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Effect of Electron Availability on Selectivity of O_2 Reduction by Synthetic Monometallic Fe Porphyrins

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Herein we report that biomimetic analogues of cytochrome *c* oxidase (CcO) couple reduction of O_2 to oxidation of a single-electron carrier, $Ru(NH_3)_6^{2+}$, under steady-state catalytic turnover. Higher Ru^{II} concentrations favor the 4-electron vs 2-electron O_2 reduction pathway. Our data indicate that the capacity of electrode-adsorbed Fe-only porphyrins to catalyze reduction of O_2 to H_2O is due to high availability of electrons and is eliminated under the biologically relevant slow electron delivery.

Dioxygen is consumed in aerobic respiration through catalytic reduction to water at the heterometallic heme/Cu site of CcO (Figure 1).[1,2] One of the more fascinating aspects of O_2 reduction by CcO is its ability to couple slow one-at-a-time arrival of external electrons from ferrocytochrome *c* to rapid 4-electron reduction of O_2. Such reactivity is made possible in part by redox cofactors within CcO that serve as electron "preloading" sites. In contrast, *monometallic* Fe-only porphyrins, when adsorbed on a graphite electrode, efficiently catalyze the 4e reduction of O_2 to H_2O under physiologically relevant conditions of pH (7) and electrochemical potential (>50 mV vs NHE).[3,4] These observations led some workers in the field to speculate that Cu_B in CcO is not necessary for *O_2 reduction*.[5] This hypothesis, however, contradicts results with Cu_B-less mutants of heme/Cu terminal oxidases[6] as well as biomimetic studies under physiologically relevant turnover-determining electron flux.[7]

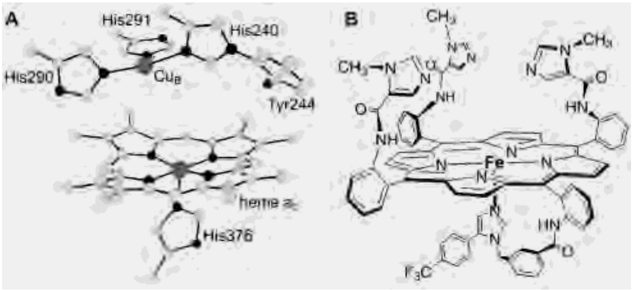

Figure 1. (A) The catalytic site of CcO.[8] (B) Fe-only catalyst **1**[10] studied in the present work.

To probe further the role of electron availability in determining the catalytic activity and selectivity of monometallic Fe-only porphyrins toward electrochemical O_2 reduction, we have studied the aerobic oxidation of $Ru(NH_3)_6^{2+}$ catalyzed by **1** (Figure 1) immobilized in a Nafion matrix at the electrode surface. When adsorbed directly on the electrode surface, **1** is a highly active and selective catalyst for reduction of O_2 to H_2O.[4] In this regime, heterogeneous electron transfer from the electrode to **1** is rapid. In contrast, direct electron transfer from the electrode to **1** dispersed in a Nafion film is significantly retarded. Unlike our previous setup involving a lipid film,[7] mobility of **1** in Nafion is extremely low.[9] Therefore, catalytic and electron transport roles must be carried out by separate species, thereby allowing a control over the availability of electrons by regulating the concentration of the electron carrier (such as $Ru(NH_3)_6^{2+}$, which is highly mobile in Nafion) in the **1**/Nafion film.

Previously, Anson, Saveant, and co-workers studied the electroreduction of O_2 by Co(tetraphenylporphyrin) immobilized in Nafion with $Ru(NH_3)_6^{2+}$ as the electron mediator.[9] Because Co(tpp) is not capable of reducing O_2 beyond H_2O_2, the redox stoichiometry (n_{av}, the average number of electrons exchanged per one O_2 molecule reduced) of their catalytic film is independent of $Ru(NH_3)_6^{2+}$ concentration. The **1**/Nafion system is more complex and more relevant to biological O_2 reduction, because of the 4e O_2 reduction activity of electrode-adsorbed **1**, the high O_2 affinity of **1**, and the relatively modest driving force for

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reduction of ferric **1** by $Ru(NH_3)_6^{2+}$ ($\Delta E < 100$ mV vs ~500 mV for the Co(tpp)/$Ru(NH_3)_6^{2+}$ couple in the Anson/Saveant system).[4,9]

The electrochemical behavior of **1** in Nafion under anaerobic conditions was studied by cyclic voltammetry (CV).[11] The electrode was covered with 5 μL of a solution of **1**/Nafion (0.1 mM/9 mM) in methanol.[12a] In the resultant film ~90% of catalytic centers are isolated from the electrode surface and are electrochemically inactive.[12b] Upon addition of $Ru(NH_3)_6^{3+}$ to the aqueous electrolyte, $Ru(NH_3)_6^{3+}$ enters Nafion by ion exchange sorption. The concentration of $Ru(NH_3)_6^{3+}$ in the film increases with time until it reaches a maximum "saturated" value (after ~10 min);[12a] all catalytic experiments were performed with such $Ru(NH_3)_6^{3+}$-saturated films.

The CV of a $Ru(NH_3)_6^{3+}$-saturated Nafion film without **1** is characteristic of thin-layer voltammetry, TLV:[11] symmetric peaks of ~95 mV width at the half wave and scan-rate-independent ~15 mV peak separation. This behavior indicates (1) Nernstian (reversible) behavior of the $Ru(NH_3)_6^{3+/2+}$ couple in the film, (2) that redox equilibration within Nafion is substantially faster than equilibration between Nafion-confined $Ru(NH_3)_6^{n+}$ and $Ru(NH_3)_6^{n+}$ in aqueous electrolyte, and (3) that reduction (oxidation) of $Ru(NH_3)_6^{n+}$ is not accompanied by significant morphological changes within the $Ru(NH_3)_6^{n+}$-saturated Nafion film. Slight deviation from the expected in TLV 90 mV width and zero peak separation is often observed for surface films, being ascribed to interactions between electroactive centers.[11,13,14]

Exposure of **1**/Nafion films to $Ru(NH_3)_6^{3+}$-containing electrolytes under N_2 results in larger areas under current−potential waves relative to those observed for pure Nafion films in contact with electrolyte of the same $Ru(NH_3)_6^{3+}$ concentration.[12] These waves retain their TLV characteristics even when 50% of the Coulombic response is due to **1**. This indicates rapid redox equilibration within the film, i.e., $Ru(NH_3)_6^{n+}$ is an efficient electron mediator between **1** and the electrode.

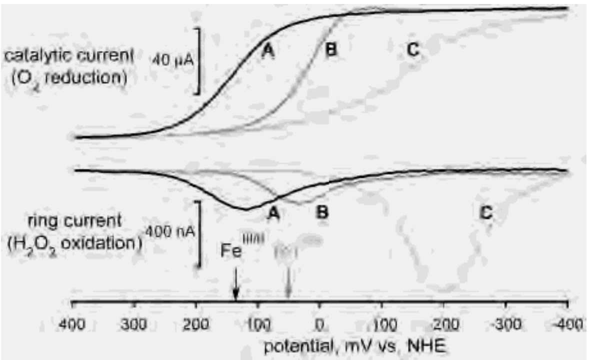

Figure 2. Rotating-ring disk voltammograms of O_2 reduction by (A) electrode-adsorbed **1**; (B) Nafion/**1** film in contact with 5 μM $Ru(NH_3)_6^{3+}$ solution; and (C) Nafion/**1** film without an electron carrier. When **1** is absent from the Nafion film, no catalytic currents are observed at potentials > -400 mV within the concentration range of $Ru(NH_3)_6^{3+}$ used in our experiments; at potentials < -400 mV reduction of O_2 on graphite is observed in the absence of **1**. The redox potentials of **1** and $Ru(NH_3)_6^{n+}$ under N_2 are indicated by arrows for comparison.

Catalytic reduction of O_2 was studied by rotating-ring disk voltammetry (RRDV)[11] with the **1**/Nafion film deposited at the edge-plane graphite disk and exposed to an air-saturated pH 7 buffered aqueous electrolyte. The Pt ring electrode was set at a sufficiently oxidizing potential so that any H_2O_2 released by the catalytic film would be oxidized and generate a ring current. In the absence of $Ru(NH_3)_6^{n+}$, very low catalytic activity is observed at $E > 0$ V (Figure 2, voltammogram C) and is ascribed to a fraction of **1** ($\leq$10%) in direct contact with the electrode.[12c] The catalytic currents increase significantly at $E < 0$ V. The O_2 reduction is accompanied by substantial release of H_2O_2 (minimum $n_{av} \sim 3$). It seems plausible that at sufficiently reducing potentials a fraction of **1** not in a direct contact with the electrode becomes electroactive, either due to through-space electron transfer or due to conformational changes in the Nafion matrix that bring catalytic sites closer to the electrode surface.[12b,c] An increase in redox stoichiometry of O_2 reduction at very reducing potentials is observed for many materials, such as graphite, Au, and Hg as well as simple Fe porphyrins.[3,4] It seems plausible that at $E < -0.2$ V the higher redox stoichiometry of O_2 reduction is due to the "two-step" mechanism, whereby free H_2O_2 released by one molecule of **1** is reduced (or disproportionated) by other molecules of **1** as they become electroactive $E < -0.2$ V.[3,4]

Upon introduction of $Ru(NH_3)_6^{n+}$ into the film catalytic currents at $E < 150$ mV increase (Ru$^{II/III}$ potential ~50 mV). The magnitude both of the current and of the catalytic selectivity[12d] correlates with the amount of $Ru(NH_3)_6^{n+}$ in the film until the $Ru(NH_3)_6^{n+}$/**1** ratio in Nafion is ~3, whereupon a further increase in the $Ru(NH_3)_6^{n+}$ content of the **1**/Nafion film does not affect the catalytic currents or selectivity. The resulting "limiting" catalytic curves (Figure 2, voltammogram B) have the same shape as those generated by electrode-adsorbed **1** (Figure 2, voltammogram A) but are displaced by ~100 mV toward more reducing potentials, as expected from ~100 mV more negative anaerobic Ru$^{II/III}$ potential compared with Fe$^{II/III}$ (Figure 2). The redox stoichiometry of O_2 reduction by **1**/Nafion with at least a 3-fold molar excess of $Ru(NH_3)_6^{n+}$ in the film is comparable to that of electrode-adsorbed **1** ($n_{av} \sim 4e/O_2$ molecule). Importantly, the "limiting" film composition of ~75% mol of $Ru(NH_3)_6^{n+}$ and ~25% mol of **1** has the same ratio of

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(12) (a) See Supporting Information for further details. (b) Electron hopping, which is responsible for fast charge propagation through films of pure **1** at the electrode surface,[4,13] is not likely to operate in the **1**/Nafion films utilized in the present work, due to high dilution of **1** by Nafion (<1% mol **1**). (c) A referee suggested that in the absence of the Ru mediator n_{av} increases at very reducing potentials because traces of metal impurities become increasingly important either in mediating electron transport between the electrode and **1** or in direct reduction of H_2O_2, or both as the fraction of such impurities in reduced states increases. (d) $Ru(NH_3)_6^{n+}$ does not appear to promote H_2O_2 disproportionation,[9] so that the decrease in the ring current in the presence of $Ru(NH_3)_6^{n+}$ most likely indicates an increase in the redox stoichiometry of O_2 reduction by **1**.

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electron storage sites to the heme as does CcO (which stores 3 electrons in Cu_A, heme a, and Cu_B per one O_2-reducing heme a_3 site).

The slope of the potential-dependent part of the catalytic waves also increases with $Ru(NH_3)_6{}^{n+}$ content of the **1**/Nafion films, which reflects changes both in the amount of electroactive **1** and in the ET mechanism. Without the mediator, reduction of **1** in Nafion is irreversible, whereas the contribution of the reversible $Ru(NH_3)_6{}^{2+}$/ferric-**1** couple to current consumption increases at larger Ru loadings.[12]

Koutecky−Levich (KL) analysis[11,14] of catalytic currents at various $Ru(NH_3)_6{}^{n+}$ content of the **1**/Nafion films gives the limiting (potential-independent) apparent catalytic second-order rate constant, k_{app}, of $(2 \pm 1) \times 10^6$ M^{-1} s^{-1}, which is larger than the $(5 \pm 1) \times 10^5$ M^{-1} s^{-1} value determined for electrode-adsorbed **1**.[4,15] The plausible origin of the faster catalysis includes lower water content around catalytic sites isolated in Nafion. The unfavorable partitioning between 6-coordinate aqua-ligated **1** and catalytically active five-coordinate ferrous-**1** decreases k_{app} for electrode-adsorbed **1**.[4] The limiting catalytic currents (e.g., those measured at the plateaus of the catalytic waves) follow the KL dependence at all examined $Ru(NH_3)_6{}^{n+}$ concentrations. However, KL plots of catalytic currents at the potential-dependent portions of the catalytic waves display deviations from linearity at intermediate $Ru(NH_3)_6{}^{n+}$ concentrations, indicating a system under a partial ET control.[14]

In summary, our results suggest that the catalytic behavior of even elaborate Fe-only porphyrins, which are highly selective 4e catalysts when adsorbed on graphite, is noticeably worse under physiologically relevant slow electron flux. This is consistent with our prior results in lipid membranes,[7] where the functions of electron transport and catalysis were performed by the same catalyst. In conjunction with these results the current data support our conjecture that Cu_B is essential in O_2 reduction by CcO. As an electron “preloading” site, it eliminates the delay in the flow of electrons to bound O_2, which would otherwise occur due to the slow, diffusional, reduction of CcO by ferrocytochrome *c*. Unlike lipids, however, the Nafion matrix is unsuitable for studies of bimetallic FeCu complexes because of the rapid loss of Cu^{n+} in the highly ionic environment of Nafion.

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Supporting Information Available: Experimental procedures and further electrochemical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The units of the apparent rate constants are determined by the following rate law: $dO_2/dt = k_{app}\Gamma_t[O_2]_\infty$, where dO_2/dt is the rate of O_2 consumption per unit area of the electrode (mol/(s × cm^2)), Γ_t is the apparent total surface coverage of the electrode by **1** (obtained by dividing the total amount of catalyst by geometric surface area, mol/cm^2), and $[O_2]_\infty$ is the O_2 concentration in bulk electrolyte (mol/cm^3). Although in **1**/Nafion the catalyst is distributed throughout the Nafion matrix, its volume concentration in Nafion is unknown, as it depends on the volume of the film, whereas the corresponding apparent Γ_t is readily available.