The Proton Pump of Cytochrome Oxidase

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Cytochrome oxidase is a membrane metalloprotein that reduces O_2 to 2 H_2O, using electrons from the oxidation of four equivalents of ferrocytochrome *c*. Variants of cytochrome oxidase are found in most aerobic organisms, and we are studying the enzyme from the bacterium *Rhodobacter sphaeroides.* The enzyme contains four redox-active metal centers. The electron from cytochrome *c* is initially transferred to Cu_A, which is a di-copper unit which functions as a 1-electron redox center. Reduced Cu_A transfers its electron to heme *a*, a six-coordinate (*bis*-histidine ligation), low spin heme buried within the protein. From heme *a*, the electron is transferred to the heme a_3-Cu_B bimetallic center, which is where O_2 binds and is reduced to water in a series of four 1-electron transfer reactions. The heme a_3-Cu_B active site is buried about half-way across the membrane. The "electron wire" ($Cu_A \rightarrow$ heme $a \rightarrow$ [heme a_3-Cu_B]) assures that all the electrons are delivered from the positive or "P side" of the membrane. There are also "proton wires" or channels which assure that all the protons needed to form H_2O are delivered to the active site from the opposite side of the membrane. This assures that the reduction of O_2 to water is mechanistically coupled to moving 4 full charges across the membrane, thus generating a proton motive force. In addition, the enzyme pumps four protons across the membrane during each turnover (per O_2). A total of 8 protons are taken up from the N-side of the membrane Hence, 8 full charges are moved across the membrane per O_2 consumed. Current evidence supports a model in which each electron transfer from cytochrome *c* to the active site is coupled to one proton being pumped across the membrane.

The primary pathway for proton delivery is the "D channel" in subunit I of the oxidase, which leads from D132 (*R. sphaeroides* numbering), at the surface of the protein in contact with the bacterial cytoplasm (negative or N-side of the membrane) to E286, near the heme a_3-Cu_B site. Water molecules, most of which are apparent in X-ray crystal structure, provide a pathway for facilitated proton diffusion from D132 to E286.

A series of mutations have been made within the D channel. Some of these mutations block or slow down proton transfer to the active site. Other mutations allow proton transfer but decouple the proton pump from the oxidase activity. These mutations will be discussed in the context of a proposed mechanism for the proton pump of the oxidase.