Heme/Cu Complexes as Functional Models for the Active Site of Cytochrome *c* Oxidase

Simona C. Puiu, Eduardo E. Chufán, Biplab Mondal and Kenneth D. Karlin

Department of Chemistry, The Johns Hopkins University, Baltimore, MD, 21218, USA

Cytochrome *c* oxidase (C*c*O) is the terminal enzyme in the respiratory cycle that catalyzes the $4e^-/4H^+$ reduction of molecular oxygen to water. The O_2 reduction takes place at an active site consisting of a heme a_3 / Cu_B site (interatomic distance Fe…Cu = 4.5 - 5 Å), but the details of the O-O cleavage mechanism are still not completely understood. It is believed that the heme-Cu reduced form of the enzyme (so-called mixed-valent) binds dioxygen at the heme a_3, giving an iron(III)-superoxo complex (Fe^{III}-O_2^-), followed by complete O-O cleavage via a single step 3 e^- reduction: one electron is donated by the oxidation of the ferric heme to give an iron(IV) oxo-ferryl species, one electron comes from Cu_B ($Cu^I \rightarrow Cu^{II}$), and the fourth electron is provided by a tyrosine residue. We are mostly interested in the second step of this mechanism, therefore, a viable approach to understanding the reductive O-O cleavage at the C*c*O heme…Cu active site is employing iron-superoxo or iron-peroxo heme a_3 synthetic models and Cu_B analogue complexes, with tridentate or tetradentate ligands (L = AN or TMPA). Three approaches were used to effect the O-O cleavage:

a) Reaction of the iron-superoxo complex $(F_8TPP)Fe^{III}(O_2^-)$ with 2 equivalents of Cu(I) complexes as reducing agents, in the presence of a Lewis base (1,5-dicyclohexylimidazole, DCHI).

b) Reaction of the iron-peroxo complex $[(F_8TPP)Fe^{III}(O_2^{2-})]^-$ with copper(I) complexes assisted by an acid and DCHI.

c) Reaction of the low-spin iron-peroxo-copper complex $[(DCHI)(F_8TPP)Fe^{III}\text{-}(O_2^{2-})\text{-}Cu^{II}(L)]^+$ with a phenol derivative as H^+/e^- donor.

All these reactions yield a product assigned as an oxo-ferryl (Fe^{IV}=O) species, that is stable only al low temperatures (-80 ˚C). At room temperature, the reaction mixture transforms to give the μ-oxo complex $[(DCHI)(F_8TPP)Fe^{III}\text{-}O\text{-}Cu^{II}(L)]^+$. Formation of the oxoferryl intermediate was proved by low-temperature UV-Vis studies (λ_{max} = 417 (Soret); 540 nm), and indirectly by its ability to oxidize phosphines or phenol.