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 (CcO) 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 CcO 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$_8$TPP)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$_8$TPP)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$_8$TPP)Fe$^{III}$-(O$_2^{2-}$)-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 at low temperatures (-80 °C). At room temperature, the reaction mixture transforms to give the μ-oxo complex [(DCHI)(F$_8$TPP)Fe$^{III}$-O-Cu$^{II}$(L)]$^+$. Formation of the oxoferryl intermediate was proved by low-temperature UV-Vis studies ($\lambda_{max} = 417$ (Soret); 540 nm), and indirectly by its ability to oxidize phosphines or phenol.