

Molecular Recognition between Human Mitochondrial Cytochromes: The Cytochrome c_1 /Cytochrome c Interaction

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The cytochrome bc_1 (Cbc_1) complex is a membrane-bound component of the mitochondrial electron transport chain. The complex, which catalyzes electron transport from ubiquinol to cytochrome c (Cc), comprises three redox centers: cytochrome b , the Rieske protein and cytochrome c_1 (Cc_1). Cc_1 is responsible for the reduction of Cc , which, in turn, results in the transfer of electrons to cytochrome c oxidase (CcO). The reaction is essential for cellular bioenergetics, insofar as it is coupled with proton translocation leading to ATP formation. Recently, our group has reported that plant Cc shows two binding sites on Cc_1 ¹. The first, or so-called *proximal* site, is suitable for electron transfer, whereas the second, or *distal* site, located near the Rieske protein, is involved in the channeling of Cc molecules towards CcO ¹⁻³.

Given this, this work aims to determine whether the Cc - Cc_1 two-binding site model is conserved through out evolution and, specifically, can be found in the interaction between the two corresponding human hemeproteins. First, in close collaboration with the *European Integrating Structure Platform (Instruct, PID1163)*, several constructions of the soluble N-terminal domain of Cc_1 were designed. Among these, a triple Cc_1 mutant lacking non-heme-coordinated cysteines and containing a bacterial periplasmic signal allowed for the expression of human Cc_1 as a recombinant protein. Physicochemical properties, including redox potential, were then analyzed. Preliminary measurements by isothermal titration calorimetry suggest that the $Cc:Cc_1$ stoichiometry of 2:1 is present in both plants and humans.

¹Moreno-Beltrán, B *et al. Biochim. Biophys. Acta-Bioenerg.* (2014) 1837: 717-729.

²Moreno-Beltrán, B *et al. FEBS Lett.* (2015) 589: 476-483.

³Louro, R. O. & Díaz-Moreno I. (eds). *Redox Proteins in Supercomplexes and Signalosomes.* CRC Press (2015).