Electron transfer and ligand binding within mixed-valence cytochrome *c* oxidase

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Mitochondrial cytochrome *c* oxidase has four redox active metal centers contained in a protein matrix consisting of thirteen subunits. Two of the metal centers are heme-based, and known as cytochrome *a* and cytochrome a_3. There are also two copper centers that are known as Cu_A and Cu_B. Each of the centers takes up one electron when the enzyme goes from fully oxidized to fully reduced. Incubation of oxidized cytochrome *c* oxidase under one atmosphere of CO yields the mixed-valence state which has two electrons, one each on cytochrome a_3 and Cu_B, and CO bound to cytochrome a_3. A half-reduced, ligand-free form is generated from the mixed-valence CO species by purging with nitrogen. The extent of cytrochrome *a* reduction following CO removal is 25-30 % as determined by optical difference spectroscopy, but less than 10% when measured at low temperature by EPR. Cyanide addition to mixed-valence CO bound oxidase gives a slow conversion to a form in which cytochrome a_3 is oxidized and cyanide bound. Cyanide addition to half-reduced, ligand-free oxidase gives a somewhat faster conversion consistent with partial electron transfer back to cytochrome *a*. These results indicate that cytochrome a_3 has a higher affinity for electrons in the half-reduced, ligand free enzyme. In EPR experiments at low temperature the extent of reversed electron transfer is less, which suggests that this affinity is temperature dependent.

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