-MS), performed on a JEOL spectrometer. The solvent composition for these measurements was 50:50 acetonitrile/water. The absolute mass was determined using polyethylene glycol as internal standard.

Product Characterization by NMR. ^{1}H NMR spectra were measured on a Bruker 400 MHz spectrometer. ^{1}H NMR (400 MHz,

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DMSO-d_6), ppm: 9.31 (d, J = 6.25 Hz, 1H, CON-**H**), 9.26 (s, 1H, Me-bpy-**H**), 8.99 (s, 1H, Me-bpy-**H**), 8.82 (d, J = 8.46 Hz, 4H, bpy-**H**), 8.75 (s, 1H, O-**H**), 8.15−8.19 (m, 4H, bpy-**H**), 7.90 (d, J = 5.88 Hz, 1H, Me-bpy-**H**), 7.79 (dd, J = 10.67 Hz, J' = 5.88 Hz, 1H, Me-bpy-**H**), 7.67−7.75 (m, 4H, bpy-**H**), 7.49−7.58 (m, 5H, bpy-**H**, Me-bpy-**H**), 7.39 (d, J = 5.88 Hz, 1H, Me-bpy-**H**), 7.07 (dd, J = 8.46 Hz, J' = 2.11 Hz, 2H, phenol-*o*-**H**), 6.64 (d, J = 8.46 Hz, 2H, phenol-*m*-**H**), 4.82−4.88 (m, 1H, phenol-CH_2C**H**CO), 4.16 (q, J = 7.20 Hz, 2H, O-C**H**$_2$CH$_3$), 3.03−3.17 (m, 2H, phenol-C**H**$_2$CHCO), 2.62 (s, 3H, bpy-C**H**$_3$), 1.22 (t, J = 7.20 Hz, 3H, O-CH$_2$C**H**$_3$). IR (KBr), cm^{-1}: 1736, 1670, 1619, 1517, 1467, 1447, 1237, 1027, 844, 765, 732, 559. ESI-MS, *m*/*z* (sample injected in a solution of chloroform and acetonitrile (50:50, v/v)): found 964 (M − PF_6^-, monocharged species requires 964), 410 (M − 2PF_6, double-charged species requires 410). Anal. Calcd for $C_{43}H_{39}N_7O_4RuP_2F_{12}$: C, 46.58; H, 3.55; N, 8.84. Found: C, 46.44; H, 3.45; N, 8.72.

[Ru^{II}(bpy)$_2$(4-Me-4′-(CONH-L-alanine ethyl ester)-2,2′-bpy)](PF$_6$)$_2$ (2). Following the procedure for **1**, we used L-alanine ethyl ester instead of L-tyrosine ethyl ester. The same workup procedure afforded a red solid (yield 75%) which had UV−vis and emission quenching spectra similar to those of compound **1**. ^{1}H NMR (400 MHz, DMSO-d_6), ppm: 9.25 (d, J = 2.95 Hz, 1H, CON-**H**), 9.08 (s, 1H, Me-bpy-**H**), 8.82 (d, J = 8.09 Hz, 4H, bpy-**H**), 8.79 (s, 1H, Me-bpy-**H**), 8.15−8.20 (m, 4H, bpy-**H**), 7.88 (d, J = 6.52 Hz, 1H, Me-bpy-**H**), 7.67−7.82 (m, 1H, Me-bpy-**H**, 4H, bpy-**H**), 7.58 (d, J = 5.88 Hz, 1H, Me-bpy-**H**), 7.49−7.55 (m, 4H, bpy-**H**), 7.41 (d, J = 5.88 Hz, 1H, Me-bpy-**H**), 4.48−4.56 (m, 1H, CH_3C**H**CO), 4.15 (q, J = 7.02 Hz, 2H, O-C**H**$_2$CH$_3$), 2.56 (s, 3H, bpy-C**H**$_3$), 1.43 (d, J = 7.35 Hz, 3H, C**H**$_3$CHCO), 1.20 (t, J = 7.02 Hz, 3H, O-CH$_2$C**H**$_3$).

Photophysical and photochemical measurements were performed at pH 7.0 and in Milli-Q water or 5 mM phosphate buffer at 20 ± 2 °C. Methylviologen dichloride ($MVCl_2$, Sigma) and $Co(NH_3)_5Cl_3$ (Aldrich, Quality "99.999%") were used as received, to quench the excited state of the ruthenium moiety in compounds **1** and **2**. For time-resolved measurements, the samples were purged with nitrogen; the concentration of **1** or **2** was 40−60 μM in the case of optical measurements and 1−2 mM for the EPR experiments.

Fluorescence Measurements. Steady-state emission spectra were recorded using a SPEX Fluorolog 2 Series spectrofluorimeter. Emission lifetimes were determined with a time-correlated single-photon-counting setup: a mode-locked, cavity dumped Nd/YAG laser was used to pump a DCM dye laser. The output from the dye laser was frequency-doubled to 327 nm and used to excite the samples with a frequency of 80 kHz. The emission around 610 nm was selected with the aid of an interference filter with 10 nm bandwidth and detected with a microchannel plate. The signals from the microchannel plate (stop signal) and a photodiode (start signal) passed through constant-fraction discriminators into a time-to-amplitude converter and further via an analog-to-digital converter and were finally stored as an emission decay curve in a multichannel analyzer. The instrumental time resolution, full width at half-maximum (fwhm), was 400 ps.

Absorbance Measurements. Absorption spectra were recorded on a HP 8453 diode-array spectrophotometer. Transient absorbance experiments were conducted with a flash-photolysis setup. An ELI-94 excimer laser operating with XeCl, λ = 308 nm, was used to pump an LT-1113 dye laser (both from the Estonian Academy of Sciences), which excited the sample in the visible absorption band maximum (λ_{exc} = 460 nm, <20 ns fwhm, 1 mJ/pulse). A pulsed Xe lamp provided the analyzing light, and the detection system employed a Techtronix 7912AD digitizer and a personal computer for conversion to absorption traces. The bleaching of the visible absorption band was followed somewhat off the band maximum, at 450 nm, in order to reduce the interference by scattered excitation light.

EPR Spectroscopic Measurements. Compound **1** or **2**, dissolved in a few microliters of acetonitrile, was added to a water solution containing 20 mM (pentaammine)cobalt(III) chloride or 50 mM sodium persulfate. The final concentration of **1** was 1−2 mM. The resultant solution was purged with nitrogen gas for 10 min. The handling of the sample was done in darkness. Field-swept CW EPR X-band spectra, or time sweeps at constant magnetic field, were made with a Bruker ESP380 spectrometer. The measurements were made in a flat cell at room temperature during illumination directly in the EPR cavity. Illumination for the field-swept spectra was made in the cavity while the spectra were recorded, either with continuous white light from a 1000 W halogen lamp or by flashing at 5 Hz with 532 nm light from a Nd/YAG laser (at 532 nm about 250 mJ, 6 ns flashes, Spectra Physics).

Time-resolved EPR measurements were made by recording the signal amplitude in the time-sweep mode after a flash with the Nd/YAG laser, at a flash frequency of 0.5−1 Hz. A personal computer and an auxiliary trigger were used to synchronize the laser pulses and EPR spectrometer sweeps. Spectrometer settings: microwave frequency 9.44 GHz, microwave power 18 mW, modulation frequency 100 kHz, modulation amplitude 4 G. The time constants were 1 ms and 66 μs for field-swept spectra and for time sweeps, respectively. The first 3 ms of the time sweep are dominated by a laser flash artifact which was independent of the magnetic field and the content of the measuring cell. This artifact is omitted from the shown kinetic traces.

Results and Discussion

To approach our aim to make a model for the water-oxidizing complex in PSII, a supermolecule was constructed where a tyrosyl residue was covalently connected to the photosensitizer Ru^{II}(bpy)$_3^{2+}$, forming compound **1**. The idea we wanted to test with compound **1** was whether electron transfer from the tyrosyl residue to oxidized ruthenium could be achieved (as depicted in Figure 2). The data available in the literature on tyrosine and Ru^{II}(bpy)$_3$ complexes indicate a difference in the redox potentials of these compounds by about 0.3 V, making the light-induced oxidation of the tyrosyl moiety by Ru^{III}(bpy)$_3$ energetically favorable. As a reference in all photochemical measurements, compound **2** was synthesized, for which the only difference is that an alanine, which is not redox active, was linked to the Ru^{II}(bpy)$_3$ moiety instead of a tyrosine. Time-resolved emission and transient absorption spectroscopy was used to follow the photoinduced oxidation and re-reduction of the Ru(II) complex. In addition, both optical and EPR techniques were used to investigate the possibility of radical formation in **1**.

The starting compound of the synthesis was ruthenium (II) tris(bipyridinemonocarboxylic acid), prepared according to the literature method.[21] The acid chloride was formed by refluxing this in thionyl chloride and then treating it with the ethyl ester of tyrosine or alanine. The crude products were purified by column chromatography and then treated with NH_4PF_6 to give the PF_6 salts **1** and **2**. The products were characterized for structure and purity by ^{1}H NMR (**1**, **2**), IR (**1**, **2**), ESI-MS (**1**), and elemental analysis (**1**).

Optical measurements. The emission lifetime from the excited Ru^{II}(bpy)$_3$ part (uncorrected λ_{max} = 640 nm) in deoxygenated water at 293 K was 370 ns for both **1** and **2** in the absence of any electron acceptors. This result shows that emission quenching by the tyrosyl moiety in **1** was negligible. When 10−20 mM of one of the electron acceptors methylviologen (MV^{2+}) or $Co(NH_3)_5Cl^{2+}$ was added, the emission lifetime of **1** and **2** decreased to 100−150 ns, due to electron transfer from the excited Ru to the acceptors.[17,22,23] About 2 μM each of Ru(III) and MV^+ was formed initially from the approximately 8 μM excited Ru(II) created in each laser flash, which is a typical value under our conditions. Note that the difference in absorption between the excited and ground states of the Ru part in **1** and **2** is smaller than for the typical Ru(bpy)$_3^{2+}$.[24]

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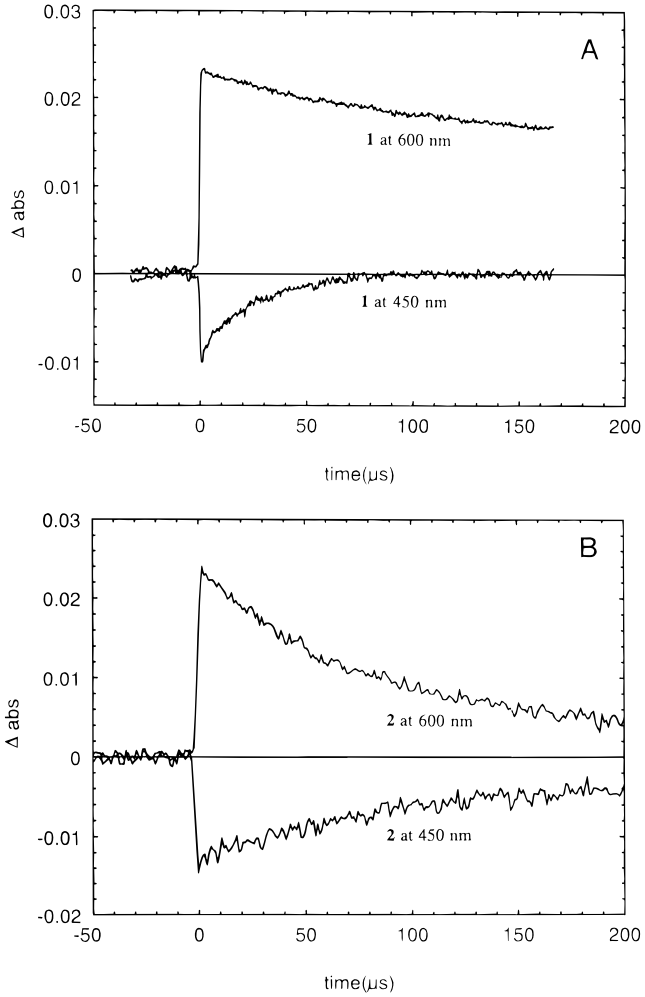

Figure 3. Transient absorption after a laser flash of (A) 60 μM **1** and 15 mM MV^{2+} and (B) 60 μM **2** and 15 mM MV^{2+}. The upper curve in each panel is the absorbance at 600 nm of the viologen radical (MV^+); the lower is the bleaching at 450 nm of the ground state absorbance from the Ru(II) complex. The Ru(II) bleaching recovery at 450 nm is faster for **1** (A) than for **2** (B), which is explained by tyrosine-to-Ru(III) electron transfer.

Light-induced electron transfer could be followed by the transient absorbance changes that occurred after excitation by a laser flash (Figure 3). The lowest energy absorption band of **1** and **2** had a maximum at 458 nm, typical for the lowest metal-to-ligand charge transfer (MLCT) transition of $Ru^{II}(bpy)_3$ complexes.[15−17]

After the flash (λ = 460 nm) a strong bleaching of the Ru MLCT band was seen, as molecules in the ground state were excited. The excited state of **1** or **2** was then quenched, and the appearance and decay of the electron transfer products could be monitored. Thus, due to formation of Ru(III) in the quenching process, the MLCT band was bleached, and the reduced MV^+ gave rise to a strong and characteristic absorbance around 396 and 600 nm (Figure 3).[25,26] The subsequent electron transfer steps were followed by observing the bleaching recovery at 450 nm (Ru(III) to Ru(II)) and the disappearance of the MV^+ absorption.

For the reference compound **2**, which do not contain tyrosine, a recombination between the Ru(III) and MV^+, re-forming the reactants, followed the initial bleaching (Figure 3B). The Ru(II) recovery signal at 450 nm and the MV^+ decay at 600 nm followed the same kinetics, within experimental error, with a first half-life ($t_{1/2}$) of approximately 80 μs (Figure 3B). The decay traces could be fitted to a second-order rate function to give a rate constant of 8×10^9 M^{-1} s^{-1}, consistent with a diffusion-controlled recombination between MV^+ and Ru(III).

With the tyrosine-containing compound **1**, the experiments yielded a quite different result. After the initial quenching, the bleaching recovery at 450 nm was much faster than the decay of the MV^+ absorbance at 600 nm (Figure 3A; compare lower and upper traces). The recovery half-life of **1** at 450 nm was $t_{1/2}$ = 15 μs, which is considerably faster than the corresponding rate in **2**. The curve could be fitted to a single-exponential function, giving a rate constant of $k_{ET} = 5 \times 10^4$ s^{-1}.[27] In addition, the first half-life of the 600 nm signal from the reduced viologen was about 250 μs, i.e. even longer than in the experiments with **2**. A second-order fit on longer time scales than shown in Figure 3A gave $k = 2 \times 10^9$ M^{-1} s^{-1}. Thus, the recovery of Ru(II) was much faster than the decay of the reduced viologen. Consequently, the oxidized Ru(III) must have received an electron from another source than MV^+, a source that is not present in **2**. Since the only additional component in **1** compared to **2** is the tyrosyl moiety, we attribute the observed increase in the Ru(II) recovery rate to intramolecular electron transfer from the tyrosyl residue to the photooxidized Ru, according to the scheme in Figure 2. Assuming this to be true, the slower decay of the MV signal at 600 nm in presence of compound **1** can be explained by a slower recombination of MV^+ with the oxidized tyrosine than with the Ru(III) in the experiment with compound **2**.

When $Co(NH_3)_5Cl^{2+}$ was used as a quencher for **1** instead of MV^{2+}, the absorbance recovery kinetics for Ru(II) at 450 nm were identical to the kinetics in the experiments with MV^{2+} and **1**, giving a k_{ET} of 5×10^4 s^{-1} (Figure 4b).[28] In contrast, when the same quencher was used with **2**, the Ru(II) absorbance did not recover on the time scale studied (0.2 ms, Figure 4a), because the reduced Co acceptor rapidly decomposed to stable Co^{2+}_{aq}, which could not recombine with Ru(III). From these results we conclude that the higher rate of Ru(III) reduction in **1** as compared to **2** was not dependent on the electron acceptors. Instead, the comparison between the kinetic traces at 450 nm for **1** and **2** strongly suggests that the photogenerated Ru(III) was rapidly reduced by electron transfer from the tyrosyl moiety in **1** (Figure 2), while the alanine moiety in **2** was unable to reduce the photooxidized Ru(III).

Strong support for electron transfer from the tyrosyl part was further obtained from transient absorbance measurements at 410 nm, where tyrosyl radicals usually exhibit an absorption maximum (ϵ = 3000 M^{-1} cm^{-1}).[29] The total photobleaching amplitude was lower at 410 nm compared to that at 450 nm, because the observations were made some 50 nm off the absorption maximum of the Ru(II) ground state. Nevertheless, the rate of the bleaching recovery of compound **1** at 410 nm was identical to that at 450 nm (compare traces b and c in Figure 4). However, when the initial bleaching of Ru(II) had decayed in compound **1**, the absorbance at 410 nm was even higher than before the laser flash. We propose that the additional absorbance is caused by the oxidized tyrosyl residue, which has a positive absorption at 410 nm. In Figure 4, curve c was normalized to the same initial bleaching as curve b. Therefore,

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(27) Since the Ru(III)−MV^+ recombination was much slower than the observed bleaching recovery in the experiments with **2** and MV^{2+}, the contribution from the recombination was ignored in the fit of the curve.
(28) Under repeated laser flashing, tyrosine in the exposed region of the optical cell was gradually irreversibly oxidized, even under efficient stirring. Thus, some oxidized fraction of **1** was accumulated, and because of this, the absorption signal at 450 nm did not return completely to the baseline (Figure 4b).
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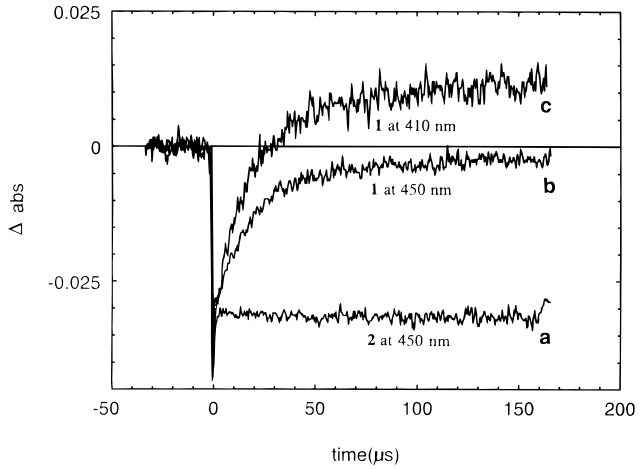

Figure 4. Transient absorption following a laser flash of 60 μM **1** or **2** with 15 mM $Co(NH_3)_5Cl^{2+}$ as acceptor: (a) trace at 450 nm for **2**; (b) trace at 450 nm for **1**; (c) trace at 410 nm for **1**. No recovery of the Ru(III) formed in the tyrosine-free compound **2** was seen on the time scale shown (trace a). In the experiments with **1**, a Ru(II) recovery was seen, presumably due to electron transfer from the tyrosine (trace b). Curve c shows the transient absorption at 410 nm, which was higher after 150 μs than before the flash. We attribute the positive absorption at the end to the formation of oxidized tyrosine. Curve c was normalized to the same initial bleaching as curve b, so that the absorption at 410 nm is directly given by the difference between traces b and c.

the absorption at 410 nm due to oxidized tyrosine is directly given by the difference between traces b and c. The relative magnitudes of the 410 nm absorption after 150 ms and the initial bleaching at 450 nm are in agreement with a 100% Ru(III)-to-tyrosine radical conversion, as judged from the pairwise comparison of many curves. Thus, the observations are consistent with the formation of an oxidized tyrosyl residue, as the result of electron transfer from the tyrosyl moiety to the photogenerated Ru(III).

The evolution of the signal at 410 nm could not be followed on longer time scales due to poor baseline stability, but time-resolved EPR measurements yielded important data on this point (see below). It should also be pointed out that the Co(III) acceptor itself did not contribute to any of the observed absorption changes and that the trace-to-trace variation at the individual wavelengths was small compared to the difference between the two wavelenghts.

Separate experiments were performed in order to prove that the proposed tyrosine-to-Ru(III) electron transfer was intramolecular, i.e. occurred between components on the same supermolecule. These were performed with $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine; the same chromophore as in **1**, but without the side arm with the tyrosine substituent), MV^{2+}, and 2 mM free tyrosine in solution. The free tyrosine was found to enhance the rate of Ru(II) recovery by a bimolecular reaction, but the pseudo-first order rate constant was only 4×10^4 s^{-1}. Thus, in the experiments with **1**, a bimolecular reaction between tyrosine and Ru(III) residing on different supermolecules would be too slow to explain the observed Ru(II) recovery when the concentration of **1** was <0.1 mM. This shows that the electron transfer between the tyrosyl and Ru moieties in **1** (Figure 1) was indeed intramolecular.

EPR Spectroscopic Measurements. In PSII, TyrZ which is involved in water oxidation, transiently forms a neutral radical when oxidized.[2,10] The TyrZ radical, which shows spectral properties similar to those of the more long-lived TyrD radical, has a characteristic EPR spectrum centered at $g = 2.0045$[30] and

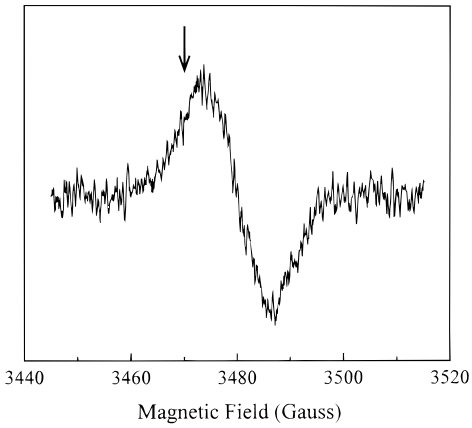

Figure 5. EPR spectrum of compound **1** recorded during illumination directly in the cavity, in the presence of $Co(NH_3)_5Cl^{2+}$ as electron acceptor. During the measurement, the sample was continuously flashed with a Nd/YAG laser working at 5 Hz. Spectrometer settings: microwave frequency 9.77 GHz, microwave power 18 mW, modulation frequency 100 kHz, modulation amplitude 5 G. The time constant was 1.3 ms, sweep time 40 s, and temperature 20 °C. The magnetic field used in time-resolved measurements, 3470 G (shown in Figure 6), is indicated by the arrow.

a peak-to-trough width of 20 G. The high g value is normal for a deprotonated tyrosyl radical.[31] The large and characteristic hyperfine splitting has been attributed to coupling between the unpaired electron on the phenol ring and the β-methylene hydrogens.[32] Oxidation of TyrZ is thought to be sustained by the deprotonation of the phenol oxygen, in that a nearby basic amino acid attracts the proton.[33]

As suggested from our optical measurements, the electron transfer from the covalently bound tyrosyl residue to the photooxidized ruthenium complex in compound **1** will possibly result in formation of a tyrosyl radical. The EPR spectrum of compound **1** was therefore recorded during illumination inside the EPR cavity, in the presence of either one of the sacrificial electron acceptors Co(III) (Figure 5) or $S_2O_8^{2-}$ (data not shown). Illumination, produced either in a continuous way or by repetitive flashing at 5 Hz, generated an EPR signal centered around $g = 2.0045$, with a peak-to-trough width of approximately 14 G (Figure 5). No EPR spectrum was detected when the sample was kept in the dark. As a control experiment, a similar measurement was made with a $Ru(bpy)_3^{2+}$ complex similar to compound **1** but without the tyrosyl moiety. In this case, no EPR signals were detected, either in the dark or during illumination. These results show that the spectrum in Figure 5 indeed originates from the tyrosyl part of **1** and strengthens the conclusion that a tyrosyl radical is formed as a result of light-induced electron transfer from the tyrosyl to the ruthenium part of **1**. This conclusion is corroborated by the high g value, 2.0045, which is very similar to the g values of other tyrosyl radicals. This g value also indicates that the tyrosyl radical in our $Ru^{II}(bpy)_3$−tyrosine compound is deprotonated when it is formed. Indeed, when the same experiment was made at elevated pH values (∼12), significantly higher amounts of the radical were induced during illumination (not shown), indicating that deprotonation promotes radical formation.

When the sample containing compound **1** was subjected to prolonged continuous illumination, the amount of the radical species formed diminished in a few minutes. This implies that

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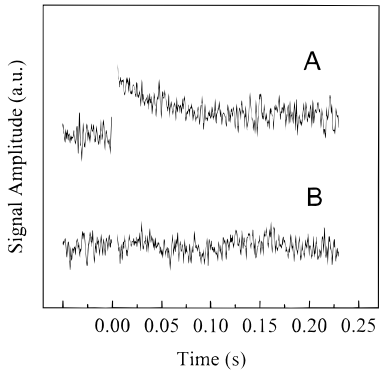

Figure 6. EPR time sweeps after a single flash, at a constant-field position of 3470 G (shown with an arrow in Figure 5). (A) Compound **1**. A nonartifactual decay with a time constant of $t_{1/2} = 50$ ms is observed. (B) $Ru^{II}(bpy)_3^{2+}$. The first 3 ms after the laser flash have been omitted in each curve, due to a laser artifact which is independent of the magnetic field. Both traces A and B are an average of 50 single-flash measurements.

1 is irreversibly consumed, possibly by some chemical reaction of the oxidized tyrosyl residue. Earlier electrochemical studies of free tyrosine have similarly indicated that tyrosine may be irreversibly depleted by so far unexplored reactions in the oxidized state,[29] which might involve dimerization of the tyrosines.

The observed signal from **1** was noticeably broader than what is normal for a radical which is freely rotating in solution. One would anticipate that the explanation for this broadening is underlying hyperfine structure, similar to that for TyrZ. The signal line shape of the spectrum from **1** was slightly asymmetric, which might corroborate this interpretation. However, the structural features were difficult to resolve, and the question of hyperfine structure cannot be fully settled with the technique presently used. Another explanation could be the presence of cobalt, which may broaden the radical by magnetic interaction with the unpaired radical spin. However, when $S_2O_8^{2-}$ was used as electron acceptor, the signal was apparently identical to that observed in the presence of the Co acceptor, weakening the argument that a specific electron acceptor is broadening the signal.

In the optical measurements, the lifetime of the presumed tyrosyl radical absorbing at 410 nm could not be monitored at longer times due to poor baseline stability. However, if the tyrosyl radical is to participate in electron transfer reactions to some extent, the lifetime of the radical is important. It was therefore measured by time-resolved EPR, by monitoring the signal amplitude at a constant field of 3470 G (Figure 6A), which is approximately at the signal maximum. The flash-induced radical signal decayed with a single-exponential decay rate, with a half-life of 50 ms. When the constant-field setting for the transient measurements was moved through the signal region, the amplitudes shifted to zero at the turning point, i.e. at zero amplitude of the derivative, and to negative amplitude in the negative region of the derivative signal (data not shown). The decay rate was constant at all of these field positions, indicating that the observed kinetics are inherent of the observed radical and that the signal represents a single paramagnetic species with an EPR signal similar to that shown in Figure 5. An analogous measurement with $Ru^{II}(bpy)_3$ yielded no observable signal traces at any magnetic field positions (Figure 6B).

Conclusions

Our measurements show that $Ru^{III}(bpy)_3$, which is produced photochemically in the presence of an electron acceptor, is capable of generating a tyrosyl radical by intramolecular electron transfer from a covalently linked tyrosyl residue. This is inferred by the enhanced rate of Ru(II) recovery after photooxidation in the presence of the tyrosyl moiety in **1**. In addition, an oxidized species is formed, which can be observed at 410 nm, where the free tyrosyl radical has an absorption maximum. EPR measurements indicate that a tyrosyl radical was present after photooxidation and recovery of Ru(II) in **1**, but not in $Ru^{II}(bpy)_3$. Compound **1** is therefore a molecule which has essential features similar to those of the central redox components in photosystem II. The Ru(II) ion is oxidized by light and extracts, analogously to the primary donor P_{680}, an electron from a nearby tyrosine. When the experiment is performed in the presence of an irreversible electron acceptor, the oxidized tyrosine has a reasonable lifetime. Since the tyrosine radical has a high redox potential (TyrZ in photosystem II has a redox potential around 0.9 V[34]), compound **1** is a promising starting point for the further development of advanced models for the water-oxidizing complex in photosystem II.

The next step of this project will be to introduce synthetic multinuclear manganese complexes that can play the role of charge storage units, in analogy with the manganese complex in PSII. Our hope is that a photogenerated tyrosyl radical will serve as a stepwise electron or hydrogen atom abstractor, generating sufficient oxidizing equivalents to oxidize first the manganese complexes and ultimately water.

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