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Proton-Coupled Electron Transfer from Tyrosine in a Tyrosine−Ruthenium−tris-Bipyridine Complex: Comparison with Tyrosine$_Z$ Oxidation in Photosystem II

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Abstract: The pH- and the temperature dependence of the rate constant for electron transfer from tyrosine to ruthenium in Ru(II)(bpy)$_2$(4-Me-4′CONH-L-tyrosine etyl ester-2,2′-bpy) 2PF$_6$ was investigated using flash photolysis. At a pH below the tyrosine p$K_a \approx 10$ the rate constant increased monotonically with pH. This increase was consistent with a *concerted* electron transfer/deprotonation mechanism. Also indicative of a concerted reaction was the unusually high reorganization energy, 2 eV, extracted from temperature-dependent measurements. Deprotonation of the tyrosine group, at pH > pK_a, resulted in a 100-fold increase in rate constant due to a decreased reorganization energy, $\lambda = 0.9$ eV. Also, the rate constant became independent of pH. In Mn-depleted photosystem II a similar pH dependence has been found for electron transfer from tyrosine$_Z$ (Tyr$_Z$) to the oxidized primary donor P680$^+$. On the basis of the kinetic similarities we propose that the mechanisms in the two systems are the same, that is, the electron transfer occurs as a concerted proton-coupled electron-transfer reaction, and at pH < 7 the Tyr$_Z$ proton is released directly to the bulk water.

Introduction

Photosynthetic energy conversion in biological systems is based on charge separation. The electron-transfer reactions involved in the charge separation often occur as a proton-coupled or proton-assisted electron transfer. In photosystem II (PS II) of oxygen-evolving organisms a tyrosine group, Tyr$_Z$, donates an electron to the primary electron donor (P680$^+$) and is believed to release its phenolic proton to a nearby base when being oxidized.[1−4] Tyrosine changes its pK_a-value from 10 to −2 upon oxidation,[5] and in water solution it can no longer hold onto its phenolic proton. The electron transfer is thus coupled to deprotonation and may occur either as consecutive electron transfer/deprotonation reactions or as a single, concerted, reaction step.[6] In PSII the Tyr$_Z$ is crucial for stabilization of the primary charge separation. The mechanism for proton-coupled oxidation and subsequent reduction of Tyr$_Z$ is currently much debated. A fundamental knowledge of the electron-transferring properties of tyrosine is therefore of great interest.

In this study a simple model compound has been used to investigate the electron transferring properties of tyrosine using light-induced reactions, thereby mimicking the PS II photochemistry (Figure 1). The ruthenium−tris-bipyridine part acts as a photosensitizer in analogy with P680, and the exited state is quenched by electron transfer to an external acceptor such as methyl viologen. An electron is then transferred to the oxidized ruthenium from the tyrosine which is deprotonated.[7] Here we provide an experimental approach to distinguish between the consecutive and concerted mechanisms through the pH- and temperature dependence of the electron-transfer rate constant. The structure of the model compound is uncomplicated and exactly known, which simplifies the interpretation of data compared to the case with PS II. Then, we use our conclusions to discuss the results and mechanism of Tyr$_Z$ electron transfer in PS II.

Experimental Section

Preparation and characterization of Ru(II)(bpy)$_2$(4-Me-4′CONH-L-tyrosine etyl ester-2,2′-bpy) 2PF$_6$ has been described elsewhere.[7] Methyl viologen (Sigma) was of highest grade commercially available. Sodium dihydrogenphosphate (Merck 99%) or NaH$_2$BO$_3$ (SigmaUltra 99.5%) dissolved in milliQ-water to a concentration of 10 mM was used as buffer solution. The pH was adjusted with concentrated sodium hydroxide or hydrochloric acid. A concentration of 40−60 μM Ru-

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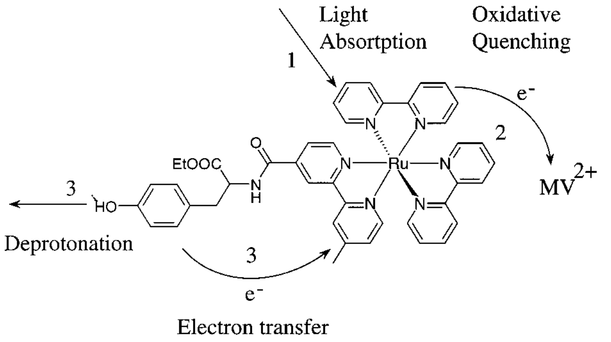

Figure 1. The supramolecular system, Ru(II)(bpy)$_2$(4-Me-4′CONH-L-tyrosine ethyl ester-2,2′-bpy)$^{2+}$. The ruthenium part absorbs light at 450 nm (step 1) and the exited electron is transferred to the acceptor (step 2). After oxidative quenching the tyrosine part donates an electron and is deprotonated (step 3).

(bpy)$_3$TyrOH and 30 mM MV^{2+} was used in the electron-transfer experiments below the tyrosine pK_a. At pH < 10 when tyrosine was initially deprotonated, the Ru(II) excited state lifetime was reduced to 50 ns, presumably by reductive quenching. To efficiently oxidize the Ru(II) excited state, and to resolve the subsequent electron transfer from tyrosine, a much higher viologen concentration, 80 mM, was used at pH > 10. A plot of the Ru(II) recovery rate constant as a function of [MV^{2+}] showed that the electron-transfer step could be resolved from the initial quenching reaction (see Supporting Information). The concentration of Ru(bpy)$_3$TyrOH was low enough so that the bimolecular reaction, between tyrosine and ruthenium moieties on different molecules, was negligible compared to the intramolecular reaction.[7]

Laser flash photolysis, with a ~10 ns time resolution (see Supporting Information), was used to investigate the electron-transfer reactions, and the rate constant for the electron transfer was extracted from recovery of the Ru(II) ground-state absorption around 450 nm.[7] The sample was exited with light from an LT-1113 dye laser ($\lambda = 460$ nm, ≈ 1 mJ/pulse) pumped by an ELI-94 eximer laser operating with XeCl, and subsequently analyzed with light from a pulsed 450 W xenon lamp. The light was detected by a side-on, P928-type photomultiplier coupled to a Tectronix 7912AD digitizer, after passing a Zeiss double prism monochromator. The electron transfer from tyrosine was investigated by following the recovery of the Ru(II) ground-state absorption after excitation. The detection was performed at 450 or 440 nm. For the investigation of the disappearance of MV^{+}, the detection was performed at 600 nm at different time domains. Before and during measurements the sample solution was purged with N$_2$ gas to de-oxygenate the solution. The sample was thermostated to 25 °C, unless stated otherwise.

Each transient curve was an average of 16 to 64 individual laser shots at low and intermediate pH. Single-shots were used at high pH. The rate constants reported at different pH values and temperatures are in turn averages from an analysis from at least seven curves recorded under identical conditions.

The 600 nm decay curves were fitted to second-order kinetics, and the 450 nm recovery transients were fitted to a first-order exponential function. At pH >8, the rate of electron transfer from tyrosine to Ru(III) was much higher than the rate of recombination with viologen radical, and recombination could be neglected. Below pH = 8, however, the first-order reactions for the Ru(II) recovery were corrected for the recombination reaction with viologen radical, using $[\mathrm{Ru(III)}] = [\mathrm{Ru(III)}]_{t=0}\, e^{-kt}\, [\mathrm{MV}^{\bullet+}]/(1 + k_{2nd}t[\mathrm{MV}^{\bullet+}])$, where k is the rate constant for intramolecular electron transfer from tyrosine and k_{2nd} is the second-order rate constant for the decay of MV$^{\bullet+}$.

Results

Electron Transfer from Tyrosine to Ruthenium. The photoinduced reactions depicted in Figure 1 were studied with laser flash photolysis. At 600 nm the MV$^{\bullet+}$ radical absorbs, and it decayed via near-diffusion controlled recombination with the oxidized tyrosine and ruthenium species. The Ru(II) ground-state recovery was much faster than the recombination rate due to intramolecular electron transfer from the tyrosine to the photooxidized Ru(III) (step 3).[7] The recovery was monitored at the Ru(II) ground-state absorption maximum around 450 nm. We have previously shown that a small, positive absorption at 410 nm from the oxidized tyrosine grows in with the same kinetics.[7] As shown in Figure 2 the Ru(II) recovery rate was pH-dependent while the MV$^{\bullet+}$ decay rate was essentially independent of pH. When fitting the Ru(II) recovery curves, correction was made for the recombination with MV$^{\bullet+}$ (see Experimental Section). Thus, the first-order rate constant for intramolecular electron transfer from the tyrosine to Ru(III) was obtained and is plotted in Figure 3a.

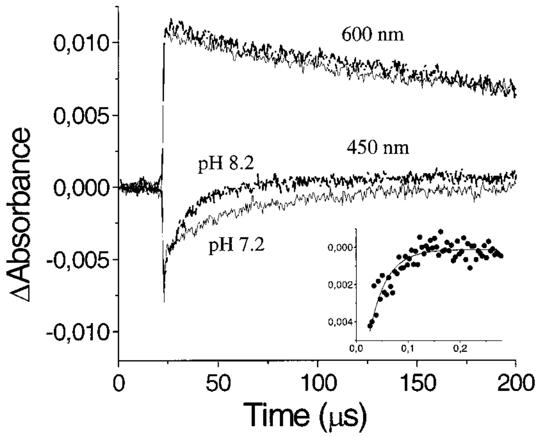

Figure 2. Transients at 450 nm, monitoring the recovery of ruthenium ground-state absorption after excitation and oxidative quenching, and at 600 nm, monitoring the build-up and decay of viologen radical absorption. The solid and dashed lines correspond to pH 7.2 and 8.2, respectively, and clearly reflect the increase in electron-transfer rate (450 nm transients) at higher pH. Inset: 440 nm transient at pH 12.0 showing the fast recovery of ruthenium II ground-state absorption corresponding to electron transfer from the tyrosinate ion.

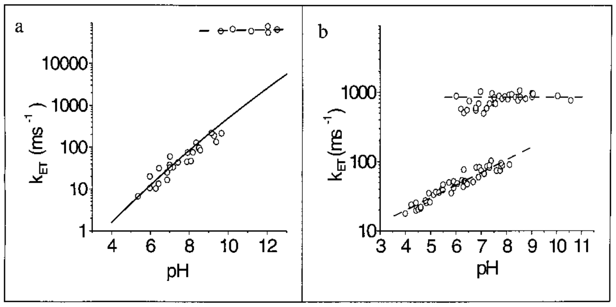

Figure 3. The rate constant for electron transfer from tyrosine to the photosensitizer as a function of pH (a) in an artificial tyrosine–ruthenium–tris-bipyridine complex. (Conditions: see Experimental Section). The solid line is a theoretical, Marcus-type function for the pH dependence of the electron-transfer rate constant; (b) in Mn-depleted PS II-particles (re-plotted from ref 12). Dashed lines are linear fits as guidance for the eye.

At pH below the tyrosine pK_a, (pH < 10), where the tyrosine group is initially protonated, the rate constant increased markedly with pH (Figure 3a). In contrast, above the pK_a the rate constant became large and independent of pH. Around the pK_a the recovery of the Ru(II) signal was double exponential with a fast component equal to the rate at higher pH (inset Figure 2), and a slow component corresponding to the data at lower pH. The lifetimes of the components differed by 2 orders of magnitude, but both could be resolved by measuring on different time scales. The activation energy (E_a) and the reorganization energy (λ) were determined by measuring the rate constant for

Table 1. Activation Energy (E_a) and Reorganization Energy (λ) for the Electron Transfer from Tyrosine to the Primary Donor in PS II from Reference 11 and in the Ruthenium−Tyrosine Complex

	pH = 6.5 < pK_a		pH > pK_a [a])	
	E_a (eV)	λ (eV)	E_a (eV)	λ (eV)
Ru−TyrOH	0.32	2.0	0.05	0.9
PS II	0.28	1.3	0.15	*b*

[a] At pH = 12 for Ru−TyrOH and pH = 8 for PS II, see text. [b] Not calculated.

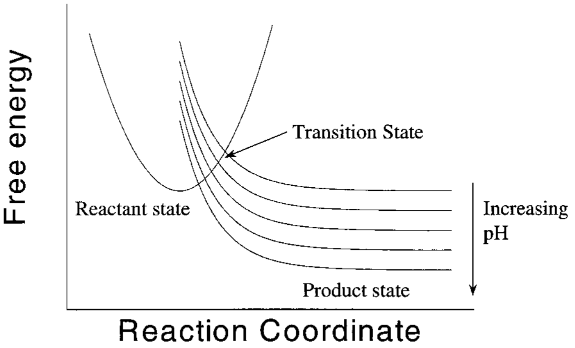

Figure 4. Schematic picture of the potential surface for the reactant and product states. The product state is repulsive in the OH-coordinate. All other contributions to the reaction coordinate are perpendicular to the plane of the figure. The free energy of the product state at equilibrium decreases with pH due to a increased entropy of mixing of the released proton. The decrease in energy is reflected in the transition state position, which gives a pH dependent reaction rate.

the electron transfer at different temperatures (10−45 °C) and by fitting the temperature dependence to the Marcus equation (eqs 1 and 2):[8]

$$k_{\mathrm{ET}} = Ae^{-E_a/k_BT} \tag{1}$$

$$E_a = \frac{(\lambda + \Delta G^0)^2}{4\lambda} \tag{2}$$

The driving force, ΔG^0, for the electron-transfer reaction was extracted from the difference between the $\mathrm{Ru(bpy)_3}^{2+}/\mathrm{Ru(bpy)}^{3+}$ standard potential, $E_{\mathrm{Ru(II/III)}}{}^0$, and the TyrOH/TyrO$^\bullet$ formal potential, $E_{\mathrm{Tyr}}{}^{0\,\prime}$. For example, at pH 7, $\Delta G^0 = -0.33$ eV. Below the pK_a the activation energy and the reorganization energy for the reaction were much larger than above the pK_a (Table 1).

The rate constant at pH = 7.0 was identical for a sample in neat water and the samples in phosphate or borate buffer at the same pH. Also, addition of 50 mM NaCl did not change the reaction rate. Thus, there were no specific buffer effects or influence of ionic strength.

Discussion

Reaction Mechanism. The kinetic data can be used to discriminate between the stepwise and the concerted mechanisms.

In the deprotonation-first mechanism the rate cannot be faster than the rate of deprotonation of the reduced tyrosine (rate constant k_{-p}).

$$\mathrm{Ru(III)-TyrOH} \underset{k_p}{\overset{k_{-p}}{\rightleftharpoons}} \mathrm{Ru(III)-TyrO^-} + \mathrm{H^+} \xrightarrow{k_e} \mathrm{Ru(II)-TyrO^\bullet} + \mathrm{H^+} \tag{3}$$

However from the tyrosine pK_a value (pK_a = 10) it follows that in water solution $K_a = k_{-p}/k_p = 1 \times 10^{-10}$ M, and even with a diffusion controlled protonation ($k_p \leq 1 \times 10^{11}\ \mathrm{M^{-1}\,s^{-1}}$) k_{-p} is limited to $\leq 10\ \mathrm{s^{-1}}$. Clearly this is far too low to agree with the experimentally observed rate constants that are on the μs time scale. Therefore, a significant reaction via electron transfer from the tyrosinate at pH < pK_a (eq 3) can be ruled out. Also, the biexponential kinetics and the large jump in rate constant around the pK_a value are consequences of a slow deprotonation and are not consistent with a reaction via the tyrosinate anion.

If the electron transfer instead occurs before the deprotonation, a steady-state approximation for the short-lived intermediate, Ru(II)−TyrOH$^{\bullet+}$, gives a rate constant for the overall reaction, $k_{\mathrm{Obs}} = k_e k'_{-p}/k_{-e} + k'_{-p}$:

$$\mathrm{Ru(III)\text{-}TyrOH} \underset{k_{-e}}{\overset{k_e}{\rightleftharpoons}} \mathrm{Ru(II)\text{-}TyrOH^{\bullet+}} \xrightarrow{k'_{-p}} \mathrm{Ru(II)\text{-}TyrO^\bullet} + \mathrm{H^+} \tag{4}$$

Since none of the reaction steps are pH dependent, the mechanism is not expected to give the pH-dependent rate constant found experimentally.

With the concerted electron transfer/deprotonation mechanism however it seems possible to explain the results:

$$\mathrm{Ru(III)-TyrOH} \xrightarrow{k} \mathrm{Ru(II)-TyrO^\bullet} + \mathrm{H^+} \tag{5}$$

The reaction driving force, ΔG^0, increases with pH due to the pH-dependent TyrO$^\bullet$/TyrOH-redox potential:

$$\Delta G^0 = -F(E_{\mathrm{Tyr}}{}^{0\,\prime} - E_{\mathrm{Ru(II/III)}}{}^0) \tag{6}$$

where F is the Faraday constant and $E_{\mathrm{Ru(II/III)}}{}^0$ is the Ru(II)/Ru(III) standard potential, $E_{\mathrm{Ru(II/III)}}{}^0 = 1.26$ V.[9] $E_{\mathrm{Tyr}}{}^{0\,\prime}$ is the pH-dependent tyrosine formal potential, $E_{\mathrm{Tyr}}{}^{0\,\prime} = (E_{\mathrm{Tyr}}{}^0 - [(RT \ln 10)/F] \times \mathrm{pH})$ V for pH < pK_a, where $E_{\mathrm{Tyr}}{}^0 = 1.34$[10] is the potential at standard conditions, that is, when the proton activity also equals unity. At pH > pK_a $E_{\mathrm{Tyr}}{}^{0\,\prime} = 0.77$ V and is independent of pH.[10] Using a pH-dependent driving force (eq 6) in the Marcus equation (eqs 1−2) it is possible to derive an expression for the pH dependence of the electron-transfer rate constant. This is justified in the following model. The electron transfer and deprotonation occur simultaneously in one reaction step, with one common transition state. For the reaction to occur the system must be in a reaction coordinate region where the free energy surfaces for the reactant and product states cross (the transition state). Since the OH-bond is broken in the reaction, the product free energy surface is repulsive in the OH coordinate, and the transition state involves an elongated OH-bond (Figure 4). This explains the large reorganization energy obtained at pH < 10 (Table 1). The entropy of mixing due to deprotonation increases with pH, causing the free energy for the product state to decrease with pH as shown in Figure 4. It is therefore likely that the activation energy for the concerted reaction decreases with pH. If one assumes a parabolic shape of the free energy surfaces in a region close to the transition state, then the effect of pH on the activation energy and the rate constant for the concerted reaction can be described as for a pure electron-transfer reaction, using the pH-dependent driving

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force of eq 6 in eqs 1−2. The resulting function is an ordinary Marcus free energy relationship and it is drawn in Figure 3a, with $\lambda = 2.0$ eV fixed according to the activation energy measurements. Only the preexponential term was allowed to vary to give the best fit to the results, and it is in good agreement with the experimentally observed pH-dependence at pH below the pK_a of tyrosine. Obviously the data cover only a part of the Marcus normal region.

Most likely $\Delta S > 0$ for the reaction and another form of eq 2 should be used that modifies the value of λ somewhat since the entropy term gives a non-temperature-dependent term in the exponent.[8] However, to be able to compare our results with results from the natural PS II, the driving force has been treated as temperature-independent. This will not affect the present discussion since the effect of mixing entropy would be very similar for PS II when the reaction mechanism is the same (see below).

The marked increase (100-fold) in the rate constant on going from the protonated form of tyrosine to the unprotonated form is mainly due to a decrease in λ; at low pH $\lambda = 2$ eV compared to 0.9 eV at high pH. The pre-exponential factor in eq 1 was even somewhat lower at high pH, opposing the effect of λ on the rate constant. We obtain a very large reorganization energy for the electron transfer at low pH that is probably an effect of a large *inner* reorganization, possibly characteristic for the concerted electron-transfer/bond-breaking reaction.[6] The only change when the tyrosine is deprotonated is the loss of the OH-bond. Therefore, it is likely that the OH-stretching motion contributes to a large part of the total reorganization energy at pH values where the tyrosine group is initially protonated. The concerted mechanism can thus explain both the pH- and the temperature dependence of the rate constant.

Comparison with Photosystem II. It is interesting to compare our results with data from Ahlbrink et al.[11] for the electron transfer from Tyr$_Z$ to P680$^+$ in manganese-depleted PS II particles that show a pH-dependence similar to our model system (cf. a and b of Figure 3). The authors report a pH-dependent rate constant below a pK_a value of 7.6 and a much faster, pH-independent rate constant above this pK_a. Furthermore the activation energies for the two systems in the low pH regime are very similar, E_a(PS II) = 0.28 eV (Table 1).[11,12] The similarities in pH dependence and in activation energy suggest that the mechanism for the electron-transfer/deprotonation process is the same in the two systems. That is, the electron transfer also from Tyr$_Z$ to P680$^+$ occurs as a concerted electron-transfer/deprotonation reaction. Both the pH-dependence and the large activation energy are consistent with a proton release from Tyr$_Z$ directly into the bulk water, as was the case in the ruthenium−tyrosine model complex.

Above pH 7.6 the rate constant in Mn-depleted PS II-particles becomes large and independent of pH, with an activation energy of 0.15 eV. The difference in rate constant and activation energy ($\Delta E_a = E_a$(low pH) − E_a(high pH)) when going to the high pH regime is smaller in the natural system than in the artificial Ru−tyrosine system: $\Delta E_a = 0.15$ eV and $\Delta E_a = 0.27$ eV, respectively.

In one model proposed to explain the results in PS II[11,13] the Tyr$_Z$ forms a hydrogen bond with a base at all pH values examined and becomes deprotonated around pH 7.6. Only the tyrosinate form is assumed to be an active electron donor to P680$^+$, so that the reaction is limited by deprotonation (a consecutive reaction).[13] Another model[11] assumes that the protonated Tyr$_Z$ is hydrogen-bonded to a base (presumably His190[2,4,13]) at pH > 7.6, which facilitates electron transfer, but that the hydrogen bond is broken at lower pH when the base is protonated.

On the basis of the kinetic similarities (Figure 2) we propose that the pH dependence of the electron-transfer rate constant in Mn-depleted PS II can be explained in the same way as in the artificial system studied here. We also propose that the electron-transfer/deprotonation reaction is concerted, *not* consecutive as currently suggested,[13] which results in a high activation energy. In line with the latter model above, we explain the higher rate at pH > 7.6 with the formation of a hydrogen bond from His190 to the phenolic proton of Tyr$_Z$. When His190 is protonated instead, below pH 7.6, the hydrogen bond is broken, and Tyr$_Z$ releases its phenolic proton directly to bulk water upon oxidation. The effect of the hydrogen bond is to weaken the force constant of the OH-bond in tyrosine and thereby decrease the reorganization energy for the concerted reaction. It also prevents the phenolic proton from being released into the bulk, so that the observed rate constant becomes independent of pH. However, since the proton is still attached to the reduced tyrosine, the rate constant is not increased as much as in the tyrosine−ruthenium complex at pH > 10, where the tyrosine is completely deprotonated. Following this reasoning we predict that a deprotonation of Tyr$_Z$ would give a larger effect on the rate and activation energy than that observed above pH = 7.6 (Figure 3b)

This model can also explain kinetic H/D-isotope effects[11−13] and the much lower rates observed at neutral pH in site-directed mutants in His190.[2,4] Above pH 8.1 in mutants to aromatic residues the rate was again higher, which was explained by direct titration of Tyr$_Z$.[4] If Tyr$_Z$ were titrated, the activation energy would decrease markedly according to our results. We suggest that measurements of the activation energy could indicate whether the reduced Tyr$_Z$ is deprotonated in the mutants at higher pH, or instead a hydrogen bond is formed to another base than His190.[14]

Conclusions

We have thus shown that the electron transfer from tyrosine to the photo-sensitizer occurs as a concerted electron-transfer/deprotonation reaction in the ruthenium−tyrosine complex studied. On the basis of the kinetic similarities we propose that this is the case also in Mn-depleted PS II particles. The concerted mechanism was supported by the pH dependence of the electron-transfer rate constant. An unusually large reorganization energy is also indicative of a concerted reaction and large reorganization energies were found both in the artificial system and in the natural system. In Mn-depleted PS II, His190 presumably forms a hydrogen bond to the phenolic proton of tyrosine when the histidine group is in its deprotonated form, and this bond makes the electron transfer more rapid by reducing the reorganization energy. Further work is in progress, aiming at a better understanding of the effect of hydrogen bonding in concerted

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electron-transfer reactions and also to include effects of entropy changes in the analysis.

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Supporting Information Available: A plot of the Ru(II) recovery rate constant at high pH as a function of [MV^{2+}] is available, showing that the electron-transfer step could be resolved from the initial quenching reaction (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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