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COMMUNICATIONS

Published on Web 04/13/2007

Valence Tautomerism and Coordinative Lability in Copper(II)−Imidazolyl−Semiquinonate Anion Radical Models for the Cu_B Center in Cytochrome *c* Oxidases

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We report that internal electron transfer (valence tautomerism) within a Cu^{II}−imidazolyl−phenolate anion system can lead to a substitutionally labile Cu^{I}−imidazolyl−phenoxyl radical system. These results inform about the potential reactivity of a Cu_B^{II}−histidine−tyrosinate intermediate in the catalytic center of cytochrome *c* oxidase (C*c*O).

In the final steps of the aerobic respiratory transfer chain, dioxygen is reduced to water within the heme a_3···Cu_B catalytic center (Figure 1) of C*c*O.[1] Free energy is conserved by coupled translocation of protons through C*c*O and across an inner mitochondrial or bacterial membrane, thereby maintaining the proton electrochemical gradient that drives ATP synthesis. The scission of dioxygen occurs in the very first step of the C*c*O catalytic cycle and requires four electrons donated under turnover conditions by Fe_{a3}^{II}, Cu_B^{I}, and Tyr244, affording the so-called P_M intermediate comprising ferryl Fe_{a3}^{IV}═O, cupric Cu_B^{II}−OH, and, more controversially, tyrosyl radical TyrO• centers.[2,3] Microsecond freeze hyperquenching experiments suggest the P_M state forms upon dioxygen scission regardless of the initial oxidation level of the enzyme.[4] In the next (and the first proton-pumping) step of the catalytic cycle, the tyrosyl radical is the electron acceptor, leading to the possibly obligatory intermediacy of a tyrosinate Cu_B^{II}−His−$TyrO^-$ species.

Mimics for Cu_B^{II}−His−TyrO• and Cu_B^{II}−His−$TyrO^-$ centers would provide valuable physicochemical information for comparison with biophysical data.[3,6,7] One major problem with the models thus far investigated is that the imidazolyl−phenoxyl radicals derived from them are only transiently stable.[3,6,7] In contrast, semiquinone anions (i.e., phenoxyl−phenoxide radicals), which may be generated by one-electron reduction of quinones or, conversely, by one-electron oxidation of hydroquinone dianions, are stable, long-lived species under anaerobic, basic conditions.[8] Hence, to avoid the limitations due to decomposition reactions of imidazolyl−phenoxyl radicals, we have targeted copper complexes of the semiquinone radical, *Im*−*sq**$^{-}$. In this work, the hydroquinone/semiquinone group acts as a surrogate for the phenol/phenoxyl radical side chain of the cross-linked tyrosine in C*c*O.

Akin to an ordinary hydroquinone, *Im*−*hq**H_2 readily undergoes two-electron oxidation to the corresponding quinone *Im*−*q** (e.g., with phenylidosodiacetate, Ag_2O, or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone). One-electron oxidation of the hydroquinone dianion, *Im*−*hq**$^{2-}$ (from *Im*−*hq**H_2 and lithium diisopropylamide (LDA, 2.0 equiv) or KOBu^t (2.0 equiv)), and one-electron reduction of *Im*−*q** are chemically reversible processes and afford the semiquinone radical anion, *Im*−*sq**$^{-}$.[9] The distinctive UV−vis and EPR spectra[10] of *Im*−*sq**$^{-}$ epitomize those of a semiquinone or phenoxyl radical;[11] in the EPR spectrum, the *N*-superhyperfine coupling is notably small (∼0.5 G), indicative for only slight inter-ring spin delocalization and in line with theoretical predictions for the histidine−tyrosyl radical in C*c*O.[3,5]

The Cu^{II}−imidazolyl−hydroquinone complex [(tpa)Cu(*Im*−*hq**H_2)](OTs)$_2$ (**1**; tpa = tris(2-pyridylmethyl)amine, OTs^- = *p*-toluenesulfonate anion) was prepared by addition of *Im*−*hq**H_2 to [(tpa)Cu(MeCN)](OTs)$_2$ in anhydrous methanol/ether.[9] Separate deprotonations of **1** were carried out under anaerobic, anhydrous conditions in two solvents, acetonitrile or tetrahydrofuran, and using two different bases, KOBu^t or LDA. Addition of 2 equiv of base results in bleaching of the *d*−*d* bands for **1** (λ_{max} 865 nm) and the appearance of the intense, characteristic bands of *Im*−*sq**$^{-}$ at 320 and 435 nm (Figure 2 and Supporting Information). In the EPR spectrum, the axial signal of **1** in MeCN is replaced by a sharp signal at $g = 2.0051$ for an organic radical. At room temperature, the EPR spectrum is identical to that for free *Im*−*sq**$^{-}$ radical (see above). The estimated conversion to the free *Im*−*sq**$^{-}$ radical anion in these experiments is 70−80%.[9] The ^{1}H NMR spectrum after double deprotonation of **1** shows sharp peaks for the Cu^{I} complex, $[Cu(tpa)(L)]^+$ (L = solvent, $HNPr^i_2$ from LDA or HOBu^t from KOBu^t),[12] the residual protio-solvent, the base, and no others.[9] Entirely analogous behavior is observed in THF solution.[9] The copper-containing species in acetonitrile solution were also characterized by ESI-FT-ICR mass spectroscopy: before and after adding KOBu^t (2.0 equiv) to **1**, the prominent ions were [(tpa)Cu(*Im*−*hq**H_2)]$^{2+}$ (*m*/*z* 306.61752 (major isotopomer); calcd *m*/*z* 306.61761) and $[(tpa)Cu]^+$ (*m*/*z* 353.08192 (major isotopomer); calcd *m*/*z* 353.08094), respectively.

In toto, these results provide unequivocal evidence for formation of the *free* *Im*−*sq**$^{-}$ radical and $[Cu(tpa)(L)]^+$ upon double deprotonation of **1**. The probable mechanism involves an internal electron transfer (valence tautomerism) producing a coordinatively

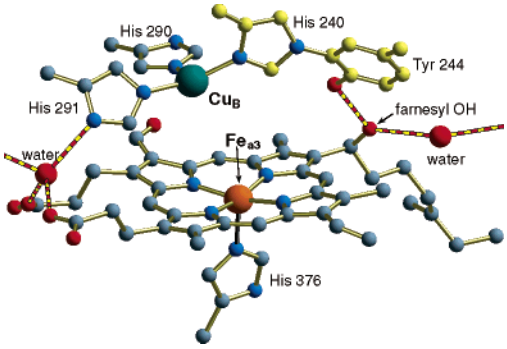


Figure 1. View of the binuclear heme a_3···Cu_B catalytic center of cytochrome *c* oxidase (coordinates and residue numbering for oxidized bovine enzyme, pdb accession no. 1V54, ref 5).

10.1021/ja068972f CCC: $37.00

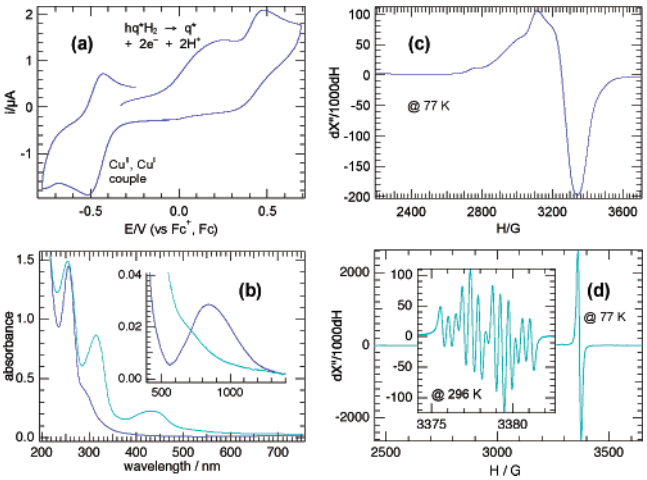
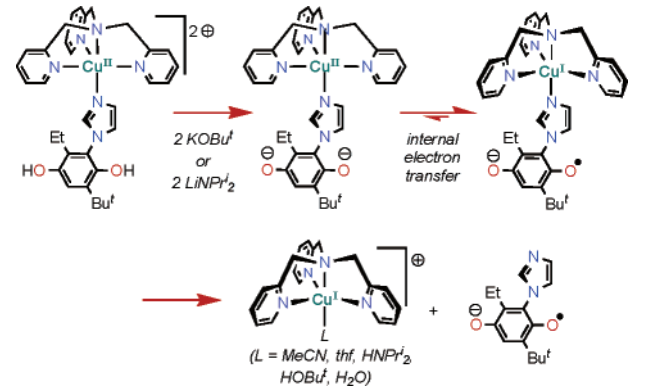


Figure 2. (a) Cyclic voltammogram of $[(tpa)Cu^{II}(Im-hq^*H_2)](OTs)_2$, **1** (2 mM) in MeCN−0.1 M $[Bu^n_4N][PF_6]$. (b) UV−vis−NIR (at 296 K) and (c, d) X-band EPR spectra for a solution of **1** (1.2 mM) in MeCN before (blue, —) and after (cyan, —) the addition of base ($KOBu^t$, 2.0 equiv). Simulation[9] of the 296 K EPR spectrum gives $g = 2.0051$, $a_H/G = 1.805$ (1 H) and 1.375 (2 H); $a_N/G = 0.490$ (1 N).

Scheme 1



labile copper(I) intermediate $[(tpa)Cu^I(Im-sq^{*-})]$ (Scheme 1). Consistent with this interpretation, cyclic voltammetry[13] reveals the Cu^{II}, Cu^I couple for **1** is −0.47 V, whereas the $Im-hq^{*2-}$, $Im-sq^{*-}$ and $Im-sq^{*-}$, $Im-q^*$ couples for uncomplexed $Im-hq^{*2-}$ are ∼ −1.5 and −0.94 V,[9] respectively. The $Im-hq^{*2-}$, $Im-sq^{*-}$ couple should be only slightly perturbed by coordination of the imidazole to a Cu^{II} center,[14] thus an internal electron transfer upon double deprotonation of **1** is expected. A phenoxyl radical, phenolate anion couple will always be higher than a closely related quinone, semiquinone couple; thus it is possible that Cu^{II}, Cu^I and phenoxyl radical, phenolate anion couples become closely matched, for example, the phenoxyl radical, phenolate anion couple of the 4-acetoxy derivative of $Im-hq^*H_2$ is −0.25 V, ∼ 700 mV higher than the $Im-q^*$, $Im-sq^{*-}$ couple and higher than the Cu^{II}, Cu^I couple in **1**.[15]

These results highlight the need to consider valence tautomerism and its possible effects in C*c*O. EXAFS studies reveal that one of the three histidine ligands to the Cu_B ion is more weakly bound than the other two and is coordinatively labile in the Cu_B^I state.[16] High-level theoretical calculations suggest that the Cu_B^{II} and the histidine−tyrosyl radical centers in the P_M intermediate have close to identical reduction potentials, that is, which center is the electron acceptor is finely balanced[17] (although a presumption that the tyrosyl radical is the electron acceptor pervades the literature[1−7,14−17]). Thus, if a tyrosinate Cu_B^{II}−His−$TyrO^-$ center occurs *anywhere* in the catalytic cycle—for example, if the reduction of the tyrosyl radical in the P_M state is not strictly concerted with the transfer of a proton to the tyrosyl oxygen atom thereby affording the tyrosinate anion[1−7,14−17]—valence tautomerism may lead to a (transient) Cu^I−His−$TyrO^\bullet$ center. Dissociation of the labile histidine ligand from the thus formed Cu_B^I center could have important ramifications such as the redox-linked opening of a pathway during turnover for egress of pumped protons and/or product waters from the $Fe_{a3}\cdots Cu_B$ catalytic center.

Acknowledgment. We are grateful for support from the Australian Research Council (Grant No. DPO557462).

Supporting Information Available: Details of preparations and measurements plus additional electrochemical, NMR, EPR, UV−vis−NIR spectra and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA068972F