Structure of the Yeast Ferroxidase, Fet3p: Implications for Function

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A subclass of multicopper oxidases (MCO) possess a specific activity towards lower valent first-row transition metal ions as substrate not shared by the majority members of this enzyme group. The most widely studied substrate for these enzymes has been Fe^{2+}; those enzymes that couple the 1-electron oxidation of $4Fe^{2+}$ to the reduction of O_2 to $2H_2O$ are known as ferroxidases. A fundamental question about these MCOs with reactivity towards Fe^{2+}, Cu^{1+}, and/or Mn^{2+} not shared by laccases or ascorbic acid oxidase is: What are the structure-function relationships that confer this specialized substrate reactivity? The 2.8Å structure of the ferroxidase, Fet3p, and structure-function, protein engineering data provide a compelling answer to this intriguing question as well as insight into the trafficking (metabolite channeling) of Fe^{3+} to Ftr1p for high-affinity iron uptake in yeast through this ferroxidase-permease complex.

The crystal structure identifies second shell residues that interact with a given Cu *via* interaction with a first shell, coordinating ligand. For example, D94, suggested to support H^+-transfer in the reduction and release of a peroxy intermediate at the T2 Cu is in an H-bond network with this metal prosthetic group. Similarly, E185 is in H-bond contact with the Nε2 NH of H489, one of the ligands to the T1 Cu. E185 and H489 have been implicated in the matrix coupling pathway that couples e^--transfer from Fe^{2+} to reduction of the T1 Cu(II).

Solution studies have taken full advantage of new insight into side chain function suggested by the structure. D409 mirrors the role of E185 in making an equivalent H-bond contact with the second of the two T1 Cu His ligands, H413; D409 is required for efficient e-transfer also, thus completing T1 Cu e^--transfer circuitry. E185, D283, D409 and Q492 appear to make up a siderophore-like Fe^{2+} binding site that sets the Fe(II)/Fe(III) potential at or below the 190 mV needed to provide the driving force for e^--transfer to a 430 mV T1 Cu(II). This "tuning-up" of the reducing capacity of the bound Fe^{2+} is what makes an MCO a metallo-oxidase. The Fet3p/Ftr1p system is the first to give us this insight as well as evidence for iron channeling.