A Biomimetic Model System for the Water Oxidizing Triad in Photosystem II

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Abstract: In plants, solar energy is used to extract electrons from water, producing atmospheric oxygen. This is conducted by Photosystem II, where a redox "triad" consisting of chlorophyll, a tyrosine, and a manganese cluster, governs an essential part of the process. Photooxidation of the chlorophylls produces electron transfer from the tyrosine, which forms a radical. The radical and the manganese cluster together extract electrons from water, providing the biosphere with an unlimited electron source. As a partial model for this system we constructed a ruthenium(II) complex with a covalently attached tyrosine, where the photooxidized ruthenium was rereduced by the tyrosine. In this study we show that the tyrosyl radical, which gives a transient EPR signal under illumination, can oxidize a manganese complex. The dinuclear manganese complex, which initially is in the Mn(III)/(III) state, is oxidized by the photogenerated tyrosyl radical to the Mn(III)/(IV) state. The redox potentials in our system are comparable to those in Photosystem II. Thus, our synthetic redox "triad" mimics important elements in the electron donor "triad" in Photosystem II, significantly advancing the development of systems for artificial photosynthesis based on ruthenium−manganese complexes.

The development of energy systems built on solar energy is of immediate interest for most sectors of society. One idea is to construct systems for fuel production using reducing equivalents from water, a sustainable and environmentally safe substrate. In our research aiming for this goal, we have adopted a strategy to develop supramolecular complexes designed on principles from the natural enzyme, Photosystem II (PSII).[1−3] PSII is a large membrane-bound protein complex, for which the detailed structure and function is not yet completely known. Synthetic compounds that mimic its detailed chemistry can consequently not be accomplished. However, also supramolecular chemistry mimicking principally important parts of the light driven reactions in PSII are important for the advancement of artificial photosynthesis. In this report we describe a major step toward the realization of such a system.

In all plants and algae, light absorption drives the electron transfer from water to carbon dioxide, producing atmospheric oxygen and providing the biosphere with an infinite source of reducing power. The light-driven oxidation of water is catalyzed by a redox "triad" in the reaction center of PSII (Figure 1A). The absorption of a light quantum by the primary donor P_{680}, which is constituted by a dimer or multimer of chlorophyll molecules, triggers charge separation and, after several electron-transfer steps, the transfer of an electron to a diffusible quinone on the acceptor side of PSII. The oxidized P_{680} retrieves an electron by oxidizing a nearby tyrosine residue, Tyr_Z, which then forms a neutral radical.[1,4] The tyrosyl radical in its turn oxidizes a tetranuclear Mn-cluster, which is bound to PSII close to the water−protein interface (Figure 1A). After four consecutive turnovers, two water molecules are oxidized, and one oxygen molecule is produced. During catalysis, the Mn-complex cycles between a series of high valence oxidation states.[1,2] The Mn-cluster is rereduced at the end of each cycle and serves to store the oxidizing equivalents throughout the four redox states. Several of the components, including $P680^+$, $Tyr_Z^\bullet$ and some of the oxidation states of the Mn-cluster, reach oxidizing potentials around $+1$ V (vs NHE), which is a requirement for accomplishing water oxidation.

The mechanism for water oxidation has long been thought to involve simple electron transfer from the Mn-complex to the oxidized Tyr_Z, while the Mn-cluster would bind and oxidize water. Recent results however, have lead to the proposal that Tyr_Z itself catalyzes water oxidation by abstracting hydrogen atoms from manganese-coordinated water molecules,[5−8] thus suggesting a crucial role for the redox active tyrosine in the oxidation of water.

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(1) Diner, B. A.; Babcock, G. T. In *Oxygenic Photosynthesis: The Light Reactions*; Ort, D., Yocum, C., Eds.; Kluwer: Dordrecht, The Netherlands, 1996; p 213.
(2) Debus, R. J. *Biochim. Biophys. Acta* **1992**, 1102.
(3) Barber, J.; Andersson, B. *Nature* **1994**, *370*, 31.

(4) Vermaas, W.; Styring, S.; Schröder, W.; Andersson, B. *Photosynth. Res.* **1993**, *38*, 249.
(5) Hoganson, C.; Babcock, G. T. *Science* **1997**, *277*, 1953.
(6) Tommos, C.; Babcock, G. T. *Acc. Chem. Res.* **1998**, *31*, 18.
(7) Gilchrist, M. L.; Ball, J. A.; Randall, D. W.; Britt, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9545.
(8) Hoganson, C. W.; Lydakis-Simantiris, N.; Tang X.-S.; Tommos, C.; Warncke, K.; Babcock, G. T. Diner, B. A.; McCracken, J.; Styring, S. *Photosynth. Res.* **1995**, *46*, 177.
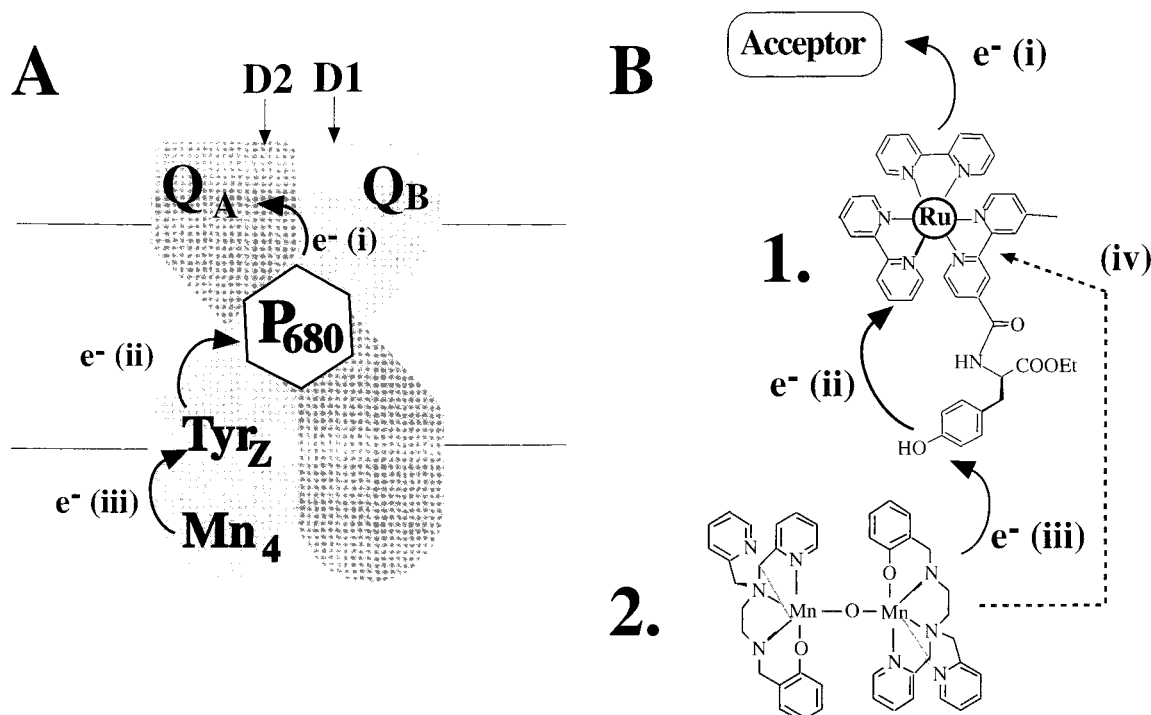
Figure 1. A. Schematic picture of the D1 and D2 reaction center proteins in Photosystem II. The arrows depict electron-transfer reactions within the water oxidizing "triad" consisting of the manganese cluster, Tyr_Z, and the donor chlorophylls P_{680}. Photooxidation of P_{680} (i) leads to oxidation of Tyr_Z (ii), which forms a neutral radical and subsequently oxidizes the Mn-cluster (iii). B. The synthetic "triad" constituted by the Ru−tyrosine compound **1** and the linear μ-oxo- Mn(III)$_2$ complex **2**. In the scheme, the light-induced electron transfer from **2** to **1** is shown: The ruthenium is oxidized by photoinduced electron transfer to an extraneous electron acceptor (i) and recapture of an electron from the tyrosyl moiety (ii).[9] The tyrosyl radical thus-formed oxidizes **2** to the mixed-valence Mn(III)/Mn(IV) state (iii). Alternatively, **2** may be oxidized by the photogenerated Ru(III) ion directly, at elevated concentrations of **2** (iv). The accurate structure of **2** is described in ref 11.

In a recent study[9] we presented a ruthenium(II) tris-bipyridyl (Ru(II)(bpy)$_3$) complex with a covalently linked tyrosyl moiety (compound **1**, Figure 1B), which was made as a model system for the part of PSII that contains Tyr_Z and P_{680}. When Ru(II) is photoexcited, it can be oxidized with the aid of an electron acceptor, typically methyl viologen (MV^{2+}) or Co(NH$_3$)$_5$Cl^{2+}. Analogous to the donor chlorophylls P_{680} in PSII, the Ru(III) ion then extracts an electron from the tyrosyl moiety by intramolecular electron transfer. EPR measurements of the photooxidized species of **1** showed that the oxidized tyrosyl moiety forms a neutral radical (Figures 1B and 4, inset, see also ref 9), in a manner similar to Tyr_Z.[1,10]

To build further on the biomimicking principle, we sought to introduce a synthetic Mn-complex of high valence as secondary electron donor. Our hope was that the photogenerated tyrosyl radical would serve as a stepwise electron or hydrogen atom abstractor (Figure 1B), generating enough oxidizing power to oxidize the manganese complex in a controlled reaction. Ultimately, using catalytically competent Mn-complexes (which to this day are extremely rare) we hope to oxidize water.

Recently, a μ-oxo-bridged Mn(III)$_2$ complex (compound **2**, Figure 1B, bottom) was synthesized, in which each of the Mn ions are provided with a phenolic oxygen ligand.[11] Among several unusual properties of compound **2**, it is reversibly oxidizable in two steps and is thus able to store two positive charges with quite high oxidation potentials, within 500mV.

The redox potential of free tyrosines in water is +0.93 V (vs NHE) at pH 7,[12] therefore it seemed feasible that the tyrosyl radical in **1** should be able to oxidize **2** and generate the mixed-valence Mn(III)/Mn(IV) state. In this study, we tested this hypothesis by subjecting compound **1** to laser flash photolysis in the presence of compound **2** and electron acceptors. Steady-state and time-resolved EPR and optical spectroscopy were used to investigate the outcome and kinetics of light-induced oxidation of **2** by **1**. Our results show that the tyrosyl radical in **1** specifically oxidizes the manganese complex **2** to the mixed-valence state. The photochemical reactions in our experiments show high analogy to reactions taking place in PSII and provide a promising new platform for the further development of Ru−Mn super complexes for artificial photosynthesis.

Experimental Section

Chemicals. Compounds **1** and **2** were prepared and checked for purity as described previously.[9,11] Methyl viologen dichloride (MVCl$_2$), Co(NH$_3$)$_5$Cl^{2+} (pentaaminechlorocobalt(III) chloride), and HEPES (*N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid)) buffer were of highest grade commercially available. Acetonitrile, Aldrich 99.8% A.C.S. reagent quality, and Millipore purified water (>18 MOhm) were used as solvents.

Electrochemistry. Cyclic voltammetry measurements of **1** and **2** were made in water and acetonitrile. Cyclic voltammetry of compound **1** was conducted using a 5 mM phosphate-borate buffer with 0.2 M KCl as electrolyte, pH was adjusted with 1 M HCl or 1 M NaOH.

For cyclic voltammetry of **2** in aqueous solution, the compound was first dissolved in acetonitrile. Measurements were made using a 10 mM HEPES buffer, pH 7.00, containing 0.1 M KCl, as electrolyte. The final concentration of acetonitrile in the electrolyte was 10%. Measurements were also performed using 0.1 M tetrabutylammonium tetrafluoroborate (Aldrich, TBABF$_4$) in acetonitrile as electrolyte. The TBABF$_4$

(9) Magnuson, A.; Berglund, H.; Korall, P.; Hammarström, L.; Åkermark, B.; Styring, S.; Sun, L. *J. Am. Chem. Soc.* **1997**, *119*, 10720.

(10) Barry, B. A.; Babcock, G. T. *Chem. Scr.* **1988**, *28A*, 117.

(11) Horner, O.; Anxolabéhère-Mallart, E.; Charlot, M.-F.; Tchertanov, L.; Guilhem, J.; Mattioli, T.; Boussac, A.; Girerd, J.-J. *Inorg. Chem.* **1998**, In press.

(12) Harriman, A. *J. Am. Chem. Soc.* **1987**, *91*, 6104.

was vacuum dried at 140 °C for 48 h. All glassware were washed and dried in a oven before use. Experiments in acetonitrile were performed in an argon-filled glovebox.

For the electrochemical measurements, a three-electrode system connected to an Eco Chemie model Autolab/GPES electrochemical interface was used. The working electrode was a freshly polished glassy carbon disk, and the counter electrode consisted of a platinum wire. The reference electrodes were Ag/AgCl in 3 M KCl(aq) used for measurements in aqueous solution and Ag/AgCl in saturated LiCl/CH$_3$CN (Merck) for measurements in acetonitrile. Both the reference and counter electrode were separated by a salt bridge from the solution in contact with the working electrode. The ferrocenium/ferrocene couple was used as the internal reference in nonaqueous media.

Flash Photolysis of 2 by 1 Studied by Low-Temperature EPR Spectroscopy. MVCl$_2$ was dissolved in acetonitrile to saturation. Compound **1** or Ru(bpy)$_3^{2+}$ was dissolved in the MVCl$_2$−acetonitrile solution. Compound **2** was added to the reagent mixture a various amounts from equimolar concentrations, down to one-fifth of the concentration of compound **1**. The solutions were prepared in dim room light and transferred to EPR tubes. Laser flashes were given at room temperature, at a repetition rate of 1 Hz from a Nd:YAG laser (about 250 mJ, 6 ns flashes, Spectra Physics) at 532 nm. The samples were given 10 subsequent flashes and transferred within 1 s to an ethanol bath cooled by liquid N$_2$ to 170 K. After being frozen, the samples were stored at 77 K. X-band EPR spectra of the samples were recorded at 78 K, before and after laser flashing.

Chemical Oxidation of 2 Studied by Low-Temperature EPR Spectroscopy. Equimolar amounts of compound **2** and Ru(bpy)$_3^{3+}$ were dissolved together in acetonitrile to a final concentration of 2 mM of each compound. (Ru(bpy)$_3^{3+}$ was produced separately by chemical oxidation of Ru(bpy)$_3^{2+}$.) The solution was added to an EPR tube and frozen to 77 K, before recording the EPR spectrum at 78 K.

Flash Photolysis of 2 by 1 Studied by Time-Resolved EPR Spectroscopy at Room Temperature. Compounds **1** and **2** were each dissolved in small volumes of acetonitrile, as stock solutions. A water solution was prepared containing Co(NH$_3$)$_5$Cl^{2+} (pentaaminechloro-cobalt(III) chloride) dissolved to saturation and 10 mM HEPES buffer adjusted to pH 7.0. From the stock solution of compound **1**, aliquots were then transferred to the buffer solution to give a final concentration of 1 mM of **1**. In measurements where compound **2** was added, the final concentration of **2** was 0.3, 0.6, or 0.8 mM. The total amount of acetonitrile in the resulting reactant mixture was 10%. A quartz EPR flat-cell containing the reaction mixture was given laser flashes at a flash repetition rate of 1/3 Hz, directly in the EPR cavity at room temperature. Time sweeps were taken by recording the signal amplitude of **1** in the time-sweep mode. The magnetic field was fixed at the signal maximum of **1** (3470 G, Figure 4, inset, and ref 9), and time sweeps were recorded after every laser flash. The amount of tyrosyl radical formed by a single flash was spin quantified versus the Tyrosine-Z (Signal II$_{fast}$) radical in Photosystem II,[2,8] which was generated after a single flash and for which the concentration is known. The different line widths of the two radicals were taken into account. A personal computer and an external trigger were used to synchronize the laser pulses and EPR spectrometer sweeps. All EPR measurements were made on a Bruker 380 spectrometer. For low-temperature measurements, this was equipped with an Oxford Instruments cryostat and temperature controller. EPR data handling was carried out with WINEPR 3.0 software for PCs.

Flash Photolysis Experiments with Transient Absorption Detection. An XeCl excimer laser ($\lambda = 308$ nm) was used to pump a LT-1113 dye laser with Coumarin 47 (both lasers were from the Estonian Academy of Sciences). The 460 nm output beam was expanded to cover a 8 × 1 mm spot, overlapping with the analyzing light beam at right angle. The energy per pulse reaching the sample was 1 mJ, and the pulse width was <30 ns. The analyzing light was provided by a pulsed Xe-lamp in a home-built spectrometer system. The analyzing light was filtered through water and a 360 nm cutoff filter before reaching the sample, and a shutter that was open for 1 ms per pulse was used in front of the sample. The light passed a monochromator used to select the observation wavelength before being detected by the photomulti-

plier. The multiplier signal was fed into a digitizer connected to a personal computer, where the transient absorbance traces were recorded and analyzed.

For the experiments in acetonitrile (Aldrich, spectroscopic grade), nitrogen-bubbled solutions with ca. 40 μM **1** and 10−20 mM MV-(PF$_6$)$_2$ was prepared. For the experiments in aqueous solutions, the preparation and conditions matched those of the EPR experiments, except that the concentration of **1** was ca. 40 μM. Thus, a stock solution of **2** in acetonitrile was prepared, and different amounts of this solution were transferred to the optical cell. Acetonitrile was added to a final concentration of 10 vol %. Compound **1** was dissolved in the acetonitrile portion before adding 10 mM HEPES, pH 7.0, that was saturated with Co(NH$_3$)$_5$Cl^{2+} (Aldrich). Air-saturated samples were used in the measurements.

Results and Discussion

Electrochemistry. The aim of our project is to enable photoinduced electron transfer from synthetic Mn-complexes to the tyrosyl radical in **1**. For this purpose, compound **2** is a useful testing compound, since the oxidation product of this compound, the mixed-valence state, is stable and possible to identify with EPR spectroscopy.[11] To investigate if a tyrosine radical in compound **1** could possibly oxidize the manganese compound **2**, we determined the redox potentials of **1** and **2** by cyclic voltammetry in both water and acetonitrile. The cyclic voltammograms for **1** and **2** in aqueous solution are shown in Figure 2. For compound **1**, the tyrosine oxidation is observed, while the peaks for the Ru(II)/Ru(III) redox couple are not possible to observe since this oxidation occurs at a higher potential (+1.26 V vs NHE) than that of water oxidation. The pH-dependence of the oxidation potential for the tyrosyl residue in **1** (inset of Figure 2A), closely follows that for free tyrosine,[12] except for a constant shift of 50 mV toward higher potentials due to Coulombic interaction with Ru(II). At pH 7.0, the tyrosine oxidation occurs over a broad potential interval (Figure 2A) and is therefore difficult to determine with precision, but can be extrapolated from the pH dependence curve to $E_{peak} = +0.97$ V vs NHE. This corresponds to a midpoint potential of about +0.93 V.[12] The observed broadening of the oxidation peak increases as the pH is lowered and might involve chemical reactions that the tyrosyl moiety in compound **1** can undergo in the oxidized state.

For compound **2** the first oxidation wave in water solution at pH 7.0 occurs at a midpoint potential of $E = +0.58$V vs NHE, while the next oxidation step is observed about 450 mV higher, at $E = 1.03$ V (Figure 2B). The reversibility of the first oxidation process was good, with a peak-split of 80 mV, whereas the peak-split for the second process was 180 mV.

In acetonitrile, the oxidation of the tyrosine in **1** is irreversible and occurs at about +0.95 V vs SCE (not shown). Compound **2** was earlier shown to exhibit two reversible oxidations in acetonitrile, with midpoint potentials $E = +0.54$ V and +0.99 V vs SCE, respectively.[11] In the first oxidation process, **2** was converted from Mn(III)/Mn(III) to the mixed-valence Mn(III)/Mn(IV) state. The second oxidation lead to oxidation of one of the phenolate ligands that coordinates to the manganese, as was demonstrated by resonance-Raman spectroscopy.[11] The oxidation potential of the tyrosyl moiety in compound **1** is therefore higher than the midpoint potential for the first oxidation event in compound **2**. On the other hand, the peak potential of **1** is lower than the midpoint potential of the second oxidation wave in **2**.

Thus, the oxidation potential of **1** in both acetonitrile and water lies between the two midpoint potentials in **2**. This shows that the oxidized tyrosyl moiety in **1** would be able to oxidize
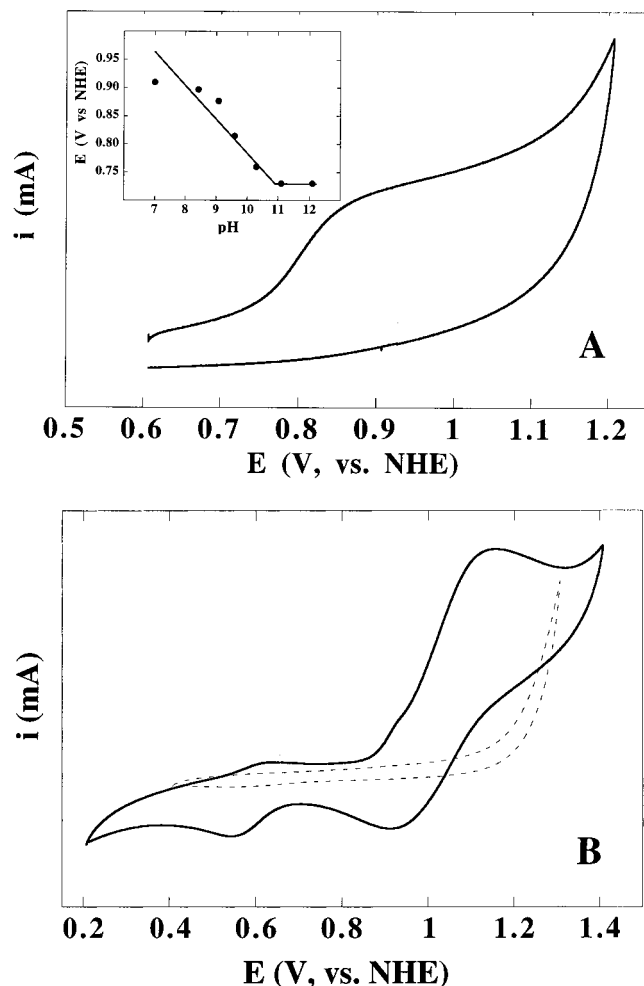
Figure 2. Cyclic voltammograms of (A) the ruthenium−tyrosine compound **1** and (B) the dinuclear Mn(III)/Mn(III) complex **2** in buffered water solution at pH 7.0. The inset in panel A shows the pH dependence of the oxidation peak potential for the tyrosyl moiety in **1** which extrapolates to +0.97 V at pH 7.0 (with a slope of 59 mV/pH unit). The oxidation of Ru(II) to Ru(III) is not observed since the potential for this reaction is higher than for oxidation of water. For compound **2**, two oxidation waves are observed (B): One with a midpoint potential of +0.58 V, and the second with a midpoint potential of +1.03 V. Scan rates were 500 mV/s in A and 100 mV/s in B. In the inset in panel A, the scan rate was 250 mV/s, except at pH 7.0 where it was 750 mV/s, to minimize artifactual side reactions that interfere with the CV at lower pH values.

2 one step, to the mixed-valence state, but not further. The Ru(III)(bpy)$_3$ moiety on the other hand should be able to generate the doubly oxidized complex **2**. This option was further investigated by EPR and transient optical spectroscopy.

Low-Temperature EPR Measurements. Compound **1** was subjected to laser flash photolysis in the presence of compound **2**, either in acetonitrile using MVCl$_2$ as electron acceptor or in aqueous solution in the presence of the sacrificial electron acceptor Co(NH$_3$)$_5$Cl^{2+}. A reaction mixture containing **1**, **2**, and MVCl$_2$ in acetonitrile was given laser flashes at room temperature and then frozen in liquid nitrogen within 1 s after the last flash. Figure 3a shows the EPR spectrum obtained after 10 laser flashes had been given to the solution. The EPR spectrum, which is approximately 1250 G wide, displays 18 hyperfine lines and is a fingerprint of the mixed-valence Mn(III)/Mn(IV) species. The spectrum is the same as the one observed when compound **2** was oxidized electrochemically to the Mn(III)/Mn(IV) state, as described previously.[11] The signal increased with an increas-
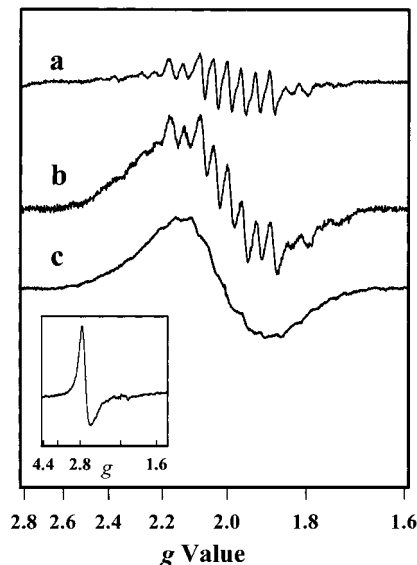

Figure 3. EPR spectra of different oxidized forms of **2**. (a) EPR spectrum of the Mn(III)/Mn(IV) state formed, as the result of 10 flashes given to a solution of **1** (2 mM) and **2** (2 mM) in acetonitrile, in the presence of MV^{2+}. Quickly after flashing, the sample was frozen to 77 K. (b) EPR spectrum recorded after oxidation of **2** (2 mM) by Ru(III) (bpy)$_3$ (PF$_6$)$_2$ (2 mM) in the dark. In spectrum b, a broad underlying signal, which is essentially absent in spectrum a, is seen together with the Mn(III)/Mn(IV) spectrum. (c) EPR spectrum recorded after 25 flashes were given to a solution of **2** (2 mM) and Ru(II)(bpy)$_3$ (2 mM), in the presence of MV^{2+}. A similar spectrum as in b is observed, with the exception that the relative amount of the broad signal, compared to that of the hyperfine resolved signal, is higher. The inset shows the EPR spectrum of Ru(III) (bpy)$_3$. This spectral species is absent from b and c, indicating that all Ru(III) was reduced when the spectra were recorded. EPR settings: $T = 78$ K, microwave frequency 9.47 GHz, modulation frequency 100 kHz, modulation amplitude 15 G (a) or 10 G (b and c), microwave power 29 mW (a) or 36 mW (b and c), conversion time 82 ms, receiver gain 5×10^3 (a), 2×10^3 (b) or 1×10^4 (c), number of scans 4 (a), 2 (b), or 8 (c). Inset: $T = 107$ K, modulation amplitude 10 G, microwave power 20 mW, microwave frequency 9.44 GHz.

ing number of flashes, reaching a maximum after 10 flashes, showing that the oxidized form of **2** accumulated upon repeated flashing. (It should be noted that the EPR spectrum displayed by the MV$^+$ radical is so narrow that it does not interfere with the EPR spectrum from the oxidized Mn compound **2**.) No manganese EPR spectrum could be observed before illumination, when laser flashes were given to a solution containing only compound **2** and methyl viologen, or when a solution only containing **1** and MV^{2+} was exposed to laser flashes. It is therefore clear that the Mn(III)/Mn(III) complex **2** was oxidized by compound **1** in a light-dependent reaction.

The question then arises: Is the manganese complex oxidized by the tyrosyl radical, or by the ruthenium moiety in **1**, in the experiment described in Figure 3a? Ru(bpy)$_3$ complexes typically have redox potentials of +1.32 V (vs SCE)[13,14] and the Ru(III)(bpy)$_3$ ion which is generated photochemically by flash photolysis, should therefore be capable of oxidizing compound **2**, without the aid of the tyrosyl moiety. To investigate this option, we allowed a chemically produced Ru(III)(bpy)$_3$ complex, without any tyrosyl moiety, to react with compound **2** at equimolar concentrations in acetonitrile solution in the dark.

(13) Bock, C. R.; Connor, J. A.; Gutierrez, A. R.; Meyer, T. J.; Whitten, D. G.; Sullivan, B. P.; Nagle, J. K. *J. Am. Chem. Soc.* **1979,** *101*, 4816.
(14) Lin, C. T.; Boettcher, W.; Chou, M.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1976**, *98*, 6536.

The EPR spectrum then recorded (Figure 3b) showed that the Mn-complex was indeed oxidized to the mixed-valence state as revealed by the typical number and width of the hyperfine lines in the spectrum. However, the EPR spectra in Figure 3a and b are not the same. Instead of the pure Mn(III)/Mn(IV) spectrum observed before (Figure 3a) indicative of a clean one-step oxidation of **2** by **1**, oxidation with Ru(III)(bpy)$_3$ in the dark produced a broad, featureless EPR signal that appeared under the manganese spectrum (Figure 3b). Since the redox potential of the Ru(III)(bpy)$_3$ ion is higher than the midpoint potential of the oxidation of the ligand molecule in **2**,[11] Ru-(III)(bpy)$_3$ alone can oxidize both the manganese and the ligand in compound **2**. It is therefore highly likely that the new broad signal originates from the oxidized ligand being weakly coupled to the paramagnetic Mn(III)/Mn(IV) dimer.

As a second control experiment, an acetonitrile solution containing Ru(II)(bpy)$_3$, compound **2** and MV^{2+} was irradiated with a series of laser flashes at room temperature, before freezing in liquid nitrogen. In this case (Figure 3c), a similar result as from chemical oxidation was obtained. The EPR spectrum again displayed the hyperfine lines typical for the Mn(III)/(IV) state, as well as the broad featureless signal. It should be noted that under these conditions the latter signal was larger in amplitude compared to the hyperfine resolved spectrum from the mixed-valence Mn(III)/Mn(IV) state (Figure 3c). This indicates that the photoinduced regeneration of Ru(III)(bpy)$_3$ ions allowed a larger part of the manganese complexes to become doubly oxidized than under our conditions for chemical oxidation (Figure 3b). Indeed, when we repeated the chemical oxidation with a higher concentration of Ru(III)(bpy)$_3$ in the dark, the relative intensity of the broad signal increased compared to the 18-line signal (not shown). The intensities of the spectra a and c in Figure 3 should not be taken as quantitative indications of the yields of the photo reactions, where for example recombination reactions may lower the apparent yield. Instead, we emphasize that the relative intensities of the two signal species in each spectrum, when the oxidant is tyrosine (spectrum a) or Ru(III) (spectrum b) are different.

We have previously shown that photooxidation of the Ru-moiety in **1** by an external acceptor in water solution, lead to oxidation of the tyrosyl residue.[9] It is difficult to monitor the tyrosyl radical in acetonitrile solution, when MV^{2+} is used as electron acceptor, due to spectral overlap with the MV$^+$ radical. We therefore made transient absorbance measurements at 460 nm in acetonitrile (using MV^{2+} to quench the excited state of the Ru(bpy)$_3$ moiety[15]) and found that the tyrosyl moiety in **1** reduced the Ru(III) ion via intramolecular electron transfer, with a first-order rate constant of $k_{ET} = 1.2 \times 10^5$ s^{-1} (not shown, but see also ref 9). Thus, photoinduced oxidation of the tyrosyl moiety is possible even in the relatively aprotic solvent acetonitrile, substantiating our conclusions from the EPR experiments in Figure 3.

The fact that the broad underlying EPR signal was not present in the spectrum after flash photolysis with compound **1**, is a very useful result. From a comparison of the different EPR spectra in Figure 3, it seems clear that compound **1** has allowed discrete and controlled oxidation of the Mn part in compound **2**, without inducing the second oxidation step. Also, since the

(15) Kalyanasundaram, K. *Photochemistry of Polypyridine and Porphyrin Complexes*; Academic Press: London, 1992.
(16) The EPR spectrum of the photoinduced tyrosyl radical in **1** (Figure 5, inset, compare also ref 9) is of similar size as the MV$^+$ radical, and the two radicals are not easily discernible. Therefore, the kinetic measurements of the formation and decay of the tyrosyl radical were performed using Co(NH$_3$)$_5$Cl^{2+} as electron acceptor, to avoid interference with the MV$^+$ spectrum while recording the changes in the tyrosyl radical spectrum.
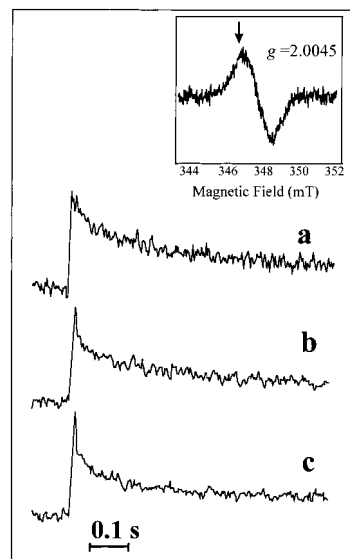

Figure 4. Time-resolved EPR measurements of the induction and decay of the tyrosyl radical signal from compound **1**, after single laser flashes in water solution at pH 7.0, in the presence of the sacrificial electron acceptor Co(NH$_3$)$_5$Cl^{2+} which prevents electron recombination.[9] Flash-induced induction and decay of the tyrosyl radical in compound **1** alone (a) and in the presence of compound **2** at 0.3 mM (b) and 0.6 mM (c) concentration, respectively. The concentration of compound **1** was 1.5 mM, and the initial concentration of tyrosyl radicals formed by a single flash was 15 ± 2 μM. Each kinetic trace represents the average of 100 flash-induced transients. EPR conditions: microwave frequency 9.76 GHz; spectral conversion time 2.56 ms; time constant 2.56 ms; modulation amplitude 3.2 G; microwave power 18 mW. The inset shows an EPR spectrum of the tyrosyl radical recorded under continuous illumination,[9] and the arrow indicates the field position for the kinetic experiments.

midpoint potential of the tyrosyl moiety in **1** lies between the two midpoint potentials in **2**, these results strongly indicate that the tyrosine radical, not the Ru(III) ion, is the oxidizing agent.

Time-Resolved EPR Measurements. To verify the hypothesis that the tyrosyl radical in **1** oxidizes the manganese complex **2**, we studied the lifetime of the tyrosyl radical, by time-resolved EPR spectroscopy, in the absence and presence of compound **2** (Figure 4). The samples were prepared in buffered water solution at pH 7.0 with 1 mM compound **1**, 0−0.8 mM compound **2**, and the electron acceptor Co(NH$_3$)$_5$Cl^{2+} dissolved to saturation.[16] Most importantly, since the sacrificial acceptor Co(NH$_3$)$_5$Cl^{2+} prevents electron recombination with the oxidation products, it also allowed a quantitative investigation of the reaction kinetics of the tyrosyl radical.

When compound **1** is given a single laser flash, the EPR signal amplitude of the tyrosine radical can be observed to rise and decay after each flash, by locking the magnetic field at the positive signal maximum and recording the amplitude as a function of time.[9] The concentration of tyrosyl radicals generated upon one flash in the experiment shown in Figure 4 was 15 ± 2 μM, corresponding to about 1% of the total amount of compound **1**. The tyrosyl radical, when generated in the absence of the manganese compound, decays in bimolecular reactions, i.e., the decay rate depends on the concentration of **1**. This decay is because the tyrosyl radicals undergo irreversible reactions, such as dimerizations, similar to what has been observed with other phenoxy radicals.[17]

When compound **2** was added at substoichiometric amounts (relative to the total amount of **1**), a fraction of the radical

(17) Denisov, E. T.; Khudyakov, I. V. *Chem. Rev.* **1987**, *87*, 113.

decayed much faster (Figure 4b,c). By an addition of 0.6 molar equiv of **2** (Figure 4c), a large fraction of the radical decayed much faster than in the absence of **2**. The fraction of fast decaying tyrosyl radical, increased with increasing concentrations of **2**, which is what we expect if the reduction of the tyrosyl radical is by electron transfer from the Mn in **2** (compare traces b and c in Figure 4). We could fit the decay curves with a double-exponential function. A double-exponential function was chosen because it was the simplest function that faithfully reproduced the data, without any intention to imply any specific reaction mechanism(s) for the radical decay. In the absence of **2**, the radical decayed with a half time of 55 ms (35% of the total amplitude) and 580 ms (65% of the total amplitude). By adding 0.3 mM or 0.6 mM of **2**, the fast decay rate increased and reached a half time of about 30 ms comprising 55 and 70% of the total amplitude, respectively. The remaining fraction had a decay rate similar to that in the absence of **2**. Therefore, the average lifetime of the tyrosyl radical decreased with increasing concentrations of **2**, strongly indicating that the decay of the formed tyrosyl radical results from direct electron transfer from **2**. It is difficult to make a detailed simulation of the process however, since not all decay paths of the tyrosyl radical are known. Furthermore, the obviously biphasic decay of the tyrosyl radical signal in the presence of the manganese complex indicates that the tyrosine radical and the manganese complex did not react in a simple, diffusion-controlled bimolecular reaction. Instead, this suggests a weak complexation between the tyrosyl moiety in **1** and the manganese complex.

It is important to note that the initial amplitude of the tyrosyl radical signal after the flash remained constant when the concentration of the added compound **2** was up to about 0.8 molar equiv of the total amount of compound **1**. Thus, the same amount of tyrosyl radical was induced by the laser flash, despite the presence of **2** at moderate concentrations. However, at higher concentrations of **2**, the initial amplitude of the flash induced tyrosyl radical signal became smaller. In this case, all of the remaining signal decayed fast (not shown). At super stoichiometric concentrations of **2**, we could not observe any formation of the tyrosyl radical at all. We can explain this decrease in tyrosyl radical formation at more elevated concentrations of **2**, by the existence of an alternative reaction pathway, where compound **2** is directly oxidized by the Ru(III) ion prior to tyrosyl radical formation (Figure 1B, iv). If direct electron transfer from **2** to the Ru(III) ion occurs, it will prevent the intramolecular electron transfer from the tyrosyl moiety to the Ru(III)(bpy)$_3$ part in **1** ($k_{ET} = 3.3-5 \times 10^4$ s^{-1} under the present conditions, see also ref 9) and thereby prevent tyrosyl radical formation. Nevertheless, even when the relative concentration of **2** to the amount of induced tyrosyl radical was up to about 50:1, the quantum yield, i.e., the amplitude, of the tyrosyl radical formation was unaltered. This indicates that the intermolecular Mn to Ru(III) reaction pathway is slower than the intramolecular electron transfer from the tyrosyl moiety to the photooxidized Ru(bpy)$_3$ up to this concentration. Instead, the manganese compound is oxidized one step by the tyrosyl radical, generating the mixed-valence state (see also Figure 3). The competing reduction of photogenerated Ru(III) ions by tyrosine or manganese complexes was further characterized by optical absorption measurements of Ru(III) decay (see below).

Photodestruction of the Tyrosyl Radical. To further establish that electron transfer occurred from the Mn-complex directly to the tyrosyl radical in **1**, we conducted an investigation of the photodestruction of the tyrosyl moiety. When **1** is photolyzed in the presence of the sacrificial electron acceptor
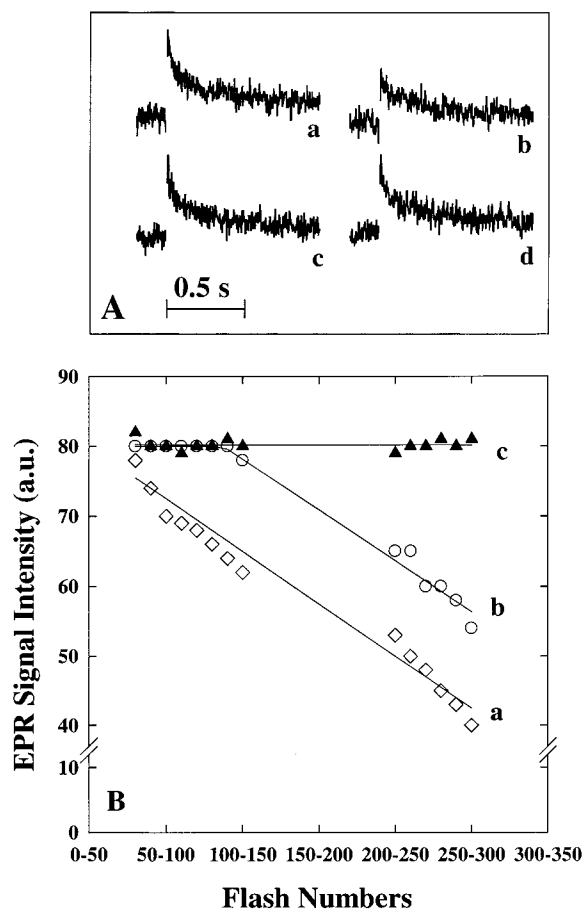

Figure 5. Protection against photodamage of the tyrosine moiety in **1** by electron transfer from **2**: (A) Formation and decay of the tyrosyl radical in **1** following a laser flash in the absence (top traces) and presence (bottom traces) of **2**. Compound **1** (1 mM in aqueous solution with 10% acetonitrile, at pH 7.0) in the presence of Co(NH$_3$)$_5$Cl^{2+} with or without 0.8 mM **2** was given saturating laser flashes at a flash repetition rate of 1/3 Hz. Traces a and c show the amplitude and decay of the tyrosyl radical induced by 50 flashes early in the flash train (flashes number 30$-$80). Traces b and d represent transients recorded later in the flash train (flashes number 250$-$300). The induced signal amplitude has clearly decreased after 300 flashes, in the absence of **2**, while it remains high in the presence of the manganese compound **2**. EPR conditions as in Figure 4. (B) Successive decrease of the tyrosyl radical amplitude in **1**, in a train of flashes, in the absence (diamonds) and presence of 0.2 mM (circles) or 0.8 mM (triangles) of **2**. Each point represents the added amplitudes of 50 flashes and is plotted such that flashes 30$-$80 is depicted at flash number 55, flashes 40$-$90 at flash number 65, and so on.

Co(NH$_3$)$_5$Cl^{2+}, the initial amplitude of the photoinduced radical signal was observed to slowly diminish with an increasing number of flashes. After 300 laser flashes had been given to a reaction mixture, the initial amplitude of the radical signal upon a flash was only about 50% of that when only a few flashes had been given to the solution (Figure 5a,b). The decrease in the amplitude of the tyrosyl radical was linear and dependent on the number of flashes given to the sample (Figure 5b, diamonds). Tyrosyl radicals are known to react with each other in irreversible cross-linking reactions.[17] It is probable that the flashnumber-dependent decrease of the radical formation reflects the decrease in active molecules that can generate tyrosyl radicals, due to the occurrence of such side reactions between the molecules. Interestingly, in the presence of compound **2**, the photodamage of the tyrosyl radical was delayed. In the presence of 0.8 molar equiv of compound **2**, the radical
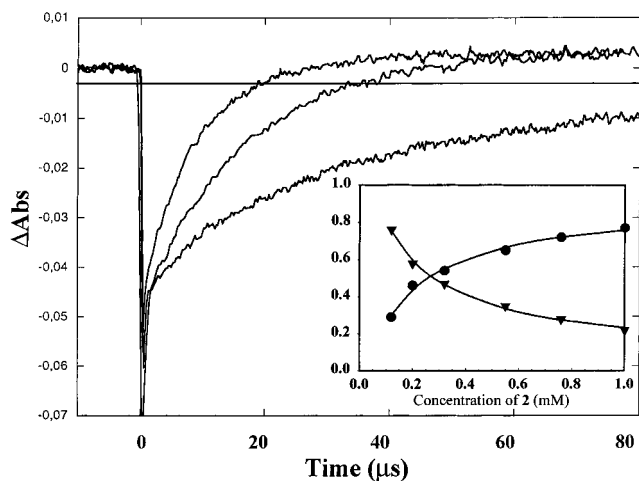
Figure 6. Transient absorption measurements at 450 nm in aqueous solution at pH 7.0 containing 10% acetonitrile and saturating concentrations of $Co(NH_3)_5Cl^{2+}$. The Ru(II) recovery in **1** (40 μM) in the presence of 0 mM (lower trace), 0.16 mM (middle trace), and 0.32 mM (upper trace) of compound **2** is observed. The Ru(II) recovery rate increases with increased addition of **2**, indicating that **2** can reduce the Ru(III) ion directly. The inset shows the fraction of Ru(III) that oxidized tyrosine (diamonds) and **2** (circles), respectively, as a function of concentration of **2**. The lines are calculated using the rate constants $k_{ET,Tyr} = 3.3 \times 10^4$ s^{-1} and $k_{Mn} = 1.1 \times 10^8$ M^{-1} s^{-1} (between **2** and Ru(III)), which is an average for several concentrations (see text for details). The points (dots and diamonds) are calculated from the individual Ru(II) recovery traces for a single concentration, by using these determined rate constants. At concentrations of **2** below 0.30 mM (corresponding to several hundred times more manganese complex than formed tyrosyl radical), Ru(III) is preferentially reduced by intramolecular electron transfer from the tyrosyl moiety.

formation was unaffected by as many as 400 laser flashes (Figure 5b, triangles). At intermediate concentrations (0.2 molar equiv) of the Mn-complex, irreversible destruction of tyrosyl radicals did occur, but at a slower rate than without any compound **2** in the solution (Figure 5b, circles). Our explanation of this result is that the Mn-complex **2** reduces the tyrosyl radical prior to its reaction with another radical, enabling it to participate in several more reaction turnovers. (It should be noted that the photodamage during the first 100 flashes that were used to induce the traces depicted in Figure 5 did not result in any significant amplitude decrease.) This result further strengthens our conclusion that the manganese compound **2** functions as an electron donor directly to the photogenerated tyrosyl radical in **1**.

Transient Absorbance Measurements. Time-resolved flash photolysis experiments were used to further investigate the reaction pathways for oxidation of **2** by the tyrosyl radical, as well as by the Ru(III)(bpy)$_3$ ion in **1**, in aqueous solution containing 40 μM **1**, 0−1 mM **2**, and a saturating concentration of $Co(NH_3)_5Cl^{2+}$ at pH 7.0. Without any addition of **2**, the Ru-(III) ion was reduced via intramolecular electron transfer from the tyrosine moiety,[9] with a rate constant of $k_{ET,Tyr} = 3.3 \times 10^4$ s^{-1} as determined by the Ru(II) absorption recovery (Figure 6, lower trace). When various concentrations of the manganese compound **2** was added to the solution, the Ru(II) recovery rate increased due to electron donation from **2** to the Ru(III) center in **1** (Figure 6, middle and upper traces). However, the reduction of the Ru(III) ion by **2** competed with the intramolecular electron transfer from the tyrosyl moiety.

To investigate to what extent the two reactions competed, we made an experiment using a Ru(bpy)$_3$ complex similar to **1** but with a redox inactive alanine moiety instead of a tyrosine.[9]

In the presence of the sacrificial electron acceptor $Co(NH_3)_5$-Cl^{2+}, electron recombination with the Ru(III) ion is prevented in this compound, and no Ru(II) recovery can be observed.[9] When compound **2** was added to the solution, Ru(II) recovery could be followed in the ruthenium−alanine complex as a result of electron transfer from **2** to the Ru(III) ion (not shown). The second-order rate constant for this bimolecular reaction was $k_{Mn} = 1.1 \times 10^8$ M^{-1} s^{-1} (Figure 6, inset).

The same dependency of the concentration of **2** was observed in the measurements with **1**, but with a constant shift of the pseudo-first-order rate constants for the reduction of Ru(III) by **2**. This was due to the competition with intramolecular electron transfer in **1**. From the observed Ru(II) recovery rates in **1** and the Ru−alanine compound, respectively, we calculated the fraction of Ru(III) reduced by each reaction pathway (Figure 6, inset). The electron transfer from **2** to the Ru(III) ion was found to be slower than the intramolecular reduction by the tyrosyl moiety, at concentrations of compound **2** lower than 0.3 mM. At moderate concentrations of **2**, the intramolecular electron transfer from the tyrosine will therefore outcompete the bimolecular reaction of Ru(III) with **2**. Under these conditions, a major part of the manganese complex **2** can be expected to be oxidized by the light-induced tyrosyl radical in **1**. In essence the results obtained by transient absorption measurements corroborate the conclusions from the EPR experiments presented in Figures 3 and 4.

Comparisons with Photosystem II. We have presented a system where an oxidized tyrosine radical is formed by intramolecular electron transfer from a covalently bound, photooxidized Ru(III)-complex. In a subsequent reaction step, the tyrosyl radical oxidizes a Mn(III)/Mn(III) complex to the mixed-valence Mn(III)/Mn(IV) state. This redox "triad" thus mimics the electron-transfer sequence of the donor side in Photosystem II. Also, the redox potentials of the different components of the triad are remarkably similar in PSII and the artificial system. In PSII, the oxidized primary electron donor P$_{680}$ reaches a potential near +1.2 V (vs NHE), which is in the same magnitude as of the Ru(II)(bpy)$_3$/Ru(III)(bpy)$_3$ couple (+1.26 V vs NHE). Also the tyrosyl radical, Tyr$_Z^\bullet$, is strongly oxidizing and has been estimated from kinetic and equilibrium considerations to have an oxidation potential of +0.95 to +0.99 V (vs NHE).[18] Similarly the oxidation peak potential of the tyrosyl moiety in **1** was measured to +0.97 V vs NHE. Interestingly, also the potentials of the Mn-complexes are similar. In compound **2**, Mn oxidation occurs at about +0.58 V vs NHE, similar to the potentials in the lower oxidation states of the Mn cluster in PSII, where the transitions from the S$_0$ and S$_1$ states involve potentials of +0.6−0.7 V.[18]

The introduction of a tyrosine as an intermediate component is not only attractive considering the proposed hydrogen abstraction model for water oxidation which would require a tyrosyl radical.[5,6] Electron transfer from a manganese will probably never be particularly fast. Therefore, efficient reduction of the photosensitizer by an intermediate component prevents fast recombination and degrading reactions. This is one of the proposed functions of Tyr$_Z$ in PSII[1] and holds for the tyrosyl intermediate in our artificial system. Furthermore, an already photooxidized manganese complex might become reduced during the subsequent photo reaction, instead of undergoing a second oxidation step. This would be an unproductive reaction, and it is prevented by the inclusion of a redox active intermediate. It has also been observed that closeby manganese may quench the excited state of the Ru(II) photosensitizer, resulting

(18) Vass, I.; Styring, S. *Biochemistry* **1992**, *30*, 830.

in a low quantum yield of the electron-transfer reactions.[19,20] This reaction is distance dependent and is also prevented by the introduction of the intermediate tyrosyl. Thus, the inclusion of a redox active component between the photosensitizer and the Mn-moiety is beneficial for several reasons, and it is probable that similar arguments hold also for the cofactors in PSII.

One may compare the reduction rate that we observe for the radical in **1** with the rate for reduction of the $Tyr_Z^\bullet$ radical in PSII. In PSII centers, which have been deprived of their manganese complex, the Tyr_Z residue is oxidized by a single flash and reduced within 100 ms in the absence of electron donors. By addition of Mn(II), which is an efficient exogenous electron donor to PSII, $Tyr_Z^\bullet$ is reduced within less than 10 ms.[21] This reduction rate is rather slow to be an electron or hydrogen atom transfer reaction and possibly reflects the ligand field rearrangements that Mn(II) has to undergo when being oxidized to the +III oxidation state. If we assume a simple bimolecular reaction for the oxidation of **2** by the tyrosyl radical in **1**, a rate constant in the range of 1×10^4 to $1 \times 10^5 \ M^{-1} \ s^{-1}$ is expected from the data presented. The time-resolved EPR data, however, suggest that **1** and **2** might form weak complexes. In that case, the reduction of the tyrosyl radical occurs with a halftime of 30 ms, corresponding to a first-order rate of $20 \ s^{-1}$. In either case, the electron transfer from the manganese compound to the tyrosine is slow, which may be explained by a large reorganization energy of the reaction, since Mn(IV) complexes normally have an octahedral coordination environment, while Mn(III) often show large Jahn−Teller distortions.

Interestingly, the protection against tyrosyl degradation by compound **2** also has precedence in the light-induced chemistry of the PSII donor side. During illumination the PSII reaction center is continuously photodamaged, degraded, and resynthe-sized *de novo*. This is a protein turnover reaction of large volume and significance in the biosphere.[22,23] In ordinary sunlight, the average lifetime of the D1 protein (Figure 1A) is less than 1 h. When the repair reactions cannot keep the pace of the damaging reactions, the plant loses its photosynthetic capacity in what is known as photoinhibition, an unavoidable and much-studied stress phenomenon in plants. There are several reactions that lead to photoinhibition of PSII and one of these, the so-called donor side induced photoinhibition,[23,24] is relevant here. When the manganese complex in PSII is inhibited or slowed—a situation which often occurs in nature—the PSII reaction center becomes extremely light sensitive. Studies of the inhibitory reaction mechanism have revealed that Tyr_Z is an early target for photoinhibition, very similar to the photodestruction of the tyrosyl radical in compound **1**. The mechanisms behind the photodamage is that the oxidizing radicals $Tyr_Z^\bullet$ and/or P_{680}^+ become long-lived when they are not efficiently reduced by the manganese cluster. Under such circumstances, they will oxidize the surrounding protein that eventually is damaged. If PSII, in the absence of a functional manganese cluster, is offered an exogenous donor, the oxidizing radicals are reduced and the Tyr_Z radical and the D1 protein are secured against photodam-age. Thus, some of the reactions involved in donor side photoinhibition are well modeled by the reactions between the Ru−tyrosine compound **1** and the manganese dimer **2**.

Conclusion

We show that a tyrosyl radical, generated by intramolecular electron transfer to a photooxidized $Ru(III)(bpy)_3$, is capable of oxidizing a binuclear manganese cluster from Mn(III)/Mn(III) to the mixed-valence Mn(III)/Mn(IV) state. The redox triad mimics electron-transfer reactions on the donor side of PSII (Figure 1A). Despite the large differences, the artificial system is similar to the PSII donor side in terms of reaction sequence, reaction rate constants, redox potentials, and the effects of photoinhibitory reactions. The inclusion of a tyrosine moiety as an interface between the ruthenium photosensitizer and the binuclear Mn center introduces a new concept in the development of artificial photosynthesis and provides, as compared to Ru(III) alone, the possibility of controlled, one-step oxidation of the dinuclear Mn-compound. Our hope is that the redox active "triad" here described will be the predecessor of a new class of supramolecular complexes containing a Ru center, a multinuclear Mn-complex, and redox active interfacing ligands.

(19) Hammarström, L.; Sun, L.; Åkermark, B.; Styring, S. *Biochim. Biophys. Acta* **1998**, *1365*, 193−199.

(20) Sun, L.; Berglund, H.; Davydov, R.; Norrby, T.; Hammarström, L.; Korall, P.; Börje, A.; Philouze, C.; Berg, K.; Tran, A.; Andersson, M.; Stenhagen, G.; Mårtensson, J.; Almgren, M.; Styring, S.; Åkermark, B. *J. Am. Chem. Soc.* **1997**, *119*, 6996−7004.

(21) Hoganson, C. W.; Ghanotakis, D. F.; Babcock, G. T.; Yocum, C. *Photosynth. Res.* **1989**, *22*, 285.

(22) Barber, J.; Andersson, B. *Trends Biochem. Sci.* **1992**, *17*, 61.

(23) Styring, S.; Jegerschöld, C. In *Photoinhibition of Photosynthesis*; Baker, N., Bowyer, J. R., Eds.; Bios. Scientific Publishers: Oxford, 1994; pp 51−73.

(24) Jegerschöld, C.; Styring, S. *Biochemistry* **1996**, *35*, 7794.