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Electrocatalytic Oxidation of Tyrosine by Parallel Rate-Limiting Proton Transfer and Multisite Electron−Proton Transfer

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Oxidation of the tyrosine phenol to its deprotonated, neutral radical is a critical step in numerous enzymatic processes.[1−5] In enzymes, loss of an electron is often postulated to occur with accompanying transfer of a proton to a neighboring base.[6] In photosystem II, for example, oxidation of Y_z is thought to occur with transfer of a proton to His190.[5,7] Knowledge of whether this proton-coupled electron transfer occurs via concerted electron−proton transfer (EPT) or sequential EPTs is of importance in understanding how these reactions occur.[2] EPT avoids the build-up of negative charge upon deprotonation of tyrosine or of positive charge upon oxidation of its protonated form. However, its microscopic demands are greater because of the requirement for moving the proton in the concerted reaction. A pathway involving initial deprotonation of the tyrosine would be controlled by the availability of a suitable base with less demand on the precise nature of the oxidant.

The oxidation of complex organic donors such as nucleobases[8] and carbon nanotubes[9] is conveniently studied via electrocatalysis utilizing metal complexes, such as $Ru(bpy)_3^{2+}$, at indium tin oxide (ITO) electrodes in neutral solution (bpy = 2,2′-bipyridine).[10] Adsorption of the phosphate buffer anion at these electrodes contributes to a high oxidative limit, rapid heterogeneous kinetics for $Ru(bpy)_3^{2+}$, negligible adsorption of the organic donor, and poor heterogeneous kinetics for direct oxidation of the organic donor.[11] Cyclic voltammograms of complexes such as $Ru(bpy)_3^{2+}$ in the presence of suitable electron donors therefore produce current enhancements that can be digitally simulated to obtain rates of electron transfer from the donor to electrogenerated $Ru(bpy)_3^{3+}$.[10] We report here application of this ITO−$Ru(bpy)_3^{2+}$ system to the electrocatalytic oxidation of tyrosine and tryptophan amino acids.[12] The electrochemical results provide new insights into the intimate details of the coupling of electron and proton transfer.

The addition of tyrosine and tryptophan to aqueous solutions of the $M(bpy)_3^{2+}$ complexes $Os(bpy)_3^{2+}$, $Fe(bpy)_3^{2+}$, $Ru(4,4'\text{-dimethyl-bpy})_3^{2+}$, $Ru(bpy)_2(4,4'\text{-dimethyl-bpy})^{2+}$, and $Ru(bpy)_3^{2+}$ produced catalytic voltammograms that were digitally simulated by assuming the mechanism in eqs 1 and 2 (see Supporting Information for fitting details). In eqs 1 and 2, X is tyrosine or tryptophan and X′ is the oxidized amino acid.

$$M(bpy)_3^{2+} \rightarrow M(bpy)_3^{3+} + e^- \quad (1)$$

$$M(bpy)_3^{3+} + X \rightarrow M(bpy)_3^{2+} + X' \quad (2)$$

For tryptophan, the dependence of k for eq 2 on the redox potential of the metal complex oxidant over the range 0.83 to 1.25 V (vs NHE) displayed a typical Marcus dependence with RT ln k increasing with $E^{o'}$ with a slope of 0.42 (Figure 1A), consistent with outer-sphere, one-electron oxidation (assuming $E_{1/2} \sim E^{o'}$).[13,14] By contrast, *oxidation of tyrosine at pH 7.5 proceeded with no detectable dependence on the redox potential of the oxidant,*

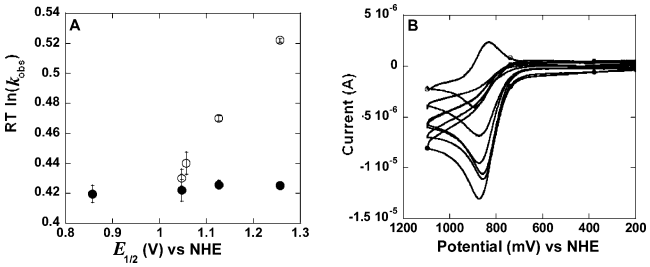

Figure 1. (A) Dependence of the overall oxidation rate constant (k_{obs}) for tyrosine (closed) and tryptophan (open) at room temperature in 0.8 M NaCl, obtained by simulation of cyclic voltammograms, on the reduction potential of the metal complex oxidant, see text. (B) Cyclic voltammograms of $Os(bpy)_3^{2+}$ (0.02 mM) in the presence of 0.1 mM tyrosine and increasing concentrations of phosphate buffer (10−50 mM) at pH = 7.5 at room temperature in 0.8 M NaCl.

suggesting that electron transfer was not involved in the rate-determining step. Further, increases in the phosphate buffer concentration increased the catalytic current (Figure 1B), with limiting currents observed at phosphate concentrations above ∼50 mM.

The reaction of tyrosine with $Os(bpy)_3^{2+}$ was investigated over a wide range of tyrosine and complex concentrations and buffer ratios. The results of this study revealed the rate law in eq 3 in which $[TyrOH]_T$ is the total concentration of tyrosine. This rate law is consistent with the mechanism in Scheme 1. In Scheme 1, oxidation of a hydrogen-bonded tyrosine intermediate occurs by parallel pathways, one involving EPT ($K'_A k_{red}$) and the other, initial deprotonation (k_1, k_2). A rate law of the same form was observed for histidine as the added base. Rate and equilibrium constants for the two bases are listed in Table 1.

$$-\frac{d[Os^{3+}]}{dt} = \left(\frac{K_A[TyrOH]_T[HPO_4^{2-}]}{1 + K_A[HPO_4^{2-}]}\right)[Os^{3+}]\left[K'_A k_{red} + \frac{k_1 k_2}{k_{-1}[H_2PO_4^-] + k_2[Os^{3+}]}\right] \quad (3)$$

A number of observations pertaining to reactivity were made consistent with the rate law in eq 3. (1) Saturation kinetics are observed at high HPO_4^{2-} concentrations, consistent with TyrOH−HPO_4^{2-} complex formation (See Supporting Information, Figure S1). (2) At high concentrations of added base, HPO_4^{2-} or histidine, and low concentrations of the conjugate acid, the k_1k_2 term dominates and the catalytic current is independent of metal complex concentration (Figure 2A). (3) At pH < 6.5 ($[HPO_4^{2-}/H_2PO_4^-]$ < 1/7), the $K'_A k_{red}$ term dominates and the dependence of the observed rate constant, k_{obs}, on $E^{o'}$ for the oxidant reappears with RT ln k_{obs} increasing with a slope of 0.25 (Figure 2B). Also, under these conditions, at high added tyrosine and base, saturation kinetics

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Table 1. Rate and Equilibrium Constants for Added Sodium Dibasic Phosphate and Histidine[a]

base	pK_a	K_A (M^{-1})	k_1 (s^{-1})	k_{-1} (M^{-1} s^{-1})	k_2 (M^{-1} s^{-1})	K_A' (M^{-1})	k_{red} (M^{-1} s^{-1})
Na_2HPO_4	7.2	30.0 ± 0.1	$3.3 \pm 0.1 \times 10^5$	$5.0 \pm 0.4 \times 10^3$	$1.7 \pm 0.3 \times 10^7$	22.2 ± 0.1	$2.3 \pm 0.5 \times 10^4$
histidine	6.6	26.3 ± 0.3	$1.4 \pm 0.1 \times 10^5$	$1.9 \pm 0.1 \times 10^3$	$1.4 \pm 0.1 \times 10^7$	37.8 ± 0.2	$6.9 \pm 0.3 \times 10^4$

[a] According to Scheme 1, at room temperature in 0.8 M NaCl.

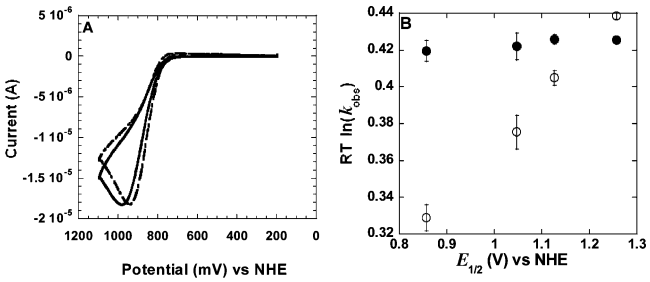
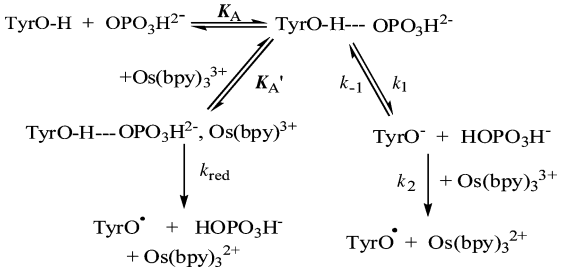


Figure 2. (A) Cyclic voltammograms of 20 μM (solid) and 40 μM Os(bpy)$_3^{2+}$ in the presence of 0.1 mM tyrosine in 50 mM phosphate buffer (pH = 8.0, [HPO_4^{2-}]/[$H_2PO_4^-$] = 15/1) at room temperatue in 0.8 M NaCl. (B) Dependence of k_{obs} on the reduction potential of the oxidant at pH 7.5 (closed) and pH 6.0 (open) for the series Os(bpy)$_3^{2+}$, Fe(bpy)$_3^{2+}$, Ru(4,4′-dimethyl-bpy)$_3^{2+}$, Ru(bpy)$_2$(4,4′-dimethyl-bpy)$^{2+}$, and Ru(bpy)$_3^{2+}$

Scheme 1



are observed in both, allowing for independent evaluation of k_{red} and K_A'. (4) When the reaction with added HPO_4^{2-} was performed in D_2O, H_2O/D_2O isotope effects of 1.7 ± 0.1 and 1.0 ± 0.1 for K_A and K_A' were observed and 2.1 ± 0.6 and 1.2 ± 0.4 for k_{red} and k_1 (Figures S3 and S4). (5) Bulk electrolysis in $HPO_4^{2-}/H_2PO_4^-$ occurs with $n = 1$, consistent with radical coupling following one-electron oxidation.

Direct oxidation of tyrosine with added phosphate at the electrode in the absence of catalyst is undetectable over the entire pH range studied. The catalytic effect of added base is considerable. Oxidation of TyrOH by Os(bpy)$_3^{2+}$, followed by spectrophotometric monitoring in 0.8 M NaCl at room temperature (pH = 7) occurs with $k \sim 1.7 \times 10^2$ M^{-1} s^{-1}, which is slower by $\sim 10^2$ than $k_{red}K_A'$ in Scheme 1 with added HPO_4^{2-} or histidine at neutral pH.

These results demonstrate that catalyzed oxidation of tyrosine in water occurs at a significant rate following association with histidine or the base form of a $H_2PO_4^-/HPO_4^{2-}$ buffer, presumably forming H-bonded association complexes such as ArOH−OP(OH)-O_2^{2-}. Once formed, the association complexes can react either via concerted loss of electrons and protons (EPT), k_{red}, or via rate-limiting proton transfer followed by electron transfer (PT-ET) oxidation of the phenoxide anion, Scheme 1.

In the EPT pathway, electron and proton transfers occur to separate acceptors, (Os(bpy)$_3^{3+}$ and HPO_4^{2-} in Scheme 1), and the reaction can be described as occurring by multisite electron−proton transfer.[5] It is important as a possible model for the tyrosine−histidine pair in photosystem II.[5,7] There is precedence for the appearance of this pathway in earlier observations on oxidation of phenols by the triplet excited state of C_{60} in the presence of added N-bases.[15]

Recent results on the oxidation of hydrogen-bonded phenols in acetonitrile also support a concerted reaction,[16] as do voltammetric results on hydrogen-bonded phenols in nonpolar solvent.[17] By contrast, in closely related studies, intramolecular oxidation of a phenol linked to a Ru(bpy)$_3^{2+}$ derivative has been found to respond to changes in the external pH and not to added buffer.[18]

The observation of competing pathways suggests that enzymes may have the ability to tune kinetic pathways based on solvent accessibility of the oxidized tyrosine.[12] Such tunability may be critical in regulating enzyme kinetics and in allowing enzyme mechanisms to respond to proton gradients.

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Supporting Information Available: Parameters, mechanisms, and methods of digital simulation as well as rate constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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