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Proton-Coupled Electron Transfers: pH-Dependent Driving Forces? Fundamentals and Artifacts

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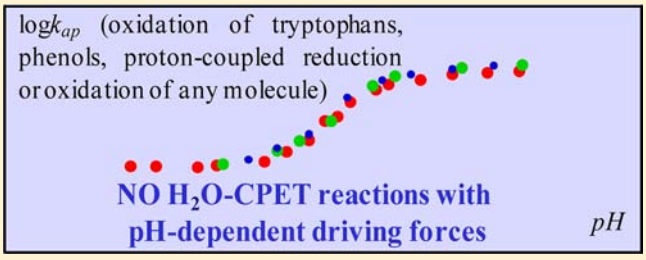
Supporting Information

ABSTRACT: Besides its own interest, tryptophan oxidation by photogenerated Ru complexes is one of the several examples where concerted proton–electron transfer (CPET) to water as proton acceptor endowed with a pH-dependent driving force has been invoked to explain the data. Since this notion is contrary to the very basic principles of chemical physics, it was interesting to attempt uncovering the source of this contradiction with an easily accessible substrate. Careful examination of the oxidation of the tryptophan (ethyl ester derivative) bearing a NH_3^+/NH_2 group showed that there is no trace of such an unconventional H_2O-CPET with a pH-dependent driving force. The reaction mechanism simply consists, with both the NH_3^+ acid and NH_2 basic forms of the tryptophan derivative, in a rate-determining electron-transfer step followed by deprotonation steps. The same is true with the ethyl ester-methyl amide derivative of tryptophan, whose behavior is even simpler since the molecule does not bear an acid–base group. No such unconventional H_2O-CPET was found with phenol, another easily accessible substrate. It may thus be inferred that the same applies to less easily available systems in which electron transfer occurs intramolecularly. These observations help to rid the road of such artificial obstacles and improve present models of H_2O-CPET reactions, a landmark towards the understanding of the role of water chains in natural systems.

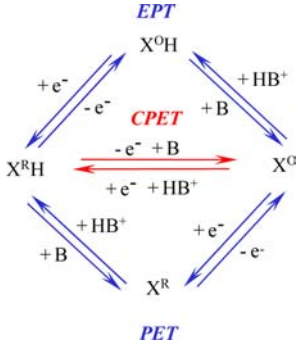


INTRODUCTION

Coupling between electron and proton transfers is ubiquitous in natural and artificial processes. It partakes in many of the schemes currently designed for the resolution of contemporary energy challenges.[1] These "PCET" reactions may go through an electron- or proton-transfer intermediate, giving rise to an EPT and a PET pathway, respectively (Scheme 1, B/HB^+ is the proton acceptor/donor couple).

In the CPET pathway, proton and electron transfers are concerted,[2] thus consisting in a single elementary step. Since they go directly from the reactant, X^RH, to the product, X^O, CPET pathways are expected to be more advantageous than stepwise pathways since they avoid the high-energy intermediates, X^OH and X^R, involved in the EPT and PET pathways, respectively. This thermodynamic advantage may however be counteracted by a kinetic penalty upon taking the CPET pathway. The main task of the mechanism analysis is therefore to distinguish between the three pathways, to determine their thermodynamic and kinetic characteristics, and to uncover the factors that govern the competition between them. Numerous theoretical and experimental investigations have been devoted to this goal.[3−9] In this framework, oxidation of phenols, particularly with water as proton acceptor, has attracted special attention in view of the importance of tyrosine oxidation in photosystem II (X^RH = ArOH, X^O = $ArO^•$).[10] From a thermodynamic standpoint, these reactions are commonly and conveniently described under the form of a Pourbaix diagram relating the apparent standard potential of the system to pH as shown in Figure 1 for tryptophan. Once a CPET pathway has been identified, analysis of the kinetics is naturally developed within the framework of an activation driving force relationship based on the same principles as the Marcus–Hush relationship for simple outersphere electron transfers. In this framework, temptation has been strong and not always fought victoriously to define the driving force of the CPET reaction as the difference between the pH-dependent Pourbaix apparent

Scheme 1



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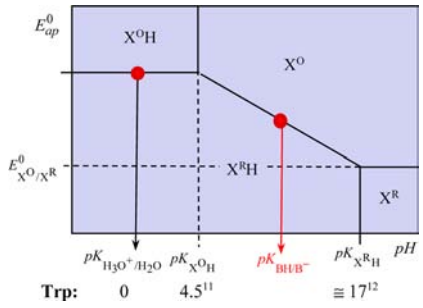

Figure 1. Pourbaix diagram (apparent standard potential vs pH[11,12]) of the PCET reaction represented in Scheme 1. pKs for reduced and oxidized form of tryptophan from refs 11 and 12.

standard potential and the standard potential of the electron donor/acceptor couple. We will see in the CPET with a pH-Dependent Driving Force section that the ensuing notion of a pH-dependent driving force is fundamentally incorrect for basic thermodynamics' reasons. We will then turn to the discussion of the experimental data that seem to give credit to the applicability of notion of pH-dependent driving forces. They essentially concern oxidation of phenols and tryptophans with water as the proton acceptor. Two series of experiments are available. One involves intermolecular systems with easily available molecules as substrates.[13−15] In the other series, requiring delicate synthesis, the electron acceptor and the substrate are linked in the same structure giving rise to an intramolecular PCET reaction.[16,17] We will thus compare the linked and intermolecular systems (Intermolecular and Intramolecular CPET Reactions section) and see that if the notion of pH-dependent driving force is faulty in the latter case, it is also faulty in the former.

It follows that we only require examining the pending intermolecular cases in which the experimental data seem to fall in line with the notion of pH-dependent driving force for reaching a general conclusion, which will apply to the linked systems as well. We were thus led to focus attention on easily available substrates, such as phenols and tryptophans. In the first case, a detailed analysis of the PCET reaction in water with and without buffer is available using a laser flash-quench technique as well as convergent electrochemical approaches.[15] There was no need then to resort to the notion of pH-dependent driving force to interpret the data and to obtain a detailed description of the particular features of water as proton acceptor in CPET reactions.[15c] The data in ref 13a, gathered in the presence of 0.1 M buffer, are compatible with this interpretation and certainly does not require to resort to the notion of pH-dependent driving force. Remains the case of tryptophans, where laser flash photolysis data compatible with the notion of pH-dependent driving force were claimed to have been obtained.[13b] In a third section, we accordingly re-examine the case of tryptophans in order to uncover the reasons behind this apparent adherence to the notion of pH-dependent driving force.

RESULTS AND DISCUSSION

CPET with a pH-Dependent Driving Force: Why Is It so Tempting? Why Is It an Incorrect Notion? In a remarkable effort to mimic the role of tyrosine in photosystem II, molecules of the type shown in Figure 2 have been synthesized. The oxidation kinetics of the tyrosine moiety by Ru^{III} laser flash photogenerated by means of MV^{2+} quenching,

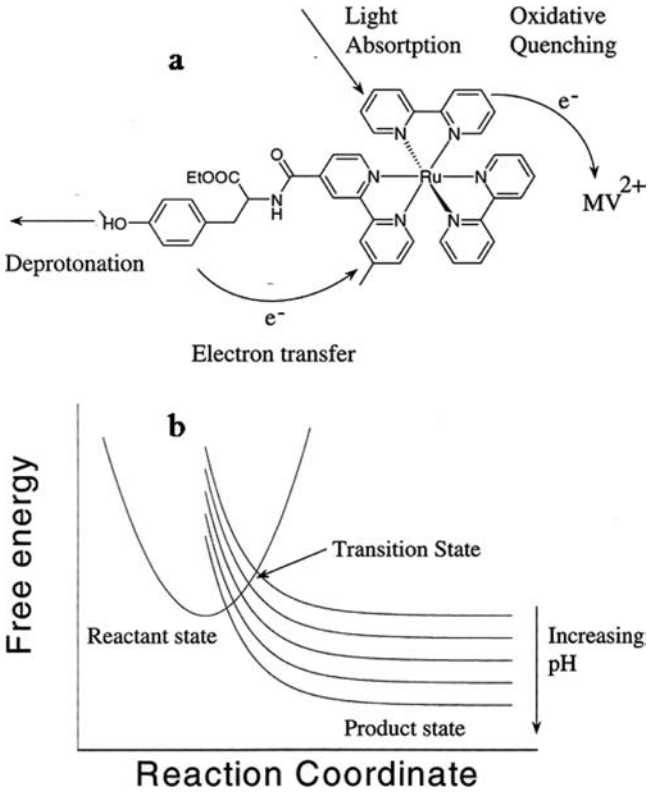

Figure 2. System investigated in reference 16b with the diagram explaining the effect on the kinetics of the H_2O-CPET reaction with the help of a pH-increasing driving force. "From Figures 1 and 4 in ref 16b. Schematic picture of the potential surface for the reactant and product states. The product 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.[16b]"

and some other quenchers were then determined as a function of the pH in a water solution containing only a small amount of buffer (Figure 2).[16b] The observed increase of the overall rate constant was interpreted as resulting from a H_2O-CPET reaction in which the driving force would be an increasing function of pH, as sketched in Figure 2b.

In the pH range where the reactant and product of the CPET reaction coexist, where the apparent standard potential E^0_{ap} varies with pH by $(RT/F)\ln 10$ (Figure 1), it is very tempting to consider that the driving force of the CPET reaction is $F(E^0_A - E^0_{ap})$(E^0_A: standard potential of the acceptor couple, here $MV^{2+}/MV^{\bullet+}$) and therefore that it is an increasing function of pH in the framework of an activation/driving force similar to Marcus−Hush-type relationship for simple outersphere electron transfers.[18,19] This notion of the driving force used in a free energy profile diagram, such as the one represented in Figure 2b, is a misconception. The driving force should not be viewed as the opposite of the free energy of the reaction but as the opposite of the standard free energy of the reaction. This well-defined thermodynamic quantity is not pH dependent. In short, the notion of pH-dependent driving force contradicts the most basic principles of chemical physics including the applicability of thermodynamics' second law. Instead, the nature of the proton acceptor ought to be specified in each case, water in the case above or others (as e.g. phosphate ions)

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as represented in Figure 1. In each case the driving force should be defined as

$$F[E^0_A - E^0_{ap}(for\ pH = pK_{proton\ acceptor})]$$

i.e. replacing the pH in the Pourbaix diagram by the pK of the proton-acceptor couple.

This misleading notion of CPET reactions with pH-dependent driving forces has been used in several other publications concerning attached[16,17] or intermolecular systems,[13,14] in spite of adverse warning.[20,21] With water being the proton acceptor in most of these cases, use of the notion of pH-dependent driving force has led to a repeated request of an analysis of the microscopic aspects of H_2O-CPET reactions,[13b,17d,e] ignoring that such a detailed analysis was already available.[21]

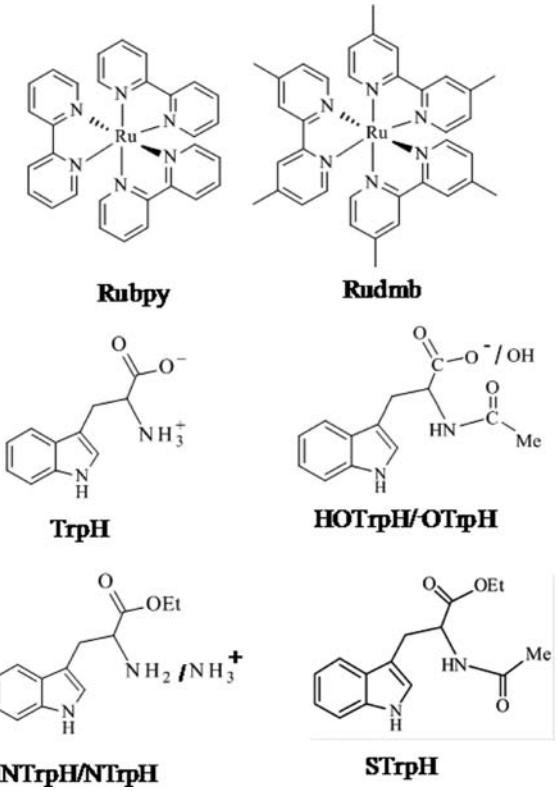
Intermolecular and Intramolecular CPET Reactions. Systems in which the electron acceptor and donor are linked together, as they are in the system depicted in Figure 2, have the advantage of avoiding diffusion problems. Also, they involve pseudo-second-order reactions as opposed to intermolecular systems, which entail termolecular reactions. The conditions should then be more favorable to successfully fight back electron transfer reactions. In fact, electron transfer between the electron acceptor and donor sites is slow and highly nonadiabatic owing to the large distance between them. Consequently, the theoretical advantage of linked systems is not so overwhelming in practice. We note in this connection that the reactions involved in the oxidation of phenols by photogenerated $Ru^{III}bpy_3$ are comfortably triggered and analyzed in spite of their termolecular character.[15a−c]

Another practical problem with the linked systems described so far is that, except for a few examples, the kinetic traces were not made available systematically,[13,16,17] which impeded testing the consistency of the set of experiments and obliged us to restrain our inquest to the easily available intermolecular systems. Having checked with the latter systems that the apparent applicability of the notion of a pH-dependent driving force results from artifacts, as shown previously for phenols[15] and evidenced below for tryptophans, we are led to naturally extend this conclusion to the linked systems.

Proton-Coupled Electron Transfer from Tryptophans in Water: Artifacts and Actual Mechanism. In the case of tryptophans we are facing a perplexing situation since experimental facts apparently exist that seem to fall in line with the faulty notion of pH-dependent driving forces.[13b] The purpose of the work described below was therefore to re-examine this reaction, detect the artifacts behind this apparent agreement, and establish the actual mechanism. The first step was the gathering of the kinetic data as a function of pH with MV^{2+} as oxidative quencher in the absence of buffer and with the two Ru complexes shown in Scheme 2 as well their comparison with the data in ref 13b. It however appeared that some doubt may be cast on these results since it was found that an adduct is formed between MV^{2+} and tryptophan that may complicate the interpretation of the kinetic data. It was therefore decided to repeat the whole set of experiments with $Ru(NH_3)_6^{3+}$ as quencher, which does not form any adduct with the tryptophan under examination.

In fact, the molecule involved in the mysterious pH-dependent H_2O-CPET reaction is not tryptophan itself but the derivative ($^+$H)NTrpH in which the carboxylic function has been esterified (Scheme 2).

Scheme 2



This molecule bears a NH_3^+ acid function whom deprotonation is likely to influence the variation of the PCET rate with pH. This possibility is absent in linked systems of the type shown in Figure 2 and in analogous systems bearing an attached tryptophan moiety.[17d] We thus investigated another tryptophan (noted STrpH in Scheme 2) that does not bear any deprotonable acid function. In all cases our line of reasoning was as follows: EPT pathways are expected to predominate over PET mechanism and conventional H_2O-CPET mechanisms because of driving force advantages deriving from the sequencing of pKs shown at the bottom of Figure 1 (the driving force advantages are ∼1.02 and 0.27 eV in the first and second cases, respectively). Since the intrinsic rate constants are expected to be larger for a simple outersphere electron transfer than for a CPET reaction (because of kinetic penalties deriving from proton tunneling), the thermodynamic advantage results in an even larger kinetic advantage. The situation is opposite for phenol since $pK_{PhOH^{\bullet+}}$ is negative (≈ −2).

The variation of the apparent rate constants with pH will thus be examined within this framework, attempting to detect the occurrence of an unconventional H_2O-CPET whose rate constant would vary with pH because its driving force would be pH-dependent or for any other mysterious reason.

Oxidation of ($^+$H)NTrpH by the Two Ru^{III} Complexes Generated with MV^{2+} as Quencher. The experimental data are thus analyzed according to reaction sequence summarized in Scheme 3, where the back electron-transfer reactions have been omitted for simplicity (the full reaction set of reactions is given in the Supporting Information (SI)). The main electron-transfer steps, in red in Scheme 3, are considered irreversible, even in the case of Rudmb where they are endergonic, because they are followed by fast proton-transfer and back electron-

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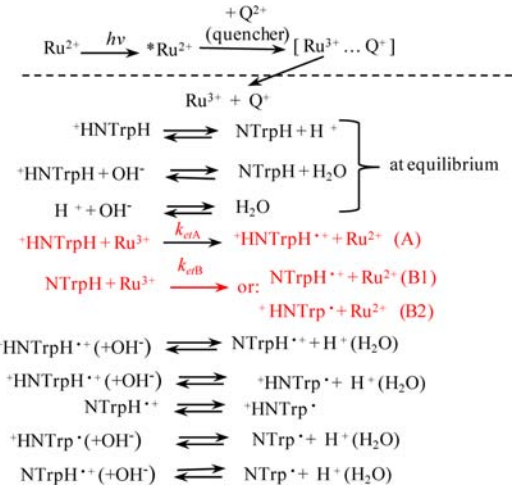
Scheme 3



transfer reactions. The decay of Ru^{3+} and of Q^+ is given by the following equations (see SI):

$$\frac{1}{[Q^+]} = \frac{1}{[Q^+]_{t=0}} + k_{bet}t \quad (1)$$

$$[Ru^{3+}] = \frac{[Ru^{3+}]_{t=0}\exp\{-(k_{ap}[(^+H)NTrpH]_{total}) \times t\}}{1 + k_{bet}[Q^+]_{t=0} \times t} \quad (2)$$

where $[(^+H)NTrpH]_{total}$ is the total concentration of the two forms of this tryptophan derivative and

$$k_{ap} = k_{etA}\frac{[^+HNTrpH]}{[(^+H)NTrpH]_{total}} + k_{etB}\frac{[NTrpH]}{[(^+H)NTrpH]_{total}}$$

The various values of k_{ap} obtained as a function of pH were derived from the fit of the decay curves with eq 2, and for MV^{2+} as quencher, eq 1, taking for $k_{bet} = 6 \times 10^9$ M^{-1} s^{-1} (see SI). Fitting of all decay curves recorded in this work may be consulted in the SI. The pH variations of k_{ap} with the two Ru complexes are displayed in Figure 3. While there is a good agreement between our results and those from ref 13b with Rubpy, the match is less satisfactory with Rudmb. It should be noted in this connection that the extraction of the apparent rate constant is less precise in the latter case than with Rubpy, since it is smaller and can hardly compete with back electron transfer, especially at the lower end of the pH range.

Another difficulty arises with MV^{2+} as quencher, namely the formation of an adduct with (^+H)NTrpH as revealed by UV–vis spectroscopy over the whole pH range of interest (Figure 4). The formation of this adduct renders uncertain the interpretation of the observed variation of the apparent rate constant with pH. To be able to browse the whole pH range in the case of Rudmb, we therefore decided to change the quencher and to use $Ru(NH_3)_6^{3+}$instead of MV^{2+}. The absence of adduct in this case is documented in the SI.

Oxidation of (^+H)NTrpH by the Two Ru^{III} Complexes Generated with $Ru(NH_3)_6^{3+}$ as Quencher. The results are shown in Figure 3 (green dots) together with those obtained with MV^{2+} for comparison. The use of $Ru(NH_3)_6^{3+}$ not only lifts the ambiguities related to the formation of an adduct with MV^{2+} but also gives rise to stronger signals thanks to a more efficient cage escape and to the possibility of using much higher tryptophan concentrations, thus favoring the reaction of interest over back electron transfer. A more reliable set of data could thus be gathered in the case of Rudmb, noting that the two sets of data are closely similar above pH 6 as expected from the discussion at the end of the preceding section.[22] This is the reason that the green dots series cover the whole pH range in Figure 3, whereas in the blue and red dot series, the data for pHs below 6 have been discarded. The various values of k_{ap} obtained as a function of pH were derived from the fit of the decay curves with eq 2, with $k_{bet} = 7.2 \times 10^9$ M^{-1} s^{-1} (see SI).

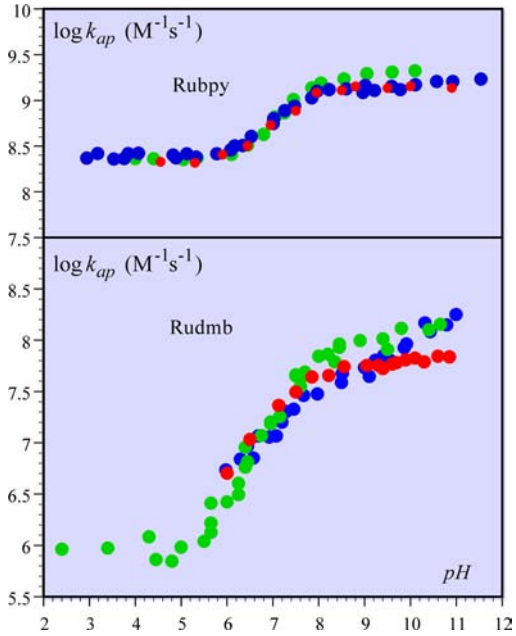


Figure 3. The pH dependence of the apparent rate constant of oxidation of (^+H)NTrpH by flash quenched generated Ru^{3+} with the two Ru complexes. Blue dots: quencher = MV^{2+}, 20 mM, from ref 13b; red dots, quencher = MV^{2+}, 40 mM, this work; green dots: quencher = $Ru(NH_3)_6^{3+}$, 20 mM, this work. In the upper diagram: (^+H)NTrpH = 1 mM in all cases. In the lower diagram: (^+H)NTrpH (mM) = 1–5 (blue dots), 1 (red dots), 1–100 (green dots).

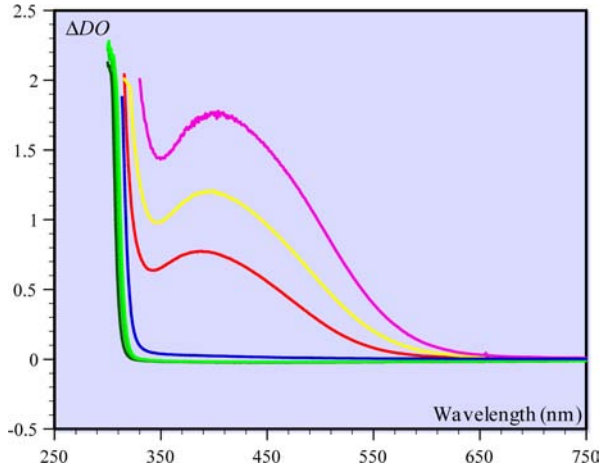


Figure 4. Spectrophotometric evidence for the formation of an adduct between MV^{2+} and (^+H)NTrpH. Mixture of 25 mM MV^{2+} and 25 mM (^+H)NTrpH at pH = 4.9 (red), 7.25 (yellow), 10.4 (magenta). Twenty-five mM MV^{2+} alone (blue line, no change with pH) and 25 mM (^+H)NTrpH (green lines, from right to left, from light to dark green, pH = 4.9, 7.25, 10.4).

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The variation of the apparent rate constant with the concentration of the Ru^{2+} complex, with $Ru(NH_3)_6^{3+}$ as quencher is negligible, justifying the irreversibility assumed for the electron-transfer steps in Scheme 3 (Figure 5).

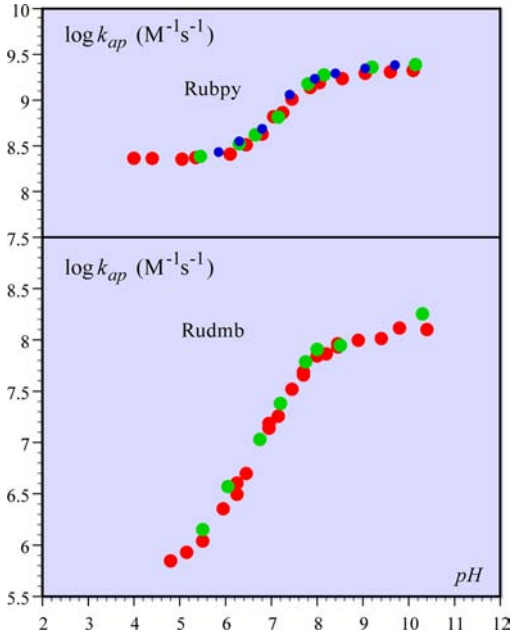

Figure 5. Independence from the Ru^{2+} complex concentration of the apparent rate constant of oxidation of ($^+$H)NTrpH (25 mM) by flash quenched generated Ru^{3+} with $Ru(NH_3)_6^{3+}$ (20 mM) as quencher. Ru^{2+} concentration (μM): 15 (blue dots), 50 (red dots), and 150 (green dots).

Does the S-Shaped Variation with pH of the Rate Constant of Oxidation of ($^+$H)NTrpH by the Two Ru^{III} Complexes Have Something to Do With a pH-Dependent Driving Force? In fact it simply results from the deprotonation of the NH_3^+ group in $^+$HNTrpH, which occurs with a pK = 7.5 as determined by mere acid−base titration (see SI) in agreement with literature data[23] but in contrast with the value of 9.3, used in ref 13b.

Then, according to Scheme 3:

$$k_{ap} = \frac{k_{etA}}{1 + 10^{pH - pK_{^+HNTrpH}}} + \frac{k_{etB}}{1 + 10^{pK_{^+HNTrpH} - pH}} \quad (3)$$

The fitting of the experimental data with this equation (Figure 6) leads to the values of k_{etA} and k_{etB} reported in Table 1 for the two oxidizing Ru complexes. Table 1 also contains an estimation of the corresponding driving forces based on the standard potentials of the redox couples that are involved.

While the estimation is straightforward for the acid form of the tryptophan derivative (see footnote d in Table 1), it is worth some comments in the case of the basic form. There are two possible oxidation pathways for the basic form as indicated in Scheme 3. Pathway B1 involves a simple outersphere electron transfer from NTrpH, which is therefore governed by the same driving force as the outersphere oxidation of $^+$HNTrpH along pathway A (Table 1). Another oxidation pathway of NTrpH may however be envisaged, in which electron transfer would be concerted with proton migration from the tryptophan nitrogen to the NH_2 nitrogen. This intramolecular CPET pathway, B2, would consequently be endowed with a driving force advantage over pathway B1 equal

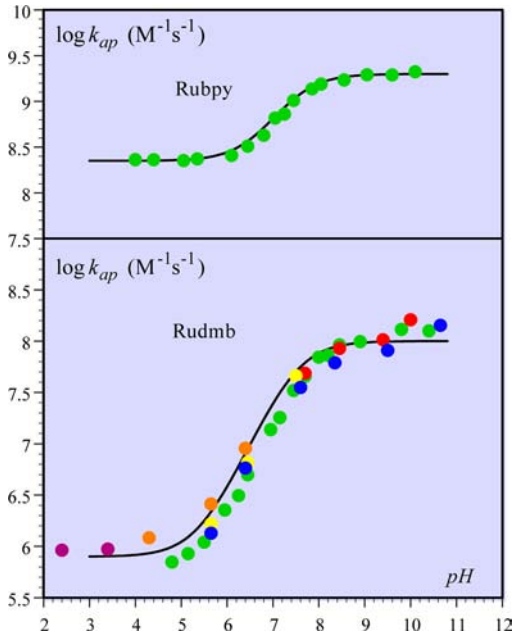

Figure 6. Fitting of the apparent rate constant of oxidation of ($^+$H)NTrpH by flash-quenched-generated Ru^{3+} with $Ru(NH_3)_6^{3+}$ (20 mM) as quencher with the predictions of Scheme 3 (see text). Upper diagram: ($^+$H)NTrpH = 1 mM and lower diagram: ($^+$H)NTrpH (mM): 1 (blue dots), 25 (green and red dots), 50 (yellow dots) 100 (orange and magenta dots).

to: $(RT \ln 10/F)(pK_{^+HNTrpH} - pK_{^+HNTrpH^{\bullet+}}) = 0.174$ eV at 20 °C, taking $pK_{^+HNTrpH} = 7.5$ and $pK_{^+HNTrpH^{\bullet+}} = 4.5$,[25] resulting in the driving force estimates reported in Table 1.

The apparent rate constant of the various pathways (A, B1, B2) is expected to obey the following equation[26]

$$\frac{1}{k_{et}} = \frac{1}{k_{dif}} + \frac{1}{k_{act}} + \frac{1}{k_{dif}\exp\left(-\frac{\Delta G^0_{et}}{RT}\right)} \quad (4)$$

where k_{dif} is the diffusion limit and k_{act}, the activation-controlled rate constant. Assuming that the symmetry factor is 1/2:[18,19]

$$k_{act} = k_0 \exp\left(-\frac{1}{2}\frac{w_R + w_P}{RT}\right)\exp\left(-\frac{1}{2}\frac{\Delta G^0_{et}}{RT}\right) \quad (5)$$

where k_0 is the rate constant at zero driving force and zero work terms. w_R and w_P are the work terms required to bring the reactants and products, respectively, from infinite to reacting distance.

Figure 7 shows the application of eqs 4 and 5 to the variation of the apparent rate constant of the various pathways with the driving force, taking into account the work terms in each case (using the estimates reported in Table 1). The work terms resulting from electrostatic interactions can be estimated as follows:

$$w_{R,A} + w_{P,A} = (3 \times 1 + 2 \times 2)w_0 = 7w_0$$

$$w_{R,B1orB2} + w_{P,B1orB2} = (3 \times 0 + 2 \times 1)w_0 = 2w_0$$

$$\text{with: } w_0 = \frac{e_0^2}{4\pi\varepsilon_0\varepsilon_S d}$$

w_0 is estimated as equal to 0.026 eV.[27]

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Table 1. Rate Constants and Driving Forces

	log k_{etA}[a]	log k_{etB}[a]	$E^0_{Ru^{3+}/Ru^{2+}}$[b]	$-\Delta G^{0\,c}_{et}$ A[d]	$-\Delta G^{0\,c}_{et}$ B1[d]	$-\Delta G^{0\,c}_{et}$ B2[e]	work terms[e] A	work terms[e] B1, B2
Rubpy	8.35	9.3	1.26	0.06	0.06	0.234	$7w_0$	$2w_0$
Rudmb	5.9	8.0	1.09	- 0.12	- 0.12	0.054		

[a] M^{-1} s^{-1}. [b] In V vs NHE, from ref 13b, checked in this work by cyclic voltammetry (see Experimental Section in SI). [c] In eV. [d] From $E^0_{Ru^{3+}/Ru^{2+}}$ and $E^0_{^+HNTrpH^{\bullet+}/^+HNTrp}$=1.21 V vs NHE. [24] [e] See text

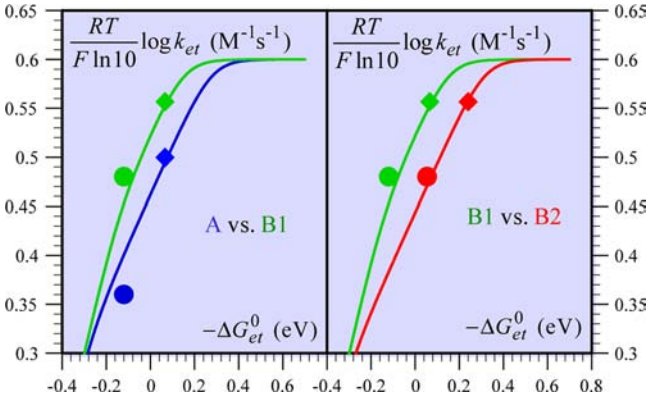

Figure 7. Application of eqs 4 and 5 to the variation of the apparent rate constant of the various pathways with the driving force. Diamonds: Rubpy, solid circles: Rudmb. Blue, green, and red symbols: pathways A, B1, and B2, respectively. Solid lines: application of eqs 4 and 5 (see text).

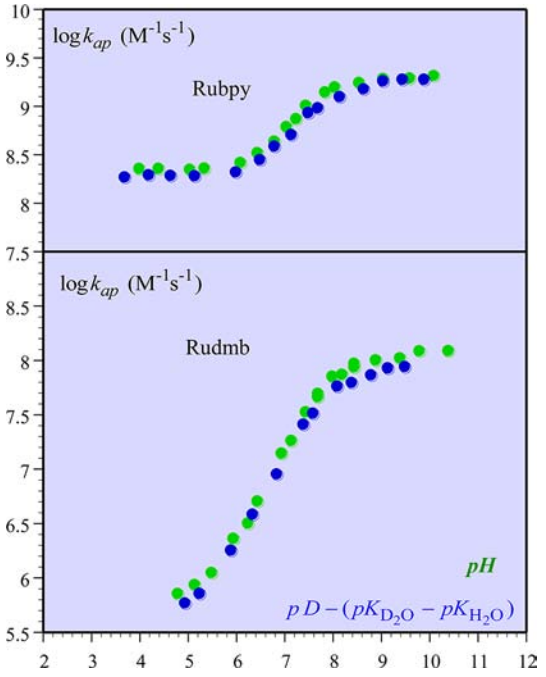

Figure 8. Apparent rate constant of oxidation of ($^+$H)NTrpH by flash quenched generated Ru^{3+} with $Ru(NH_3)_6^{3+}$ (20 mM) as quencher (same conditions as in Figure 6). Green dots: H_2O. Blue dots: D_2O.

In the application of eqs 4 and 5, leading to the solid lines in Figure 7, k_{dif} was taken as equal to 10^{10} M^{-1} s^{-1}, k_0 to 1.4×10^9 M^{-1} s^{-1} for pathways A and B1 and to 0.7×10^9 M^{-1} s^{-1} for pathway B2. The data for the oxidation of the acid form of the tryptophan derivative are consistent (left-hand Figure 7) with a rate-determining electron transfer in the framework of a simple EPT sequence as shown in Scheme 3. The data for the oxidation of the basic form of the tryptophan derivative are both consistent with pathways B1 and B2. It is indeed perfectly conceivable that the self-exchange rate constant for the intramolecular CPET B2 pathway is half that of the simple electron-transfer B1, due to kinetic penalties associated with the CPET reaction such as proton tunneling.[3]

In order to discriminate between pathways B1 and B2, we repeated the experiments in D_2O so as to detect a possible H/D kinetic isotope effect, taking into account that the pK of interest is 8 in D_2O instead of 7.5 in H_2O (see SI). The results are shown in Figure 8. After the variation of pK has been taken into account, it is seen that no H/D kinetic isotope effect is detected within experimental error. We may thus conclude that the oxidation of the basic form of the tryptophan derivative (H) follows an EPT pathway as in the case of the acid form.[28]

Oxidation of STrpH by the Two Ru^{III} Complexes Generated with MV^{2+} and $Ru(NH_3)_6^{3+}$ as Quenchers. The upper limit of the pH rang was restrain to 10 in order to avoid an observed irreversible transformation of the substrate, presumably a hydrolysis of the ester function. Within this pH range two quenchers could be used: MV^{2+} since, unlike the case of ($^+$H)NTrpH, no adduct is formed (see SI), and $Ru(NH_3)_6^{3+}$ shown previously to be stable within the pH range considered here.

With Rubpy (Figure 9), the rate constant does not vary with pH and appears to be diffusion limited, reaching the same value as with NTrpH at high pHs. With Rudmb (Figure 9), the rate constant is also pH independent. Its value (log k_{ap} (M^{-1} s^{-1}) = 7.5) is clearly below the diffusion limit and also lower than with NTrpH at high pHs.

In both cases, the reaction follows an EPT pathway with a rate-determining electron transfer. As compared to ($^+$H)-NTrpH, the lack of pH variation of the rate constant for STrpH is simply related to the absence of an acid−base moiety in the molecule. With Rudmb, the driving force of the rate-determining electron-transfer step is less with STrpH than with NTrpH as can be inferred from the cyclic voltammetry of these two compounds shown in Figure 10. The cyclic voltammetric traces are far from reversibility and thus unable to provide a precise estimate of the respective standard potentials.[29] They nevertheless indicate that STrpH is less oxidizable than NTrpH by ~50 mV. Knowing that a 60 mV shift of standard potential indicate a change of a factor of 10 in terms of equilibrium constant, the factor 3 found in the rate constant is perfectly compatible with the decrease in driving force expected when passing from NTrpH to STrpH. We may thus conclude that an EPT pathway is followed in this case too, with no trace of a H_2O-CPET pathway whose driving force would depend of pH. There is no reason that the same should not apply when the tryptophan is attached to the Ru complex structure as in reference 17d.

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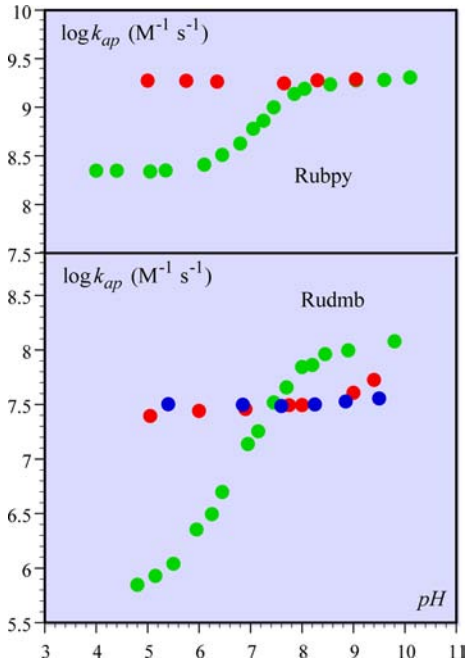

Figure 9. pH dependence of the apparent rate constant of oxidation of STrpH (1 mM) by flash quenched generated Ru^{3+} with the two Ru complexes. Blue dots: 40 mM MV^{2+} as quencher; red dots: 20 mM $Ru(NH_3)_6^{3+}$ as quencher; green dots: recall of (^+H)NTrpH oxidation with $Ru(NH_3)_6^{3+}$ (20 mM) as quencher (from Figure 3).

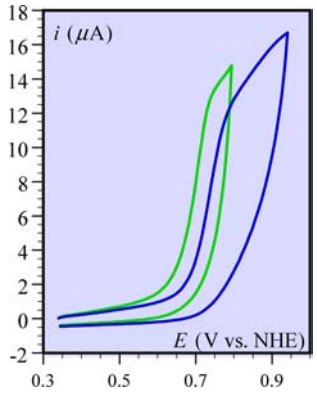

Figure 10. Cyclic voltammetry of 1 mM STrpH (blue) and NTrpH (green) in a pH 10, 0.1 M KNO_3 aqueous solution.

CONCLUDING REMARKS

Oxidation of the tryptophan derivative (^+H)NTrpH follows, under its acid as well as its basic form, a simple EPT pathway with the initial electron transfer as a rate-determining step. There is no trace of any unconventional H_2O-CPET pathway whose rate constant would vary with pH because its driving force would be pH dependent or for any other mysterious reason. The mistake in previous interpretations derived from an inaccurate estimation of the pK of the neighboring NH_3^+/NH_2 couple (9.3 instead of the actual value of 7.5).[30] The same is true for STrpH, whose behavior is even simpler since the molecule does not bear an acid−base group.

We may note that with easily accessible substrates, such as phenol and now tryptophan, there is no trace of any H_2O-CPET pathway endowed with a pH-dependent driving force, according to what is expected from the very basic principles of chemical physics. We may infer, as discussed in the Intermolecular and Intramolecular CPET Reactions section, that the same should hold for structures such as those shown in Figure 2 with an intramolecular electron transfer from a tyrosine or a tryptophan moiety.[16,17] In these cases, the careful search for artifacts that cause the misleading evidence of a H_2O-CPET pathway endowed with a pH-dependent driving force should be preferred to repeated calls for a nonconventional microscopic model.

Microscopic models based on correct basic principles have indeed been developed and validated on sound experimental data. They evidenced the remarkable properties of water as proton acceptor in CPET reactions both as concerns the structure of the water cluster involved and the association between a Grotthuss-like proton displacement and the CPET pathway.[15c] Much remains to be investigated in this area, having particularly in mind the role of water chains in natural systems. The findings of the present work rid the road of artificial obstacles, such as H_2O-CPET pathways endowed with a pH-dependent driving force.

ASSOCIATED CONTENT

Supporting Information

Chemicals, experimental procedures, derivation of equations, pK measurements, full sets of data, fittings and residues and data tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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