Investigating the Tyrosine-Histidine Linkage in the Cu_B Site of Cytochrome c Oxidase: Model Studies Utilizing a Zinc Imidazole-Phenol Containing Complex

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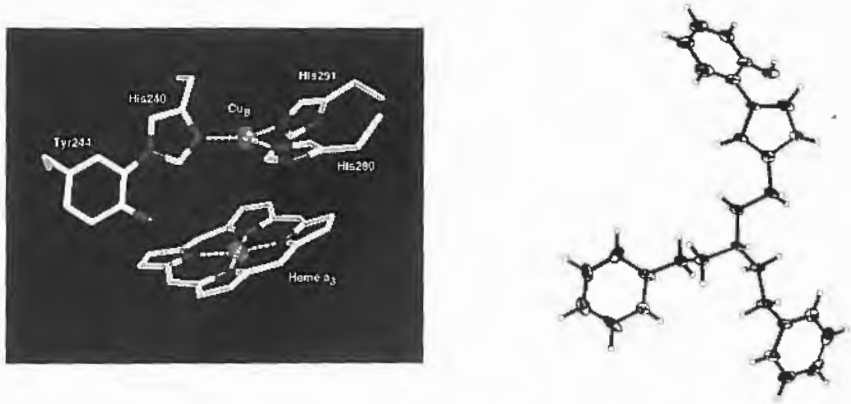
Cytochrome c Oxidase (CcO) is the final enzyme complex in the electron transport chain embedded in the inner membrane of mitochondria or bacterial membranes in organisms that participate in aerobic respiration. CcO catalyzes the thermodynamically favorable four-electron reduction of molecular oxygen to water, which results in the generation of a proton gradient, as denoted by H_O^+ below.[1]

$$4\ e^- + 8\ H_I^+ + O_2 \rightarrow 4\ H_O^+ + 2\ H_2O$$

Three of the four electrons used in the reduction of oxygen in mixed valence CcO are proposed to arise from the reduced metals in the bimetallic center of the Cu_B site, Cu(I) and Fe(II). The source of the fourth electron has been a matter of debate for several years. In 1998, Yoshikawa and coworkers reported a high resolution x-ray structure of the active site of CcO.[2] The Cu_B site was found to contain a unique post-translational modification that covalently linked a Cu bound histidine to a tyrosine (Figure 1A).[2,3] Recent evidence suggests the existence of a tyrosyl radical near the Cu_B site during turnover of CcO.[4] Thus, tyrosine could provide the necessary fourth electron in the reduction of oxygen in mixed valence CcO.[4,5]

To elucidate the physicochemical effects of the imidazole substitution on the phenol ring, three constitutional isomers were synthesized: ortho, meta, and para imidazole-phenol. Spectrophotometric titrations were carried out to measure the phenolic pK_A of each isomer. In comparison to phenol (pK_A = 10.1), the imidazole substituted phenol derivatives all had lower pK_A values, suggesting the enhanced stability of the phenolate anion upon imidazole substitution. p-Imidazole-phenol had the highest pK_A (9.61) followed by meta (9.01) and finally ortho (8.69). The trend is consistent with an inductive effect, as the phenolate is more stabilized as the imidazole is positioned closer to the phenolate oxygen. Oxidation potentials of each isomer were measured at high pH. All imidazole substituted isomers had higher peak potentials compared to the parent phenolate (+187 mV vs. $Fe(CN)_6^{-3/-4}$), providing further evidence of a strong inductive effect upon imidazole substitution. p-Substituted imidazole had the lowest oxidation potential of the three isomers (+258 mV), followed by the ortho substituted isomer (+326 mV) and the meta derivative (+399 mV).

A tertiary amine based chelating ligand (BPAIP) was synthesized incorporating an ortho substituted imidazole-phenol moiety along with pyridine groups to mimic the coordination sphere found in the Cu_B site (Figure 1B).



A B

Figure 1 (A) Cu_B site of CcO and (B) chelating ligand (BPAIP).

A Zn(II) complex was synthesized by the addition of $ZnBr_2$ to BPAIP•HBr in MeOH, which was characterized by ^1H NMR and mass spectroscopy. The Zn(II) complex was water soluble, and was observed to exhibit pH dependent stability as observed by ^1H NMR spectroscopy (Figure 2).

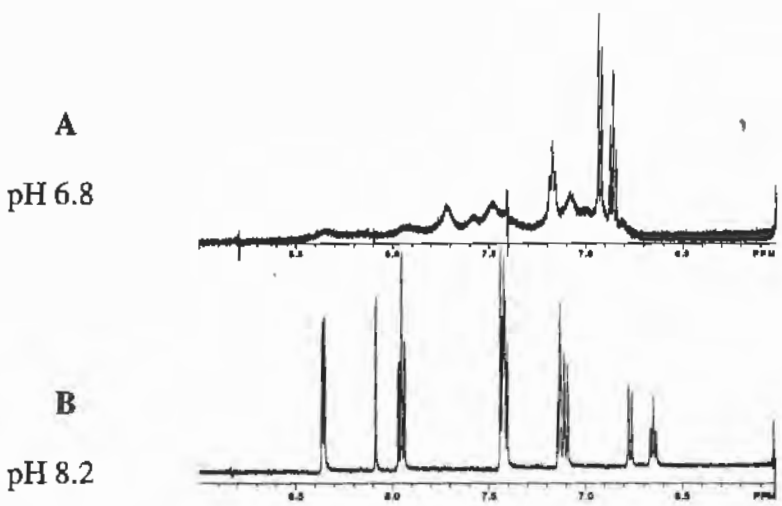


Figure 2 $[Zn(BPAIP)(Br)]^+$ in (A) pH 6.8 and (B) pH 8.2 buffered D_2O.

The ^1H NMR spectrum (aromatic region) at pH 6.8 suggests an equilibrium (exchange process) existing between several Zn(II) species in solution. At pH 8.2, the $[Zn(BPAIP)(Br)]^+$ complex exhibits sharp, resolved proton peaks. The coordination of Zn(II) to BPAIP was found to lower the pK_A of the phenol from the $pK_A = 8.61$ in the unbound ligand to 8.24 found in the Zn(II) complex, $[Zn(BPAIP)(Br)]^+$. Thus, the pH in which the Zn(II) complex becomes stable coincides with the experimentally determined pK_A of the phenolic proton of the imidazole-phenol ligand. In addition, the oxidation potential of the deprotonated phenol (phenolate) was found to increase by nearly +50 mV

compared to the unbound BPAIP phenolate oxidation potential, consistent with an increased inductive effect upon Zn(II) coordination.

Density Functional Theory (DFT) was utilized to find the optimized geometry of $[Zn(BPAIP)(Br)]^+$ in the gas phase. In agreement with ^1H NMR and electrochemical studies, the optimized structure was found to have distorted trigonal pyramidal geometry (equatorial pyridine, imidazole ligands), with an axial bromide and a weakly or unbound tertiary amine defining the z-axis (Figure 3). Gas phase calculations suggest a significant distortion in the complex resulting from deprotonation of the phenol. A contraction of the $Zn\text{-}N_{Im}$ bonding distance coincides with an increase in the $Zn\text{-}N_{am}$ of over 0.2 Å. Overall, the increased donating ability of the imidazole phenolate ligand results in a Zn(II) complex that is further distorted toward tetrahedral geometry. This may contribute to the increased stability of $[Zn(BPAIP)(Br)]^+$ at high pH.

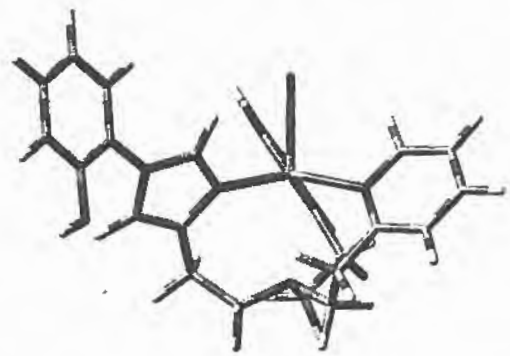


Figure 3 Optimized gas-phase structure of $[Zn(BPAIP)(Br)]^+$.

References

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