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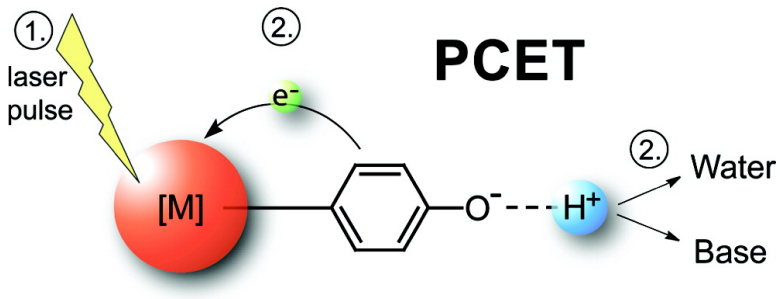

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Proton-Coupled Electron Transfer of Tyrosine Oxidation: Buffer Dependence and Parallel Mechanisms

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Proton-coupled electron transfer (PCET) in chemical reactions is of great interest as an elementary reaction step that is frequently encountered in biological systems.[1] The proton and the electron need not transfer as a hydrogen atom to be coupled.[2] An example of this type of mechanism, in which the proton and electron are transferred between one donor and separate acceptors, has been termed MS-EPT[3] or bidirectional PCET.[1b,4] This reaction is exemplified by Y_Z in photosystem II, in which tyrosine oxidation is coupled to proton transfer to a hydrogen-bonded H190 residue.[5] Y_Z oxidation could proceed by either a stepwise (ETPT or PTET; pathway 1 and 2, respectively, in Scheme 1) or concerted electron−proton transfer (CEP) mechanism, the latter defined as occurring with a single transition state. Our previous studies on systems with Y appended to $Ru(bpy)_3^{2+}$ (**RuY**),[6a] $Ru(bpy\text{-}4,4'\text{-}COOEt)_2(bpy)^{2+}$ ($\mathbf{Ru}_{ester}\mathbf{Y}$),[6b] and $Re(phen)(CO)_3(PPh_3)^+$ (**Re**(**P**−**Y**))[6c] (Chart 1) showed a pH-dependent rate constant for the PCET oxidation of Y. The nature of this pH-dependence has recently been of great debate.[3a,7]

The **RuY** system was previously treated within a Marcus framework for electron transfer (ET)[8] with the driving force defined by the pH dependent Y•/Y reduction potential: $E°'(Y•/Y) = E°(Y•/Y^-) + 0.059 \log(1 + 10^{pK_a(Y)-pH})$ (V). This analysis yielded a slope of 0.4−0.5 in the $\log(k_{PCET})$ versus pH plot, consistent with the expected slope of a Marcus free energy plot at low driving force ($\partial \log k/\partial \Delta G^0 = -118$ meV^{-1} for $\Delta G^0 = 0$, at 298 K).[8] Tyrosine oxidation in this system was thus interpreted as occurring with a CEP mechanism with bulk solvent as proton acceptor.[6a] However, a pH dependence in Y oxidation may also be explained by a PCET reaction with OH^- or the basic form of buffer as proton acceptor,[3a,7] which may call into question our previous interpretations.[6] In this manuscript, we clearly distinguish the contributions of these two mechanisms from the rate of Y oxidation in **RuY**, $\mathbf{Ru}_{ester}\mathbf{Y}$, and **Re**(**P**−**Y**). We explicitly show that a pH dependence in the rate of tyrosine oxidation can arise in the *absence* of buffer and that the basic form of the buffer acts as a proton acceptor only at high buffer concentrations. Analysis of the series of compounds reveals parallel PCET mechanisms, the relative contribution of which varies with the oxidant strength.

The rate for Y• generation in **RuY** and $\mathbf{Ru}_{ester}\mathbf{Y}$ was measured *directly* by nanosecond transient absorption spectroscopy. Following flash-quench oxidation of the $^3[Ru^{II}]^*$ metal-to-ligand charge transfer (MLCT) excited-state with methyl viologen (MV^{2+}), the oxidizing Ru^{III} species is reduced by the appended Y residue (Scheme 2).[6a,b] Tyrosine oxidation was monitored by the recovery of the Ru^{III} absorbance bleach at 450 nm concomitant with the production of the Y• absorption feature at 410 nm.[6b] Time-resolved emission was used to calculate the rate of Y oxidation for **Re**(**P**−**Y**), in which the excited **Re**-unit is directly quenched by Y (Scheme 2). Comparison of the quenched **Re**(**P**−**Y**) emission lifetime with that of the phenylalanine control complex, **Re**(**P**−**F**), yields the rate of PCET.[6c] Experimental details for these reactions are provided in the Supporting Information.

Scheme 1. Mechanisms of PCET, Where B is Solvent or Base Form of the Buffer: Stepwise ETPT (pathway 1) or PTET (pathway 2) and CEP Mechanisms

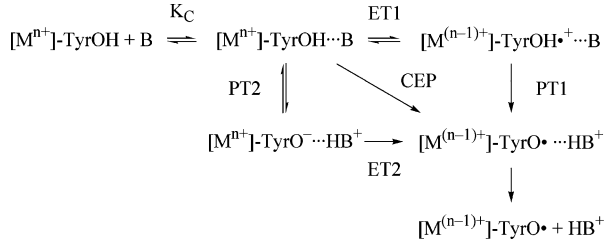
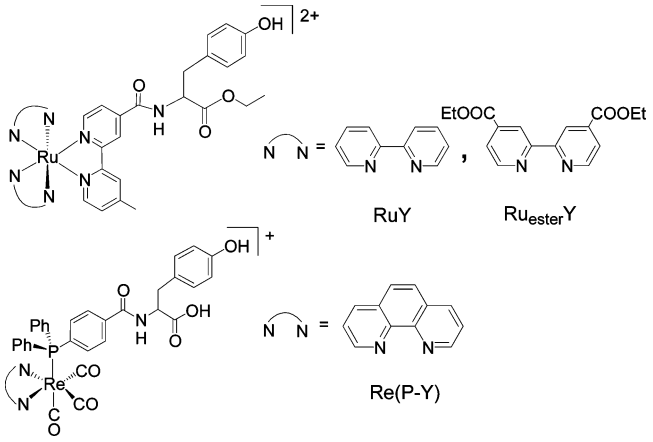


Chart 1. Structures of Complexes Described Herein



Scheme 2. Mechanisms of Y• Generation Employed Herein

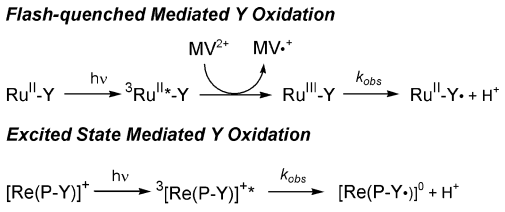


Figure 1 plots the observed rate constant of Y oxidation, k_{obs}, in **Re**(**P**−**Y**) and $\mathbf{Ru}_{ester}\mathbf{Y}$ as a function of buffer concentration and pH. The k_{obs} is independent of buffer at low concentrations ($<10^{-3}$ M) and increases linearly with buffer at high concentrations. The buffer concentration dependence becomes more pronounced as the pH is increased up to pH ≈ 8.5. These data are consistent with an $H_2PO_4^-/HPO_4^{2-}$ titration ($pK_a = 7.2$) where HPO_4^{2-} acts as the proton acceptor at high buffer concentrations, as previously

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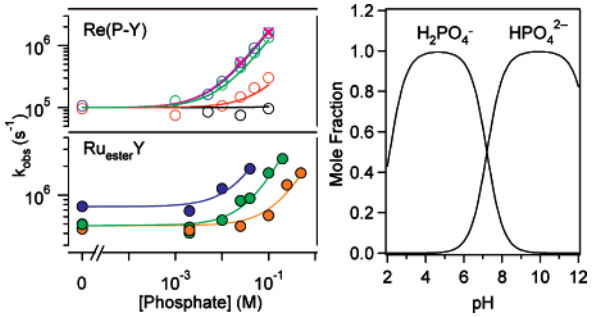

Figure 1. (Top left) Phosphate buffer dependence of k_{obs} with fits to eq 1 for **Re**(**P**−**Y**) at pH 4.5 (black circle), 6.1 (red circle), 7.5 (green circle), 8.3 (blue circle), and 9.2 (×). (Bottom left) Corresponding data for **Ru**$_{ester}$**Y** at pH = 9.9 (blue dot), 7.7 (green dot) and pH = 6 (yellow dot) (T = 298 K). (Right) Mole fraction (f_b) of relevant buffer species as a function of pH.

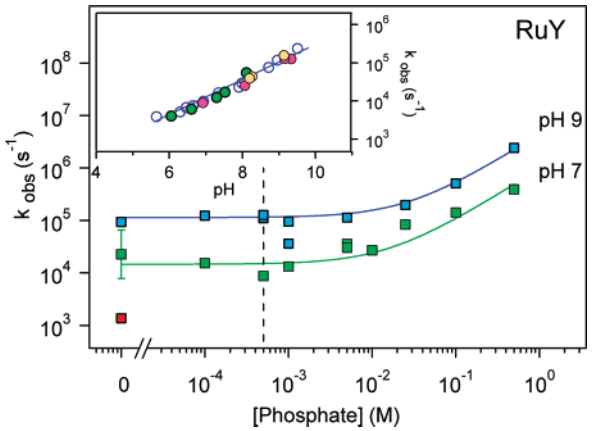

Figure 2. Phosphate buffer dependence of k_{obs}, for **RuY** and fits to eq 1 at pH 7 (green), pH 9 (blue) and pH 3 (red). The inset shows the pH dependence of k_{obs} in **RuY** at 0.5 mM buffer concentration (dashed line in main figure) with MES (green dots, pK_a = 6.2), phosphate (red dots, pK_a = 7.2), borate (yellow dots, pK_a = 9.1) and borate/phosphate mixture (circles) (T = 298 K). Unless otherwise illustrated, standard deviations are smaller than the point size.

described for Y oxidation in other systems.[3a] The entire data set for each complex can be fit to

$$k_{obs} = k_w + f_b[\text{buffer}]k_b \quad (1)$$

where k_w is the PCET rate constant obtained in the absence of buffer (with solvent as proton acceptor), f_b is the fraction of the basic form of the buffer in solution (HPO_4^{2-}), and k_b is the bimolecular PCET rate with proton transfer to HPO_4^{2-}. Similar effects were observed for **Re**(**P**−**Y**) with imidazole and pyridine as the buffer (Figure S3; Table S2).

Figure 2 plots k_{obs} for the **RuY** system as a function of phosphate buffer concentration at pH 7 and 9. A fit to eq 1 results in a pH-dependent k_w, as illustrated by the offset in the pH 9 (blue line) and pH 7 (green line) data. Sufficiently precise measurements of pH for unbuffered solutions near neutral pH were not feasible, therefore we used the measurement of k_{obs} at a low buffer concentration (i.e., in the flat region of Figure 2) to approximate k_w. The inset of Figure 2 plots the pH dependence of k_{obs} (= k_w) at 0.5 mM concentration of different buffers with various pK_a values. The linear correlation in the inset shows a genuine pH dependence of k_w that is independent of buffer identity and pK_a. The rate constant does not level out at pH > pK_a of MES (2-[*N*-morpholino]-ethanesulphonic acid) and [$H_2PO_4^-$], as would be the case if the base form of the buffer were the proton acceptor. The data at 0.5 mM exhibit a pH-dependence similar to that previously reported

Table 1. Rate Constants and Kinetic Isotope Effects for Y Oxidation in Three Separate Systems with Water or Water-Containing HPO_4^{2-}

	k_w (10^5 s^{-1})	$k_{w,H}/k_{w,D}$[a]	k_b (10^7 M^{-1} s^{-1})	$k_{b,H}/k_{b,D}$[a]	k_{ET2} (10^7 s^{-1})
RuY	0.1[b,c]	2.2−2.5[d]	0.3	1.8−2.0	5[c]
Ru$_{ester}$**Y**	4.4[b]	2[d], >10[f]	3.0	N/A	>10[e]
Re(**P**−**Y**)	1.0	<3	1.7	3.0	>10

[a] In H_2O vs D_2O. [b] pH-dependent, at pH 7. [c] Reference 6a. [d] Reference 1a. [e] Reference 6b. [f] pH = 10.

using 10 mM buffer (Supporting Information, Figure S2).[6a] Importantly, the data obtained in the absence of buffer in Figure 2 (at the approximate pH values indicated) span the same range of rates as the data in the inset.

The pH-dependence for Y oxidation in **RuY** in the buffer independent region of Figure 2 cannot be explained by a PTET mechanism with proton transfer to H_2O, OH^- or the basic form of the buffer (Scheme 1), or by a reversible PCET reaction, which all would show a slope of either 0 or 1 in the log(k_{obs}) versus pH plot (see Supporting Information for kinetic derivations). Instead, we assign the observed slope of ca. 0.5 (along with the kinetic isotope effect in Table 1) as arising from a CEP reaction with proton transfer to the bulk, as previously discussed.[6a] The pH of the bulk solution obviously affects the rate of this reaction, potentially via the pH-dependent driving force for the overall tyrosine oxidation (see above).

The excited-state of **Re**(**P**−**Y**) is a stronger oxidant than the Ru^{III} species in **RuY**, and the pH independence of k_w for the former system is consistent with a stepwise, ETPT mechanism for Y oxidation (pathway 1, Scheme 1). In **Ru**$_{ester}$**Y**, the Ru^{III} species is of intermediate oxidant strength, and as such, k_w is first pH-independent but becomes pH-dependent at high pH (see data at pH 9.9 in Figure 1). This has been attributed to a switching of the redox mechanism from ETPT at low pH to CEP with proton transfer to bulk at high pH.[6b] As previously described, ETPT has lower reorganization energy compared to CEP;[6b,11] thus the ETPT rate constant increases more steeply with increasing $-\Delta G^0$ and can outcompete CEP for stronger oxidants.[6b] The higher k_H/k_D for the CEP reactions (**RuY** and **Ru**$_{ester}$**Y** at pH 10, Table 1) compared to the pure ETPT reactions with tyrosine (**Ru**$_{ester}$**Y** at pH 7 and **Re**(**P**−**Y**), Table 1) or ET from tyrosinate[6] support these mechanistic assignments.

In contrast, at higher buffer concentrations the rate is first order in [HPO_4^{2-}] for all compounds studied, indicating a buffer-assisted PCET reaction. Saturating kinetics was not observed up to the solubility limit of phosphate, and we found no evidence for a rate dependence on [$H_2PO_4^-$] or [PO_4^{3-}] (see Supporting Information). We now consider the potential mechanisms for this PCET reaction.

PCET via an ETPT Mechanism: As the pK_a for the tyrosine radical cation is ca. −2, its deprotonation in aqueous media is very rapid ($k \approx 1 \times 10^{13}$ s^{-1}).[1a,6a] Therefore, the ETPT reaction will be ET-limited and not expected to depend on either pH or buffer concentration; we know of no reports that the Y•/Y potential should be significantly affected by phosphate buffer.

Diffusion-Controlled PTET with Rate-Limiting ET. This mechanism is illustrated by the following reaction:

$$[M^n]\text{-TyrOH} + HPO_4^{2-} \underset{k_{-PT}}{\overset{k_{PT}}{\rightleftharpoons}} [M^n]\text{-TyrO}^- + H_2PO_4^- \xrightarrow{k_{ET}} [M^{n-1}]\text{-TyrO}\cdot + H_2PO_4^-$$

Assuming a diffusion-controlled deprotonation of $H_2PO_4^-$ by TyrO$^-$ ($k_{-PT} \approx 1 \times 10^{10}$ M^{-1} s^{-1}) and an equilibrium constant (k_{PT}/k_{-PT}) of $10^{-\Delta pK_a}$ (ΔpK_a = pK_a(TyrOH) − pK_a($H_2PO_4^-$) = 2.8), a rate-limiting ET requires that $k_{ET} \ll k_{-PT}$ [$H_2PO_4^-$]. We calculate k_{-PT}

$\times$ [$H_2PO_4^-$] $\approx 1 \times 10^7$ s^{-1} and measure $k_{ET} \geq 5 \times 10^7$ s^{-1} (k_{ET2} in Table 1), thus this condition does not hold for any of the systems studied.

Diffusion-Controlled PTET with Rate-Limiting PT. Following the assumptions made above, the calculated rate constant for the rate-limiting PT mechanism is equal to $k_{PT} = 1.6 \times 10^7$ M^{-1} s^{-1}, which is comparable to the values of k_b that we measure for **Re**(**P**−**Y**) and **Ru**$_{ester}$**Y**. We used deuterium kinetic isotope effects to further investigate this reaction. The pK_a of $D_2PO_4^-$ is 7.8[9] in D_2O, while we measure the pK_a of TyrOD at 10.6 in D_2O (data not shown). The ΔpK_a between monobasic phosphate and tyrosine is therefore the same in H_2O and D_2O and, with the above reaction scheme, there should be no deuterium isotope effect for the PTET mechanism. The bimolecular rate constant for quenching of **Re**(**P**−**Y**) emission with DPO_4^{2-} in D_2O was measured as 5.7×10^6 M^{-1} s^{-1}, yielding a deuterium isotope effect (k_H/k_D) of 3.0 (Table 1). For **RuY** and phosphate buffer, $k_H/k_D = 1.8$ to 2.0 in the buffer-dependent region (ca. 50 mM). These experiments therefore do not support the mechanism of diffusion controlled PTET with rate-limiting PT. Separate experiments with pyridine and imidazoles as buffers are also consistent with this analysis. In these cases ΔpK_a is larger so that PTET is even inconsistent with the high rates observed (see Supporting Information).

PTET within a Hydrogen-Bonded Complex. For PTET within a hydrogen-bonded complex (pathway 2 in Scheme 1), the steady-state approximation can be used to derive an expression of the rate constant for the reaction

$$k_b = \frac{k_d k_{PT2} k_{ET2}}{k_{-PT2} k_{-d} + k_{ET2} k_{PT2}} \qquad (2)$$

With a diffusion controlled complexation rate constant, $k_d = 1 \times 10^{10}$ M^{-1} s^{-1}, and an association constant[10] $K_C = 0.5$ s^{-1}, the dissociation rate constant (k_{-d}) can be calculated as 2×10^{10} s^{-1}. The rate constant of tyrosine protonation within the complex (k_{-PT2}) cannot be faster than the frequency factor of 6×10^{12} s^{-1} given by absolute rate theory. Assuming that Δp$K_a = 2.8$ does not change within the complex, the rate constant for deprotonation (k_{PT2}) can then be calculated as 1×10^{10} s^{-1}. The rate constant for electron transfer (k_{ET2}) was measured as 5×10^7 s^{-1} for **RuY** (Table 1). Substituting these values into eq 2, k_b is estimated as 4×10^4 M^{-1} s^{-1}, which is 2 orders of magnitude slower than the observed value of 3×10^6 M^{-1} s^{-1} for **RuY**. Because of the similar buffer-dependent behavior of the **RuY**, **RuY**$_{ester}$, and **Re**(**P**−**Y**) compounds, we have no reason to believe that the mechanism for PCET within an H-bonded complex between Y and B would be different for the three systems.

CEP with Buffer as Proton Acceptor. On the basis of the discussion above we exclude the stepwise mechanisms. Instead we assign the buffer-assisted PCET to a CEP mechanism with ET to metal oxidant and proton transfer to basic form of the buffer. ET oxidation of tyrosine occurs with a large thermodynamic barrier owing to the large reduction potential of the tyrosine cation radical (E^0(Y$^{\bullet+}$/Y) = 1.46 V vs NHE[6b]). Thus CEP with proton transfer to base in solution avoids the formation of this intermediate. A single water molecule is a poor proton acceptor (pK_a(H_3O^+) ≈ -1.7), and the driving force for CEP may be increased by the replacement of water with the basic form of the buffer. Moving beyond thermodynamic considerations, hydrogen-bonding bases may further enhance the rate of CEP reactions in aqueous media by increasing the proton vibrational wavefunction overlap and/or by decreasing the reorganization energy for CEP.[4,11]

We have thus revealed a pattern of reactivity in the **RuY**, **Ru**$_{ester}$**Y**, and **Re**(**P**−**Y**) series with at least three competing PCET mechanisms: (1) CEP with the solvent as proton acceptor, which does indeed show a pH-dependent rate constant that is independent of buffer; (2) pH-independent ETPT; (3) a buffer-assisted CEP that is a general phenomenon at higher buffer concentrations (Figures 1 and 2). The pH-dependence of mechanism 3 follows the titration of the buffer.[12,13] The relative importance of mechanism 2 increases systematically with oxidant strength.

The pH-dependence of CEP with proton transfer to water, identified for the first time for **RuY**[6a], has been questioned on theoretical grounds[7,14] and proposed to arise from reactions with the buffer.[3a] Here we have experimentally confirmed that the pH-dependence is genuine and cannot be explained by buffer-assisted reactions or simple reaction schemes with first-order dependencies on OH^- or H_3O^+, as has been suggested.[3a,7] The rate-dependence on pH phenomenologically follows the Marcus equation for pure ET, which cannot be explained by models based on bulk reversibility (see Supporting Information). However, a detailed mechanism connecting the bulk property of pH to its effect on the rate-determining steps of the CEP reaction (e.g., with microscopically reversible steps) remains to be developed. Our results underpin the mechanistic richness of PCET and serve as a model for discussion of PCET reactions in more complex systems such as radical-based enzymes.

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Supporting Information Available: Detailed description of experimental procedures; additional kinetic data for **Re**(**P**−**Y**) with imidazole and pyridine bases; data of Figure 2 in table form; additional kinetic treatments of various PCET mechanisms. This material is available free of charge via the Internet at http://pubs.acs.org.

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