Close Structural Analogues of the Cytochrome *c* Oxidase Fe$_{a3}$/Cu$_B$ Center Show Clean 4e$^-$ Electroreduction of O$_2$ to H$_2$O at Physiological pH

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The heteronuclear heme a_3/Cu_B metal center of Cytochrome *c* Oxidase (C*c*O) is a biologically unique structural motif in which a myoglobin-type iron center combines with a tris-histidine copper moiety to form an efficient catalyst for the reduction of O$_2$ to H$_2$O.[1] This 4e$^-$, 4H$^+$ reduction is assisted by homo-dinuclear Cu$_A$ and mononuclear heme *a* metal centers which facilitate fast electron transfer from cytochrome *c* into the heme a_3/Cu_B active site. The reduction proceeds without leakage of toxic peroxide or superoxide intermediates and is coupled to a proton pumping mechanism that generates a proton gradient across the inner mitochondrial membrane.[2] The structural features of the Fe$_{a3}$/Cu$_B$ center are now known in detail thanks to two atomic resolution X-ray structures obtained for bacterial[3] and mammalian[4] cytochrome *c* oxidases.

Considerable progress has been made in unraveling the mechanism of O$_2$ reduction to H$_2$O at the binuclear Fe$_{a3}$/Cu$_B$ site of C*c*O.[1] Nonetheless, many issues remain controversial: the role of Cu$_B$ and Tyr residue in O$_2$ binding and reduction, and the nature of the peroxo-level intermediate have been contentious topics in many previous[1] and recent studies.[5] The construction of *functional* biomimetic model compounds that closely resemble the native enzyme active sites is important to the elucidation of the catalytic mechanism. This approach also allows for the study of structure−catalytic activity relationships, which in turn may lead to the design of efficient catalysts for the 4e$^-$ reduction of O$_2$ to H$_2$O.

Several structural analogues of the oxidized Fe$_{a3}$/Cu$_B$ form of C*c*O have been prepared by a synthetic self-assembly approach.[6] Although this strategy proved to be useful for structural and spectroscopic characterization of various Fe^{III}(X)Cu^{II} (X = CN$^-$, O^{2-}, OH$^-$, F$^-$, HCO$_2^-$) assemblies, their use in mechanism studies as electrocatalysts is limited due to the facile dissociation of the Fe/Cu pair. To obviate this problem, construction of covalently linked structural models is desirable. Several structural models with covalently attached "copper binding" sites have appeared recently in the literature,[7] but these still lack a covalently attached axial ligand.[8] In our first generation of the fully covalently linked structural models of the Fe$_{a3}$/Cu$_B$ active site of C*c*O, the Cu

Scheme 1
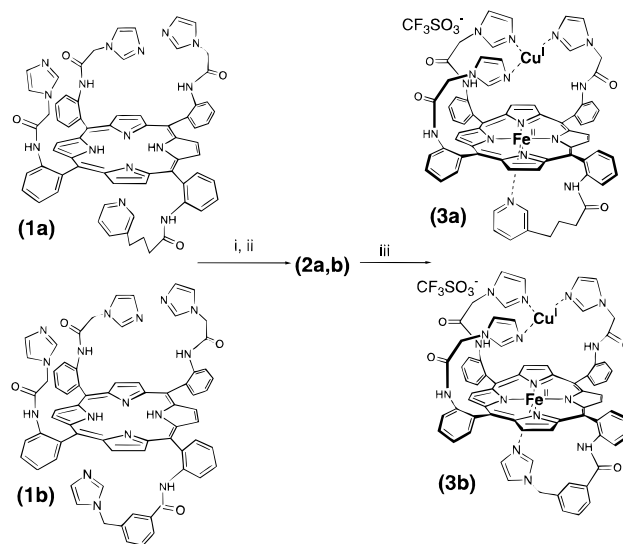
(i) FeBr$_2$ (THF, 24h at RT); (ii) CH$_2$Cl$_2$/H$_2$O+EDTA extraction; (iii) Cu(CF$_3$SO$_3$)

coordination has been provided by the triazacyclononane (TACN) or *N,N′,N″*-tribenzyl-tris(aminomethyl)amine (TBTren) ligands.[9] Most of these model compounds show predominantly 4e$^-$, 4H$^+$ electroreduction of O$_2$ at physiological pH with minor leakage of H$_2$O$_2$.

In our continuing effort to construct closer structural analogues of the Fe$_{a3}$/Cu$_B$ active site of C*c*O, we have focused on the design and synthesis of systems where the Cu coordination site is provided by three imidazole ligands. Herein we describe the synthesis, characterization, and electrocatalytic activity of two such synthetic models. We are delighted to report that these new biomimetic model compounds show clean (i.e. no leakage of peroxide) electroreduction of O$_2$ to H$_2$O over a wide pH range.

Two closely related ligands **1a** and **1b** used in this work are depicted in Scheme 1. Both contain three distal imidazole ligands tethered to the porphyrin core via acetamide linkages, but they differ in the covalently attached axial base.[10] Our molecular-modeling studies suggest that the acetamide linkages provide enough flexibility to the distal imidazole ligands, and they are short enough to keep Cu···Fe separation within 4.5−5.5 Å range, but not to coordinate to the iron atom.

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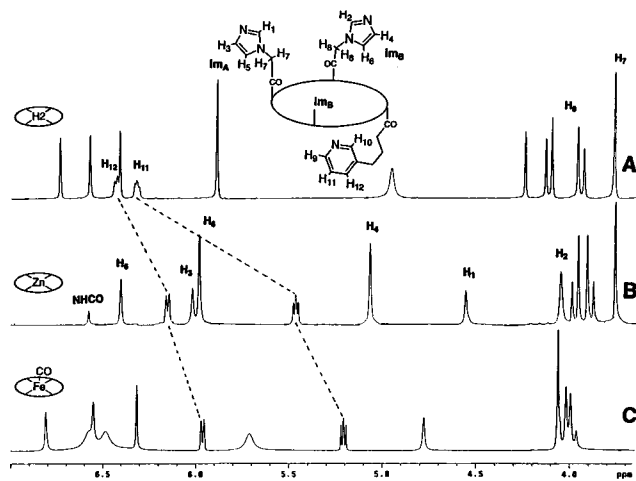
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(8) The use of a covalently attached axial ligand ensures the structural integrity of the complex during the catalytic cycle and eliminates the possible interference of excess external base with copper atom. This advantage is most important for electrocatalytic studies−all our attempts to achieve efficient electroreduction of O$_2$ to H$_2$O with complexes having an external axial base have been unsuccsesfull.[9a,c]

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Figure 1. ^{1}H NMR spectra (CDCl$_3$-*d*) for the 7.0−3.5 ppm spectral window for (A) free base **1a**, (B) Zn(II) porphyrinato complex of **1a**, and (C) Fe^{II}(CO) porphyrinato complex **2a**. The dashed lines indicate an upfield shift of the pyridine ligand resonances upon coordination to the metal ion.

In addition to standard characterization of the iron(II) porphyrin complexes (**2a,b**) by UV−vis spectroscopy and mass spectrometry,[11a] their identity and purity have been confirmed by ^{1}H NMR spectroscopy of the diamagnetic CO-bound complexes. A comparison of the solution ^{1}H NMR of the free base **1a**, its zinc derivative,[11b] and the CO-bound Fe(II) complex is shown in Figure 1 for the most definitive part of the spectrum. In this window we can see clearly all resonances from the imidazole "picket" groups as well as resonances from the axial ligand. In both the zinc and iron(II) CO complexes, we observe the expected upfield shift of the coordinated axial ligand resonances (dotted lines). The most dramatic shift is observed for the pyridine *ortho* hydrogens (H$_9$ and H$_{10}$, resonances not shown in Figure 1) which for the Zn complex move from 7.68 and 7.95 ppm upfield to 2.05 and 2.60 ppm, respectively. In the Fe(CO) complex these resonances are found at even higher field (1.36 and 1.53 ppm), which is consistent with stronger binding of this ligand to iron. The final Fe(II)/Cu-(I) complexes **3a,b** are prepared by the reaction of **2a** (or **2b**) with Cu(I) triflate.[11c]

The electrocatalytic activities of complexes **3a** and **3b** have been studied by rotating ring-disk electrochemistry (RRD) as previously described.[8a,12] The results for both compounds at pH 7 are illustrated in Figure 2 (Supporting Information), which also shows the catalytic wave of a 2e$^-$ electrocatalyst for comparison. Both Fe/Cu compounds (**3a**, **3b**) show 4e$^-$ reduction of O$_2$ to H$_2$O with no detectable peroxide leakage under these conditions. The catalytic wave for the imidazole-tailed compound **3b** is found at a more positive potential than that for the pyridine-tailed compound **3a** as well as our previous TACN-based model compounds.[9c,d]

The onset of the electrocatalytic reduction of O$_2$ correlates with the middle position of the voltammetric responses of the adsorbed electrocatalysts **3a** and **3b** in the absence of oxygen (Figure 2). The position of the surface wave (under N$_2$) becomes more negative with increasing pH; the catalytic wave follows this shift.[13] Over the 3.5−8.5 pH range there is no significant decrease in catalytic currents for either electrocatalyst **3a** or **3b**. These new Fe/Cu complexes also show electrocatalytic reduction of H$_2$O$_2$ at potentials ∼50 mV more positive than that of O$_2$. It appears

that the onset of the electroreduction of O$_2$ is determined by the "regeneration" of the electrocatalyst **3a** or **3b**. Addition of chloride, bromide, or carboxylate anions to the pH 7 buffer solution has no effect on O$_2$ reduction. This lack of response to these anions suggests that it is the water-derived ligands (e.g. aquo-hydroxo or hydroxo ligands) which determine the redox potential of the Fe/Cu pair during the catalytic cycle.[14] Addition of cyanide (∼1 mM) to the pH 7 buffer solution results in complete inhibition of the electrocatalytic activity at $E > -0.4$ (vs SCE) for both Fe/Cu complexes. On the other hand, the presence of CO does not significantly affect the electrocatalytic reduction of O$_2$ by these compounds—behavior very similar to that of native C*c*O.[15]

As with our previous TACN-based model compounds, the porphyrinato iron(II) complexes **2a,b** react reversibly with oxygen in aprotic solvents, forming myoglobin-type complexes. Introduction of copper(I) to the "distal" site results in irreversible oxygen binding due to the formation of an apparent peroxo-Fe^{III}(O$_2$$^{2-}$)-Cu^{II} intermediate. The lifetime of this "peroxy" species is very short for compounds **3a** and **3b**. This may seem to contradict the argument about the large free energy of the Fe^{III}(O$_2$)$^{2-}$-Cu^{II} formation,[14] but the stability of this intermediate toward peroxide leakage does not necessarily correspond to the stability of the oxygen−oxygen bond. On the contrary, the increased stability of the iron(III)−oxygen and copper(II)−oxygen bonds could result in a weaker oxygen−oxygen bond, which is exactly what promotes clean and efficient 4-electron electrocatalytic reduction of O$_2$ to water. Studies are ongoing into the nature of these intermediates during electrocatalysis on a graphite electrode and in solution.

In conclusion, this is the first example of the synthesis and characterization of fully covalently linked model compounds of the binuclear Fe/Cu active site of C*c*O that have tris-imidazole coordination of the copper ion. We have demonstrated that the model compound **3b** which most closely resembles the geometry of this center also shows clean 4e$^-$ reduction of oxygen to water at the most positive potential of any Fe/Cu model compounds studied to date. The structural and catalytic relevance of these new model compounds to the Fe/Cu active site of C*c*O makes these and similar future studies a key part in a broad effort to elucidate the mechanism and underlying thermodynamic and kinetic principles of the reduction of oxygen to water by the C*c*O family of enzymes.

Supporting Information Available: References 11a−c containing experimental details and Figure 2 giving electrocatalytic activities for **3a** and **3b** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

(13) The plot of $E_{1/2}$ vs pH shows a linear relationship in the studied pH range with a slope of ∼30 mV. This is consistent with a 2e$^-$, 1H$^+$ redox process as a potential-limiting reaction. Due to the broadness of the surface wave, it is not possible to determine with certainty which redox center is reduced first under these conditions. Our voltammetric studies with Fe-only and Zn/Cu forms indicate that the two redox couples are separated by less than 30 mV with the Fe^{III}/Fe^{II} redox couple being more positive.

(14) It is important to note that the electroreduction of oxygen occurs at a more positive potential than the $E°$ of the O$_2$/H$_2$O$_2$ redox pair at pH 7. As proposed previously,[9c] this situation is possible only if the free energy released upon the formation of Fe^{III}(O$_2$)Cu^{II} peroxy intermediate is greater (at pH 7) than that for the formation of H$_2$O$_2$. This makes the release of peroxide unfavorable, resulting in a clean 4e$^-$ reduction of O$_2$ to H$_2$O.

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