1P.15 Knock-down of cytochrome *c* oxidase structural subunits in HEK293 cells

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Background: Mammalian cytochrome *c* oxidase (CcO), the terminal enzyme of respiratory chain, is a multiprotein complex of approximately 200 kDa and composed of 13 subunits. CcO assembly within the inner mitochondrial membrane is a sequential and relatively slow process. The aim of our study was to analyze an impact of downregulation of selected structural CcO subunits on CcO assembly. We chose Cox4 and Cox5a subunits, both of which enter the initial stage of the process and Cox6a subunit taking a part in a terminal step of CcO holocomplex assembly. **Methods:** The down-regulation of selected subunits was performed using RNA interference. We constructed 33 derivatives of pCMV-GIN-ZEO plasmid coding for hairpins targeted to different positions of *COX4I1, COX5A* and *COX6A1* mRNA, respectively. RNAi inducing transcript of pCMV-GIN-ZEO plasmid contains GFP and neomycin phosphotransferase coding sequences situated in tandem, upstream of the miR30-like hairpins. The recombinant plasmids were transfected to the HEK293 cells and the stable polyclonal cell populations were selected in a medium containing G418. **Results:** The depletion of Cox4, Cox5a and Cox6a was confirmed by SDS-PAGE immunoblot analyses. In selected cells with the lowest residual amounts of Cox4 and Cox6a subunits, transcripts of both tissue-specific isoforms were quantified by qRT-PCR. The depletion of Cox4, Cox5a and Cox6a subunits in HEK293 cells was accompanied by diminished amount of CcO holoenzyme and an altered assembly pattern. Lower content of CcO correlated with decreased CcO activity. Isolated CcO deficiency also manifested at the level of supercomplexes. The high-resolution respirometry showed normal normoxic maximally stimulated respiration after FCCP treatment (state 3u) in knockdown cells with Cox5a and Cox6a1 depletion but increased P50 values. **Conclusion:** Our results argue for importance of Cox4, Cox5a and Cox6a subunits for CcO biogenesis.

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1P.16 Structural and functional characterization of respiratory supercomplex containing complex III and IV from hyperthermophilic eubacterium *Aquifex aeolicus*

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Respiratory chain consists of several complexes named complex I to V according to their redox potentials during electron transfer. Complex I, III and IV couple the electron transfer to proton translocation and form a proton gradient across membrane which is used by complex V to produce ATP. The organization of these complexes has been studied for years and the term "supercomplex", representing several complexes in direct contact, was suggested. Here we report a supercomplex mainly containing fully assembled complex III and complex IV isolated from native membranes of *A. aeolicus*. Later a full complex IV and an incomplete complex III could be isolated from this supercomplex. All these three proteins were identified and characterized. The supercomplex mainly contains cytochrome *b* (*petB*), cytochrome c_1 (*cyc*), Rieske iron–sulfur protein (*petA*), cytochrome *c* oxidase subunit I (*coxA2*) and subunit II (*coxB2*). Isolated complex IV consists of subunit I and II. The incomplete complex III lost Rieske iron–sulfur protein during isolation. EPR spectra of the supercomplex showed signals from low-spin cytochrome b_H, b_L and cytochrome c_1 as well as signals of an intensive copper and a high-spin heme. Copper and high-spin heme signals were also detected in the complex IV. Metal content measurement indicates four coppers in each supercomplex and complex IV. The supercomplex catalyzes cytochrome *c* oxidation and reduction, quinol oxidation and oxygen consumption; complex IV catalyzes cytochrome *c* oxidation, quinol oxidation and oxygen consumption; and the incomplete complex III is able to catalyze cytochrome *c* reduction without Rieske iron–sulfur protein. Stoichiometry of the supercomplex was analyzed by LILBID (Laser Induced Liquid Bead Ion Desorption), which indicates two molecules of complex III and one molecule of complex IV in each supercomplex. To summarize all these results, possible models and electron transfer schemes of this supercomplex are proposed.

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1P.17 Localizing the ubiquinone binding site of the *Escherichia coli* complex I

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The NADH:ubiquinone oxidoreductase, the respiratory complex I, is the entrance point of electrons into the respiratory chain. Complex I transfers two electrons from NADH to ubiquinone and couples this reaction with the transport of four protons across the membrane. The position and structure of the ubiquinone binding site within the complex is not known. We generated several variants of the enzyme by *Lambda*-Red Recombineering bearing cysteine residues at suitable positions on the surface of the protein. To these cysteine residues (1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl)-methanethiosulfonate (MTSL), a spin-probe, was attached. In addition, a derivative of pentyl- and decyl-ubiquinone was synthesized containing the MTSL label at the distal position of the alkyl-chain. In experiments with the spin-probe bound to decyl-ubiquinone and bound to the protein, distances between the spin-probes were measured pairwise by pulsed EPR spectroscopy (ELDOR). The positions of the cysteine residues were chosen that they allow localizing the quinone binding site via triangulation. First results have been obtained in cw-experiments using the NuoB R112C variant in combination with the two different ubiquinone derivatives demonstrating that the method is suitable for measuring distances within the protein and between distinct protein sites and the quinone binding site.

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1P.18 Sodium interaction of the complex I antiporter-like subunits NuoL, M and N from *Escherichia coli* studied by ^{23}Na NMR

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