

*A novel protein-protein interaction detected in Cyt.c-Cytochrome oxidase complex*

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Mitochondrial cytochrome c oxidase (CcO) transfers electrons from cytochrome c (Cyt.c) to O<sub>2</sub> to generate H<sub>2</sub>O, a process coupled to proton pumping. Although a significant amounts of data have accumulated regarding electron transfer (ET) from Cyt.c to CcO, and the X-ray structures of mammalian CcO (PDB 5B1A) and Cyt.c have been determined at high resolution, the underlying mechanism of ET remains incompletely understood. To elucidate the ET mechanism, we determined the structure of the mammalian Cyt.c-CcO complex at 2.0-Å resolution and identified an electron transfer pathway from Cyt.c to CcO [1], which was consistent with previous chemical modification studies of Cyt.c. The complex structure indicates a novel protein-protein interaction mode. The specific interaction between Cyt.c and CcO is stabilized by only a few electrostatic interactions between side chains. We compared the interaction scheme of the Cyt.c-CcO complex with those of the Cyt.c-CcP [2] and the Cyt.bc1-Cyt.c complex [3]. The shortest distance between two Ca atoms of Cyt.c and CcO is 8.2 Å. By contrast, those in the Cyt.c-CcP (PDB 4GED) and Cyt.bc1-Cyt.c (PDB 3CX5) complexes are much shorter, 5.3 and 5.6 Å, respectively. Furthermore, the contact surface areas of the Cyt.c-CcO complex (222.8 Å<sup>2</sup>) is approximately one-third that of the Cyt.c-CcP complex (615.2 Å<sup>2</sup>), and less than one-fourth that of Cyt.bc1-Cyt.c (1008.7 Å<sup>2</sup>).

Between the Cyt.c and CcO are three water layers with a long inter-molecular span, one of which lies between the other two layers without significant direct interaction with either protein. More water molecules are present between Cyt.c and CcO than between Cyt.c and CcP or Cyt.bc1 and Cyt.c, and there are a total of 14 non-interacting water molecules in the Cyt.c-CcO complex. By contrast, the Cyt.c-CcP and Cyt.bc1-Cyt.c complexes each have only four and two non-interacting water molecules, respectively. When Cyt.c docks with CcO, both proteins retain water molecules on their surfaces, and they interact with each other via the long arms of side chains. On the other hand, the docking of Cyt.c and CcP or Cyt.bc1 leads to the exclusion of water molecules from the surface of each protein. B-factor distribution in Cyt.c-CcO complex indicates that Cyt.c undergoes large structural fluctuations, using the interacting regions with CcO as a fulcrum.

These features of the protein-protein interaction at the docking interface represent the first known example of a new class of protein-protein interaction, which we term "soft and specific". This interaction is likely to contribute to the rapid association/dissociation of the Cyt.c-CcO complex, which facilitates the sequential supply of four electrons for the O<sub>2</sub> reduction reaction.

## References

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**Keywords:** [electron transfer complex](#), [membrane protein](#), [protein-protein interaction](#)