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Direct Evidence of a Tryptophan Analogue Radical Formed in a Concerted Electron−Proton Transfer Reaction in Water

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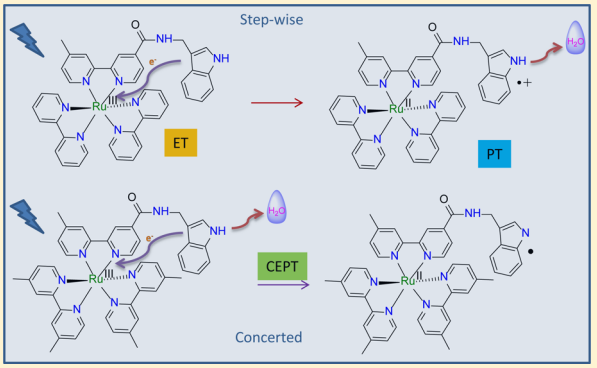
Supporting Information

ABSTRACT: Proton-coupled electron transfer (PCET) is a fundamental reaction step of many chemical and biological processes. Well-defined biomimetic systems are promising tools for investigating the PCET mechanisms relevant to natural proteins. Of particular interest is the possibility to distinguish between stepwise and concerted transfer of the electron and proton, and how PCET is controlled by a proton acceptor such as water. Thus, many tyrosine and phenolic derivatives have been shown to undergo either stepwise or concerted PCET, where the latter process is defined by simultaneous tunneling of the electron and proton from the same transition state. For tryptophan instead, it is theoretically predicted that a concerted pathway can never compete with the stepwise electron-first mechanism (ETPT) when neat water is the primary proton acceptor. The argument is based on the radical pK_a (∼4.5) that is much higher than that for water (pK_a(H_3O^+) = 0), which thermodynamically disfavors a concerted proton transfer to H_2O. This is in contrast to the very acidic radical cation of tyrosine (pK_a ∼ −2). However, in this study we show, by direct time-resolved absorption spectroscopy on two $[Ru(bpy)_3]^{2+}$−tryptophan (bpy = 2,2′-bipyridine) analogue complexes, that also tryptophan oxidation with water as a proton acceptor can occur via a concerted pathway, provided that the oxidant has weak enough driving force. This rivals the theoretical predictions and suggests that our current understanding of PCET reactions in water is incomplete.



INTRODUCTION

Proton-coupled electron transfer (PCET) reactions are common elementary reactions in enzymatic reactions and other catalytic processes.[1−6] For example, the coupled transfer of electrons and protons avoids charge accumulation at catalytic centers,[7,8] or may lead to conversion of an electrical potential to a proton gradient.[9−11] The potential of a redox couple is also influenced by PCET and depends strongly on the proton acceptor/donor. Water is an abundant proton acceptor/donor in the form of bulk water, interfacial water, or water clusters in the protein interior. Therefore, understanding the PCET reactions with water as reactant is of great interest.

PCET reactions can proceed either via a stepwise or concerted pathway. In a concerted electron−proton transfer (CEPT), both electron and proton tunnel in a single kinetic step, thus avoiding the formation of high energy, charged intermediates formed by pure electron transfer (ET) or proton transfer (PT).[12,13] This may result in a lower reaction barrier for the CEPT pathway. At the same time, the kinetic pre-exponential factor is often smaller because of the double tunneling requirement (weaker vibronic coupling between the reactant and product states).[14−16] The precise determination of the mechanistic pathway in a particular case is not straightforward and specific experimental data is essential to delineate the mechanism.

Tyrosine (Tyr) and tryptophan (Trp) constitute the class of amino acids most commonly involved in protein PCET reactions.[17,18] Furthermore, PCET reactions involving tyrosine have reached a working understanding due to the definitive identification and characterization of participating tyrosyl radical.[19,20] In many such reactions, the kinetic, thermodynamic, and spectroscopic data available for the tyrosyl radical are consistent with a concerted mechanistic pathway. In contrast, the work on tryptophan has primarily demonstrated $Trp^{\bullet}H^+$ and $Trp^{\bullet}$ species as a result of pure ET or stepwise, electron-first (ETPT) events.[21−23] One interesting example is DNA photolyase, where a photoexcited flavin chromophore induces single electron transfer in a chain of three tryptophan residues after which the ultimate, surface-exposed tryptophan radical ($Trp^{\bullet}H^+$) deprotonates to the bulk water.[24,25]

It is even predicted on theoretical grounds that tryptophan cannot undergo a concerted PCET with water as proton acceptor,[26,27] a prediction that has become generally accepted.[28,29] The argument is that, in contrast to tyrosyl radical cation $Tyr^{\bullet}H^+$ (pK_a ≈ − 2), the pK_a value of $Trp^{\bullet}H^+$ (pK_a ≈ 4.5) is much higher than for a proton in water (pK_a of H_3O^+ = 0). This makes the driving force for CEPT smaller than the one for ET, as shown in the following. Equations 1−3 give

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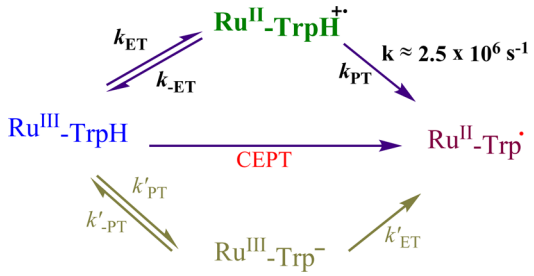
the reaction free energies for the different steps of ETPT and CEPT as a function of the oxidant potential ($E^0_{ox/red}$), see Scheme 1.

$$\Delta G^0_{ET} = -F(E^0_{ox/red} - E^0_{Trp^{\bullet}H^+/TrpH}) \quad (1)$$

$$\Delta G^0_{PT} = -ln(10)RT(pK_a(H_3O^+_{(aq)}) - pK_a(Trp^{\bullet}H^+)) \quad (2)$$

$$\Delta G^0_{CEPT} = \Delta G^0_{ET} + \Delta G^0_{PT} \quad (3)$$

Scheme 1. Possible Stepwise or Concerted (CEPT) Pathways in the PCET Reactions Studied for 1 and 2[a]



[a]The stepwise, ET-first pathway (ETPT) via $Trp^{\bullet}H^+$ follows the upper part of the scheme, with ΔG^0_{ET} and ΔG^0_{PT} given by eqs 1−2, while ΔG^0_{CEPT} is given by the sum of these steps (eq 3).

Because $\Delta G^0_{PT} > 0$, the direct oxidation of TrpH to $Trp^{\bullet}$ has significantly lower driving force than pure ET to form $Trp^{\bullet}H^+$, by an amount equal to that for the uphill proton transfer from $Trp^{\bullet}H^+$ to water (eq 2). In addition, the prefactor is expected to be lower for a CEPT reaction (see above). Therefore, it is predicted that tryptophan oxidation in water will always follow a stepwise, ETPT mechanism via formation of the $Trp^{\bullet}H^+$ intermediate. Note that, after formation of $Trp^{\bullet}...H_3O^+_{(aq)}$ via either ETPT or CEPT (eqs 1−3), subsequent dilution of the excess proton stabilizes $Trp^{\bullet}$ according to the solution pH, but this should not affect the PCET rate constant.[26−28]

Prior to this work, we reported a set of data that indirectly suggests that CEPT can nevertheless occur from tryptophan where water acts as a proton acceptor.[30,31] The concerted mechanism was supported by a substantial kinetic isotope effect (KIE = $k_H/k_D \approx 3.5$) in D_2O. A weak pH dependence of the PCET rate constant was also observed, which has previously been associated with CEPT reactions of tyrosine derivatives.[30,32,33] For comparison, a reference complex that reacted by stepwise ETPT gave KIE ≈ 1 and showed no pH dependence of the rate at pH < 10.

In the present study, we report the first direct evidence that a tryptophan analogue (Trp) can be oxidized directly to the deprotonated radical $Trp^{\bullet}$ without forming a $Trp^{\bullet}H^+$ intermediate, with water as primary proton acceptor. This is evidence against a stepwise ETPT, and for a CEPT pathway. Time-resolved absorption spectroscopy measurements allow clear observation of $Trp^{\bullet}H^+$ and $Trp^{\bullet}$ spectral signatures[34−37] from intramolecular oxidation by an appended $[Ru(bpy)_3]^{3+}$ complex, as shown for complex **1** (Figure 1). In contrast, complex **2**, which has a weaker Ru^{III} oxidant, shows no signal of a $Trp^{\bullet}H^+$ intermediate. Importantly, the formation of $Trp^{\bullet}$ by Ru^{III} oxidation in **2** is rapid enough compared to $Trp^{\bullet}H^+$ deprotonation ($\tau \approx 400$ ns) to exclude transient formation of $Trp^{\bullet}H^+$. The strong evidence for a CEPT reaction of

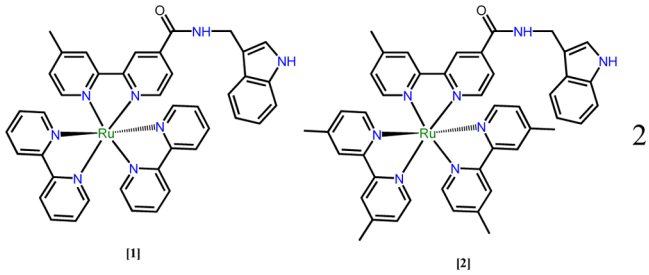


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Figure 1. Structures of $Ru(bpy)_3$−Trp molecules investigated in the study.

tryptophan in water challenges our understanding of this important class of reactions.

RESULTS AND DISCUSSION

In Figure 1, two similar metal complexes are shown. In complex **1**, a $[Ru(bpy)_3]^{2+}$ photosensitizer is covalently connected to a tryptophan analogue (TrpH) and in complex **2**, bpy ligands bearing electron donating methyl groups are used instead of unsubstituted 2,2′-bpy to decrease the $Ru^{III/II}$ redox potential of the Ru metal center. The $Ru^{III/II}$ potentials for the Ru-units of **1** and **2** are 1.26 and 1.10 V vs NHE, respectively, while the $Trp^{\bullet}H^+/TrpH$ potential is 1.16 V.[30] Due to complex, multielectron oxidation of the TrpH unit under voltammetric conditions, electrochemical data was taken from previous studies. We have previously shown that the $Ru^{III/II}$ potential is unaffected by linking to a Trp or Tyr unit.[30,32,33] Compared to the Ru−Trp complexes of previous studies,[30,32] **1** and **2** have a shorter link by one methylene unit, which is expected to increase the electronic coupling between the units and therefore increase the PCET rate. This turned out to be crucial in order to conclude whether the intermediates of stepwise reactions have been formed or not, as shown below.

The PCET reaction was initialized by photooxidation of the Ru center with a 10 ns laser flash at 460 nm in the presence of 40 mM of $[Ru(NH_3)_6]^{3+}$ as an external electron acceptor ($k_q[Ru(NH_3)_6]^{3+} = 2.30\ (\pm 0.01) \times 10^7\ s^{-1}$; Figure S2). The subsequent intramolecular PCET reaction where Ru^{III} oxidizes the appended TrpH can be monitored by following the kinetics for Ru^{II} absorption recovery at 450 nm, as well as the 510 and 580 nm absorption of $Trp^{\bullet}$ and $Trp^{\bullet}H^+$, respectively.[30,32,34−37]

Scheme 1 shows the three different mechanistic pathways for the PCET reactions of complexes **1** and **2** in water: (top) electron transfer generating the protonated $Trp^{\bullet}H^+$ followed by its deprotonation to water (ETPT); (middle) the concerted transfer of electron and proton (CEPT); and (bottom) TrpH deprotonation followed by its oxidation (PTET).

The time dependent transient spectra shown in Figure 2 and the corresponding decay traces at 510 and 580 nm shown in Figure 3 provide a conclusive picture of the transient species involved. Figure 2a shows the data for **1** at pH = 3: the initial Ru^{II} bleach at 450 nm and the broad absorption at >500 nm from the Ru^{III} and remaining $*Ru^{II}$ species are replaced by a broad absorption band around 580 nm. The latter is ascribed to a $Trp^{\bullet}H^+$ radical formed by ET from TrpH to the oxidized Ru^{III} metal center. Fits to kinetic traces at 450 nm gave the rate constant $k_{ET} = 5.7\ (\pm 0.1) \times 10^6\ s^{-1}$. As pH < pK_a of $Trp^{\bullet}H^+$, this species remains protonated until it recombines with the reduced acceptor $[Ru(NH_3)_6]^{2+}$ on a much slower, 100 μs time scale. Therefore, the TrpH oxidation is obviously a pure ET at a pH below $pK_a(Trp^{\bullet}H^+)$. Control experiments with [Ru-

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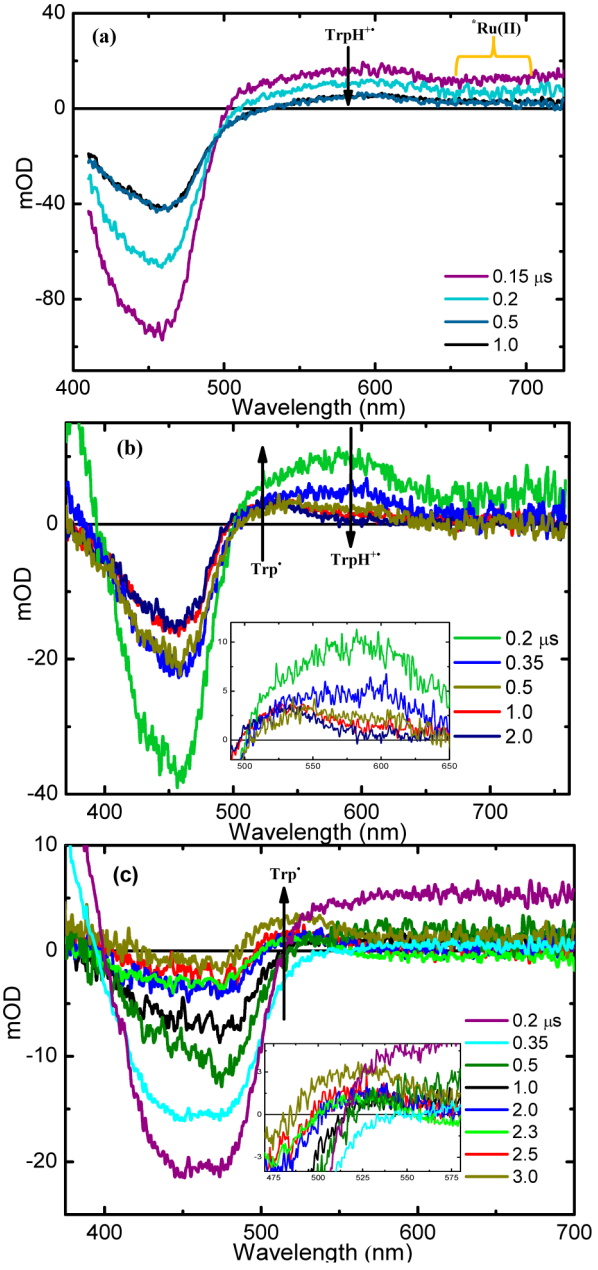

Figure 2. Transient absorption spectra after exciting **1** or **2** at 460 nm with a 10 ns laser pulse; solution conditions: nitrogen saturated 0.5 mM phosphate-borate buffer at 298 ± 2 K in the presence of 40 mM of $[Ru(NH_3)_6]^{3+}$ as an external electron acceptor; (a) complex **1** at pH 3 where only a protonated $Trp^{\bullet}H^{+}$ radical is seen at 580 nm; (b) complex **1** at pH 7.2 where the initial product is the protonated radical at 580 nm followed by the subsequent formation of a neutral radical at 510 nm; (c) complex **2** at pH 7.2 where a neutral radical appears directly at 510 nm without forming a protonated radical. The insets focus on the region of $Trp^{\bullet}H^{+}$ and $Trp^{\bullet}$ absorption where appearance of the corresponding radical species can be noticed. The legends in the graphs give delay times after the laser pulse, and the arrow indicate the time evolution of the spectra.

$(bpy)_3]^{2+}$ that lacks the TrpH unit showed no reaction of the Ru^{III} species on the time scale of a few microseconds (Figures S1 and S7); instead the Ru^{II} bleach remained until recombination with the reduced $[Ru(NH_3)_6]^{2+}$ acceptor, on the 100 μs time scale. Figure 2b shows the results for **1** at pH 7.2 instead. Following the formation of the $Trp^{\bullet}H^{+}$ species (k_{ET}

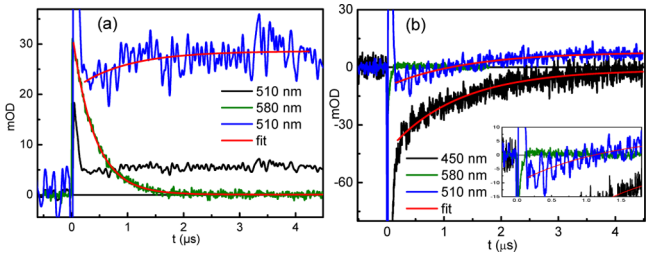

Figure 3. (a) Decay of the $Trp^{\bullet}H^{+}$ signal at 580 nm (green) and concomitant appearance of $Trp^{\bullet}$ at 510 nm (black) in complex **1**; for clarity, the 510 nm signal is also multiplied by 5 (blue). The fits give the rate constant of $Trp^{\bullet}H^{+}$ deprotonation as k_{PT} = 2.5 (±0.2) × 10^6 s^{-1}. (b) Recovery of the $[Ru^{II}]$ absorption at 450 nm (black) and concomitant growth of the 510 nm absorption from $Trp^{\bullet}$ (blue) in complex **2**. The fits give rate constants of $k_{(450\ nm)}$ = 1.1(±0.1) × 10^6 s^{-1} and $k_{(510\ nm)}$ = 8.3(±0.3) × 10^5 s^{-1}. The trace at 580 nm (green) shows no signal changes after the $[*Ru^{II}]$ emission has decayed (<100 ns). The inset is a zoom of the green trace. The samples were excited at 532 nm in N_2 saturated 0.5 mM phosphate-borate buffer at pH 7.2 with 40 mM $[Ru(NH_3)_6]^{3+}$ as quencher.

= 5.7 (±0.1) × 10^6 s^{-1}), a comparatively less intense band in the 490−550 nm range appears and this band can be attributed to the $Trp^{\bullet}$ species.[34−36] The appearance of the $Trp^{\bullet}$ species is concomitant with the disappearance of the previously formed $Trp^{\bullet}H^{+}$ species, which deprotonates to form the neutral radical species: Ru-$Trp^{\bullet}H^{+}$ → Ru-$Trp^{\bullet}$ (Figures 2b, 3a, and S5; the rise at 510 nm is relatively small as also $Trp^{\bullet}H^{+}$ absorbs at this wavelength). This allows us to determine the rate constant for deprotonation of $Trp^{\bullet}H^{+}$ in water to k_{PT} = 2.5 (±0.2) × 10^6 s^{-1}, in good agreement with previous results[30] and with what is expected for an Eigen acid with pK_a ≈ 4.5.[38,39]

The data for **2** reveals a strikingly different behavior (Figure 2c), where no $Trp^{\bullet}H^{+}$ absorption in the 520−620 nm range was observed. Instead, as the Ru^{II} bleach recovers the direct formation of $Trp^{\bullet}$ radical absorption in the 490−530 nm range is evident, with k_{obs} = 1.1 (±0.1) × 10^6 s^{-1} (at 450 nm). This result is clearly illustrated by the kinetic traces in Figure 3b: the 450 and 510 nm traces are parallel, with essentially the same rate constant, while there is no sign of $Trp^{\bullet}H^{+}$ absorption at 580 nm. The yield of $Trp^{\bullet}$ from $[Ru^{III}]$ is quantitative, within experimental uncertainty, as judged from the initial $[Ru^{III}]$ signal (≈ −35 mOD; see fit in Figure 3b) at 450 nm ($\Delta\varepsilon$ = −1.0 × 10^4 M^{-1} cm^{-1})[30−33] and the $Trp^{\bullet}$ signal at 510 nm (≈ 7 mOD; ε = 2.2 × 10^3 M^{-1} cm^{-1})[37,40] after 4 μs. In the following paragraph, we show that these results give direct evidence that TrpH is oxidized in a CEPT reaction in complex **2** and that the stepwise mechanisms can be excluded.

First, a proton-first mechanism (PTET) from TrpH in **2** can be excluded at pH = 7 due to its high pK_a value of ∼17.[31] For all of the available proton acceptors, i.e., H_2O, OH^-, or base forms of buffer, the maximum PTET rate constant is at least 10^6 times smaller than the observed rate constant of k_{obs} = 1.1 (±0.1) × 10^6 s^{-1}. This maximum value is calculated assuming a diffusion-controlled formation rate constant, the difference in pK_a between TrpH and the conjugate acid, and the concentration of acceptor pH = 7: k_{PT} = 10^{10} × $10^{\Delta pK_a}$ [acceptor].

Second, an ETPT can be excluded because the observed reaction rate constant is close to that for deprotonation of the $Trp^{\bullet}H^{+}$ species (k_{PT} = 2.5 (±0.2) × 10^6 s^{-1} as determined for **1**; the same value can be expected for **2** as the TrpH unit is the same) and yet there is no observable signal from any

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intermediate $Trp^{\bullet}H^{+}$ species. The extinction coefficient for $Trp^{\bullet}H^{+}$ at 580 nm (3000 M^{-1} cm^{-1}) is larger than for $Trp^{\bullet}$ at 510 nm (2200 M^{-1} cm^{-1}).[37,40] A consecutive reaction with the rate constant values reported here (k_{obs} and k_{PT}) would have given a signal of at least 3 mOD at 580 nm that then would have decayed (see Supporting Information for details), but the green trace in Figure 3b shows <1 mOD absorption over the entire time range (see Figure S6 for a larger version of the same figure). Therefore, this excludes both the case of rate limiting ET followed by PT, and that of reversible ET (pre-equilibrium) followed by PT (see Supporting Information). On the basis of these observations, it can be concluded that, while the oxidation of tryptophan in complex **1** occurs via a stepwise, ETPT pathway, in complex **2** it occurs via a concerted (CEPT) pathway.

We point out that this conclusion can be reached thanks to the shorter link in **1** and **2** compared to the previously reported Ru–Trp complexes.[30,32] This increases the rate constant of TrpH oxidation to be comparable with that for $Trp^{\bullet}H^{+}$ deprotonation, and thus, we can exclude the formation of a $Trp^{\bullet}H^{+}$ intermediate for **2**. With a slower oxidation an intermediate is not seen even for a stepwise reaction because it would deprotonate much faster than it is formed. Therefore, the evidence for a CEPT reaction in the previous studies was indirect, based on KIE values and a difference in the pH-dependence of the rates.[30,32]

The kinetic isotope effect by replacing TrpH with TrpD can nevertheless be used as another piece of evidence for a mechanistic difference between the two complexes. The H/D exchange occurred in situ with D_2O instead of H_2O as solvent. For **1** in D_2O, the rate constant for $Trp^{\bullet}H^{+}$ formation decreased to k_D = 4.2 (±0.1) × 10^6 s^{-1} at pD 7.2. This compares to k_H = 5.7 (±0.1) × 10^6 s^{-1} at pH 7.2 in H_2O resulting in a modest KIE = k_H/k_D of 1.4; weak solvent isotope effects for pure ET in water are often observed (see ref 33, and references therein). In the case of **2**, the PCET rate constants were k_H = 1.1 (±0.1) × 10^6 s^{-1} in H_2O and k_D = 2.3 (±0.1) × 10^5 s^{-1} in D_2O giving a quite significant KIE of 4.3 (Figure 4c). The lower KIE in the case of **1** is consistent with an ETPT mechanism in H_2O/D_2O,[41] while the much larger KIE exhibited by **2** supports a concerted (CEPT) pathway where PT between two electronically nonadiabatic potential energy surfaces occurs via proton tunneling.

The PCET rate constant was also examined at different pH values over a wide range (Figure 4 and Table S1). For **1**, no appreciable change was observed in the rate constant for $Trp^{\bullet}H^{+}$ formation and Ru^{II} recovery below pH 10, as expected for an ETPT reaction. For **2** instead, log(k_{obs}) showed a weak, approximately linear increase with pH in the range of pH 5.5–10, with a slope of ~0.15. This behavior is consistent with the previous reports where CEPT reactions for tryptophan and tyrosine in water showed a weak pH-dependence,[33,42,43] although this behavior is not understood. The increase in observed rate constant at pH > 10 may be due to OH^- as proton acceptor, and is therefore not the topic of the present study.

A key point to emphasize is that water is the only primary proton acceptor available in this PCET reaction. First, we have used only a low concentration of buffer (0.5 mM) to allow for precise setting of the solution pH while not interfering kinetically. Control experiments at different buffer concentrations show that this is the case (Figure S9), as was also shown by Zhang et al. for related systems.[30,31] Second, at pH = 7 also the concentration of OH^- (10^{-7} M) is too low to allow for rate constants of 1.1 (±0.1) × 10^6 s^{-1} in bimolecular reactions. Finally, for OH^- and buffer to be the primary proton acceptor, the reaction would be first order in concentration of base or OH^- with a slope = 1 instead of a ~0.15 slope in the plot of log(k_{obs}) vs pH. Thus, we can conclude that water (H_2O) is the primary proton acceptor in the CEPT reaction of **2**.

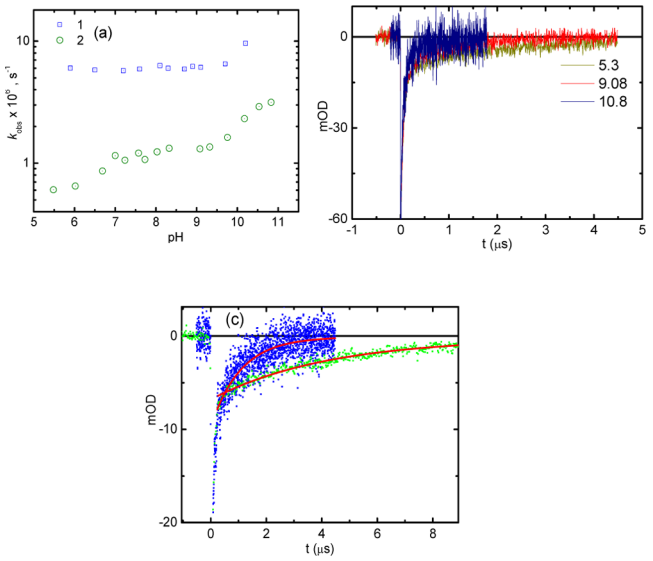

Figure 4. (a) pH dependence of the observed first-order rate constants in the PCET reaction where oxidation of tryptophan by $*Ru^{II}$ occurs (determined from the 450 nm absorption recovery). Squares and circles represent experimental data points for **1** and **2**, respectively. (b) Transient absorption traces at 450 nm for **2** at different pH's indicated in the figure. (c) Transient absorption traces at 450 nm for **2** at pH = 7.2 in H_2O (blue) and D_2O (yellow-green) buffers, respectively. Data acquired in N_2 saturated phosphate-borate buffer (0.5 mM) with 40 mM $[Ru(NH_3)_6]^{3+}$ as quencher.

Our results give evidence that the PCET mechanism changes between the structurally very similar complexes **1** and **2**. This can be attributed to the difference in oxidant strength between the two Ru^{III} centers (ΔE^0 = 160 mV). It has been shown for some amino acid derivatives and metal complexes that the PCET mechanism can be switched: strong oxidants tend to favor an ETPT mechanism, while weaker ones favor CEPT.[14,30,32,33] This can partly be understood from simple kinetics and Marcus-type analysis for ET and PCET.[44,45] With a weak oxidant the initial ET of ETPT is sufficiently uphill that the rate constant will be determined by an ET pre-equilibrium, thus decreasing by an order of magnitude for each 59 meV further decrease in oxidant strength. In contrast, the driving force dependence of single-step CEPT follows a Marcus-type expression (eq 4 for a nonadiabatic reaction; the index i represents either ET or CEPT),[12] thus decreasing by at most one natural logarithmic step per 59 meV decrease in oxidant strength around ΔG^0 = 0 (eq 5).

$$k_i = A_i \exp\left(-\frac{(\Delta G_i^0 + \lambda_i)^2}{4\lambda_i RT}\right) \quad (4)$$

$$\frac{\partial \ln k_i}{\partial \Delta G_i^0} = -\frac{1}{2RT}\left(1 + \frac{\Delta G_i^0}{\lambda_i}\right) \quad (5)$$

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Also in the case where ET is rate limiting ($k_{obs} = k_{ET}$) and follows the same type of driving force dependence as CEPT (eqs 4 and 5), it has a lower driving force than CEPT and is therefore still more strongly dependent on the variations in ΔG^0 (cf. eq 5). The change in mechanism between **1** and **2** is consistent with the notion that CEPT is a more "energy conservative" mechanism, i.e., that it may occur with a lower reaction barrier than the stepwise reactions, when the overall driving force is small.[13,14] Note that other factors may complicate this picture so that it cannot safely be generalized to all cases. These may include differences in reorganization energy between CEPT and ETPT, and a possible driving force dependence of the proton tunneling probability.

Deprotonation of an acid in water occurs in several microscopic steps:[38,39,46] Transfer of the proton to one water molecule is driven by solvent fluctuations to stabilize the charge redistribution (eq 6, step 1). The subsequent dissociation and cage escape of the proton then completes the reaction, forming a solvated excess proton ($H_3O^+_{aq}$) that is uncorrelated with its geminate base (for simplicity, the Ru^{II} unit is not indicated).

$$H_2O\cdots Trp^{\bullet}H^+ \rightleftharpoons H_3O^+\cdots Trp^{\bullet} \rightleftharpoons H_3O^+_{aq} + Trp^{\bullet} \quad (6)$$

The initial proton transfer of a weak acid ($pK_a \gg 0$) is endergonic, because $pK_a(H_3O^+) = 0$, but a quasi-steady state fraction of $H_3O^+\cdots Trp^{\bullet}$ will be formed, a fraction that depends on the acid pK_a, that may dissociate completely. For a CEPT reaction, a similar model should hold, but the initial step involves PT with a concerted oxidation of the acid:

$$H_2O\cdots TrpH{-}Ru^{III} \rightleftharpoons H_3O^+\cdots Trp^{\bullet}{-}Ru^{II} \rightleftharpoons H_3O^+_{aq} + Trp^{\bullet}{-}Ru^{II} \quad (7)$$

In **1**, the initial ET step of the ETPT reaction is exergonic ($\Delta G^0_{ET} = -0.10$ eV) so that $Trp^{\bullet}H^+$ can be rapidly formed and then deprotonated in a following step (eq 6). In **2** instead, $\Delta G^0_{ET} = +0.06$ eV. Apparently, this slows down the ET step enough that CEPT is favored. The change in mechanism between **1** and **2** follows the trend explained above (eqs 4 and 5). However, formation of the initial dissociation product $H_3O^+\cdots Trp^{\bullet}$ of CEPT in **2** is still much more uphill than for ET in the same complex. Following the argument by Krishtalik and others (*vide supra*) it is not clear what factor would compensate for the lower driving force for CEPT in this comparison, and for the presumably weaker vibronic coupling, to make CEPT the faster reaction. It seems that our models and understanding of PCET reactions with water as primary proton acceptor are not complete.

CONCLUSIONS

To conclude, we have presented direct spectroscopic and kinetic evidence for a concerted PCET from the tryptophan analogue in **2** with water as primary proton acceptor, in contrast to theoretical predictions. The Ru−TrpH complexes **1** and **2** were designed such that the rate of TrpH oxidation is faster than, or nearly as fast as, that for deprotonation of the intermediate $Trp^{\bullet}H^+$. Thereby we have been able to directly observe the $Trp^{\bullet}H^+$ intermediate of an ETPT reaction for **1** as well as to show that **2** produces $Trp^{\bullet}$ in a direct CEPT reaction, without forming the $Trp^{\bullet}H^+$ intermediate. This challenges our understanding of PCET in water, and more work is needed to fully understand these fundamentally important reactions of chemistry and biology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b08294.

Detailed synthetic and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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