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Commentary

The currents of life: The terminal electron-transfer complex of respiration

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Aerobic organisms derive most of the energy needed for life processes by the burning of foodstuffs with the molecular oxygen in air, as first suggested in 1789 (1) by Antoine Lavoisier (1743–1794). In the first part of the respiratory process, hydrogen atoms are extracted from organic molecules. The hydrogen carriers are later regenerated in the respiratory chain located in cell organelles, mitochondria, or, in bacteria, in the cell membrane. These chains consist of a series of membrane-bound protein complexes in which the hydrogen atoms are split into protons and electrons. The electrons are passed down the chain and reduce molecular oxygen to water, whereas the protons are left behind on one specific side of the membrane. In addition, the electron transfer (ET) or "current" through the chain is coupled to a pumping of additional protons from water to the same membrane side. Thus, the two proton currents lead to an increased positive charge and decreased pH on this side—i.e., an electrochemical potential across the membrane, analogous to a storage battery. This potential drives the synthesis of ATP, the universal energy currency in living cells, by a chemiosmotic mechanism formulated by Peter Mitchell (2), who was awarded the Nobel Prize in Chemistry in 1978.

In the terminal reaction, cytochrome-*c* oxidase takes electrons from a soluble, iron-containing ET protein, cytochrome *c*, and passes them on to O_2. This is probably the best understood of the redox-driven proton pumps (see ref. 3 for a review), but the exploration of its mechanism has been severely hampered by a lack of structural information. The structures of membrane proteins are much more difficult to determine than those of soluble proteins, such as hemoglobin, because the method of choice is x-ray diffraction. This technique requires the growing of highly ordered crystals, which must diffract the x-rays to 3 Å or better, and growing of such crystals with membrane proteins has generally been hampered by lack of homogeneity and the hydrophobic nature of the protein surface. Until this year, only one structure of a redox-linked membrane protein had been determined to high resolution, that of a bacterial photosynthetic reaction center (4), leading to a Nobel Prize for Deisenhofer, Huber, and Michel in 1988. Therefore, it was a nice surprise when the structures of two cytochrome-*c* oxidases were announced at conferences this year and published within one day of each other in *Nature* (5) and *Science* (6).

The structure of the oxidase from the bacterium *Paracoccus denitrificans* was described (5) by a group headed by Michel, the investigator who had earlier crystallized the photosynthetic reaction center. His group used a complex with an antibody fragment to aid in crystallization. The other group, led by Yoshikawa, crystallized the enzyme from bovine heart (6) and found that the choice of detergent was critical; in fact, as late as June, 1994, Yoshikawa reported at a conference that his best crystals diffracted to only 8 Å. Apparently, his crystals improved in 1995; indeed, it would appear that this will be remembered as a good year for oxidase-related crystals, because, in addition to Michel's and Yoshikawa's work, Wilmanns, Saraste, and coworkers, in this issue of the *Proceedings* (7), report their determination of the crystal structure of a quinol oxidase fragment containing an engineered copper center (8), Cu_A, to a resolution of 2.5 Å. The structure determination of this soluble domain of the oxidase is a very important achievement because the results allow a closer look at the Cu_A redox center, whose optical and, to some extent, EPR spectra cannot be observed in the intact oxidases because of interference from the strongly absorbing heme redox centers that are present.

Two linkages of the Cu_A center to subunit I of the oxidase are shown in Fig. 1. The structures show that Cu_A is a binuclear complex, and EPR has established that the complex has one unpaired electron that is completely delocalized over the two Cu nuclei (9). A binuclear mixed-valence site had, in fact, been suggested in 1962 by Beinert *et al.* (10), who first described the unique EPR spectrum of this center. The visible spectrum of Cu_A can be recorded only with the soluble domain, because it is completely masked by the heme absorptions in the whole oxidase. Despite the fact that amino acid sequence alignment (11) shows Cu_A to be related to blue Cu proteins, such as plastocyanin, the visible spectra are quite distinct (8, 12). Instead of one strong sulfur-to-copper charge-transfer band at roughly 600 nm, there are two bands of almost equal intensity in the 500-nm region. The band pattern accords with the binuclear copper structure, because there should be two components in the charge-transfer system of a Cu–Cu-coupled chromophore (13, 14).

From Cu_A the electron is transferred to the heme of cytochrome *a* and then to the binuclear cytochrome a_3–Cu_B center, where O_2 is reduced. The rate constant for electron transfer from Cu_A to cytochrome *a* is remarkably high ($1.8 \times 10^4\ s^{-1}$; ref. 15) given that the metal centers are separated by 19 Å (6) and the reaction driving force is just 50 meV (15). Inspection of the cytochrome-*c* oxidase structure reveals that there is a direct ET pathway from Cu_A to heme *a* consisting of 14 covalent bonds and 2 hydrogen bonds (Fig. 1). The coupling efficiencies of the 14 covalent bonds can be estimated from experimental work on Ru-modified proteins such as azurin and cytochrome *c* (16, 17). In these (16, 17) and other (18, 19) experiments, however, it has been found that hydrogen-bond couplings can vary over a wide range. Taking reasonable values for hydrogen-bond interactions, we estimate that the maximum (driving force-optimized) ET rate from Cu_A to cytochrome *a* will fall in the range 4×10^4 to 8×10^5 s^{-1}. Assuming these limits, the $Cu_A \rightarrow$ cytochrome *a* ET reorganization energy (λ) must be between 0.15 and 0.5 eV. Typical λ values for protein ET reactions are between 0.7 and 1.3 eV (17, 20).

The relatively small reorganization energy for $Cu_A \rightarrow$ cytochrome *a* ET may be related to the binuclear structure of Cu_A (13). The advantage of a binuclear copper site is the potential ability to delocalize an electron over a large region of space. This delocalization can be achieved by enforcing a three-coordinate ligand geometry about both copper centers that disfavors trapping the electron at a single metal site. It apparently is the combination of a low reorganization energy and an efficient ET pathway that allows electrons to flow rap-

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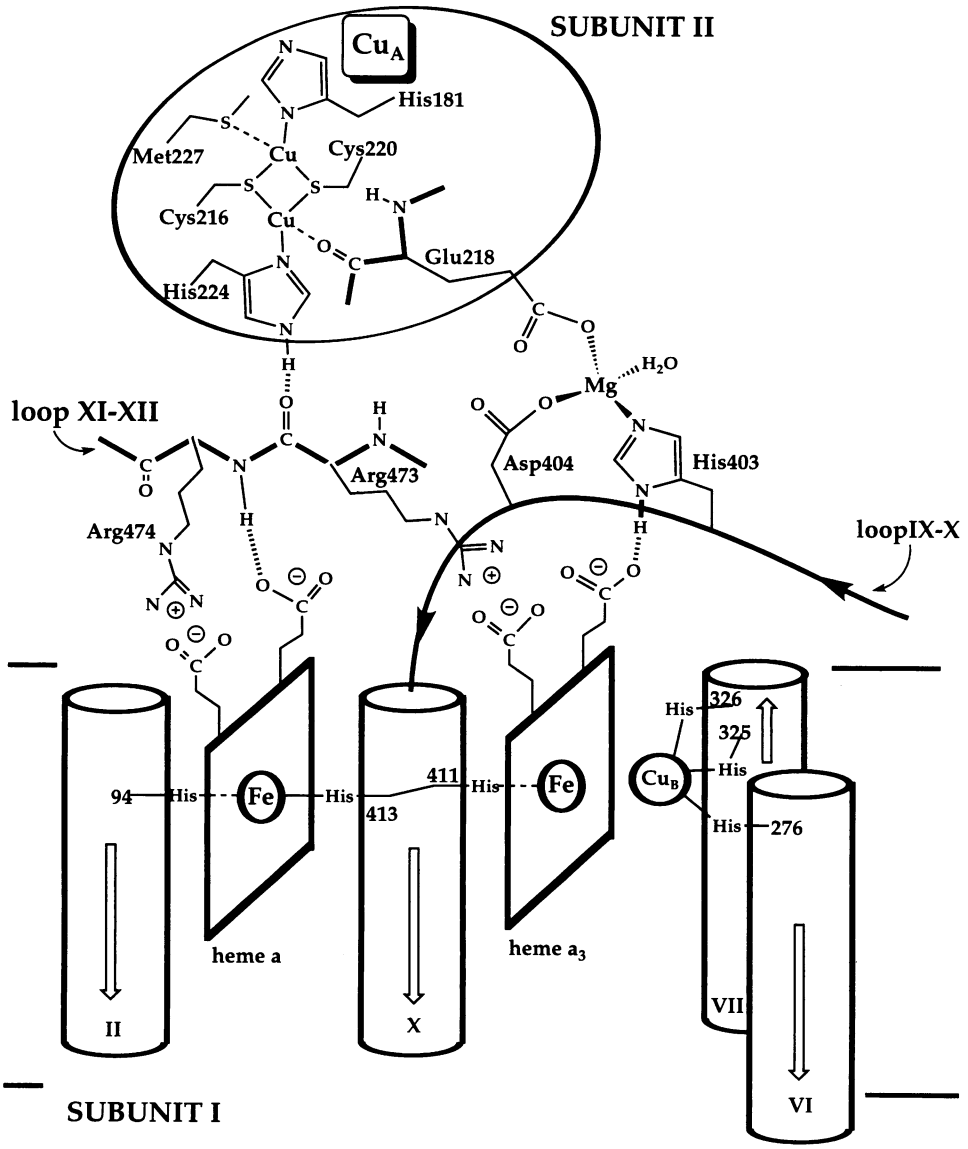

FIG. 1. Diagram showing several linkages between subunits I and II in cytochrome-*c* oxidase. The oval at the top highlights the soluble or exposed domain of subunit II that contains the Cu_A center. Cylinders are transmembrane α helices of subunit I (arrows indicate the direction of the peptide chain). The porphyrin rings of the hemes of cytochromes *a* and a_3 are drawn as squares with the propionate groups highlighted. Two loops (loop IX–X and loop XI–XII) connecting helices in subunit I are also shown. Hydrogen bonds to H—N and C=O of a peptide unit in loop XI–XII connect subunits I and II and form a good $Cu_A \rightarrow$ heme *a* ET pathway from the imidazole of a histidine (His-224) to one of the heme propionates. Two arginines (Arg-473 and Arg-474) form salt bridges with propionates of hemes a_3 and *a*, and a Mg complex is linked to both Cu_A and heme a_3 and could serve as a communicator between subunits I and II. The amino acid numbers refer to the *Paracoccus denitrificans* enzyme (5).

idly with only a small change in free energy from the Cu_A center of subunit II to cytochrome *a* in subunit I.

Research on cytochrome-*c* oxidase has been actively pursued since the 1920s, when it was pioneered by David Keilin in Cambridge (21, 22) and Otto Warburg in Berlin (23, 24), but we are still far away from understanding the mechanism of this redox-linked proton pump (25). This function was, in fact, discovered as late as 1977 (26). The structures will be of great help, but it should be remembered that they largely confirm earlier proposals for the nature of the metal sites based on spectroscopic investigations (see ref. 3). Furthermore, the arrangement in the membrane was predicted quite closely on the basis of mutagenesis studies (27). One important finding in the structure of the bacterial oxidase is the probable location of two proton channels, and, indeed, a new pump mechanism has already been formulated (5). With the structural data now available, it will be much easier to plan and interpret mutagenesis experiments to test this proposal as well as other proton-pump mechanisms that are currently under discussion.

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