Structural characterization of respiratory complexes in potato tuber

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of soluble electron carriers may have significant functional consequences since they would introduce macroscopic heterogeneities in the chain. The expected signature of such heterogeneities is essentially kinetic calling for new methods allowing the time-resolved analysis of the electron transfer sequence associated with the mitochondrial respiration *in vivo*. We are currently developing such a method based on the flash-induced photolysis of CO in the presence of O_2, as fruitfully conducted for the mechanistic dissection of complex IV. The advantages and pitfalls of the approach will be described and preliminary results will be presented and discussed.

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S13.19 A role for sodium ions in the respiratory chain of *Rhodothermus marinus*

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Rhodothermus marinus is a strictly aerobic and thermohalophilic organism isolated from submarine hot springs in Iceland and Açores. Its respiratory complexes have been studied and include a complex I (NADH:menaquinone oxidoreductase), a complex II (succinate: menaquinone oxidoreductase), a novel complex III and at least three different dioxygen reductases. Since it is a halophilic organism, and because a proton/sodium antiporter gene was found among its complex I genes, a possible role of sodium ions in *R. marinus* bioenergetics was investigated. We prepared inside-out vesicles from *R. marinus* and demonstrated that the vesicles maintained an electrochemical K^+ potential imposed by K^+/valinomycin. The membrane potential driven by the addition of substrates NADH and succinate to *R. marinus* membrane vesicles was followed using the sensitive dye oxonol V. It was observed that the NADH-driven membrane potential was sodium ion dependent, while the build-up of a membrane potential during succinate oxidation seems not to be influenced by Na^+. To investigate the mode of Na^+ transport during NADH respiration, ^{23}Na in membrane vesicles was followed by NMR spectroscopy.

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S13.20 Structural characterization of respiratory complexes in potato tuber

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The aim of this study was to determine the structures of potato respiratory supercomplexes. Therefore, mitochondrial inner membranes from potato tuber cells were isolated, mildly solubilized with digitonin and the respiratory supercomplexes were separated by sucrose gradient ultra centrifugation. Finally, content of sucrose gradient fractions was inspected with Blue Native electrophoresis and electron microscopy. Single particle analysis of our data revealed several projection maps of complex I, monomeric and dimeric ATP synthase, supercomplex III_2+IV_1, supercomplex I+III_2 and larger unassigned supercomplexes. In some side-view projection maps of complex I the structure of carbonic anhydrase shows its trimeric features. Furthermore, one projection map revealed an extra unknown density at the intermembrane side of complex I. Top-view projection maps of I+III_2 supercomplex showed similar features found in other plant species including the presence of carbonic anhydrase. Besides the top-views, two different side-views and several angular views of the I+III_2 supercomplex were revealed which allowed a better assignment of interaction between complex I and III_2 within the supercomplex. The side-views of the largest supercomplex most likely do not represent the structure of the I+III_2+IV_1 supercomplex, also known as the respirasome. The largest particles represent probably a supercomplex composed of two copies of complex I and one copy of complex III_2.

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S13.21 Production, characterization, and determination of the real catalytic properties of the 'succinate dehydrogenase' from *Wolinella succinogenes*

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The genomes from both of the ε-proteobacteria *Wolinella succinogenes* and *Campylobacter jejuni* contain operons (*sdhABE* operons) that encode for hitherto uncharacterized enzyme complexes annotated as 'non-classical' succinate dehydrogenases. In the framework of a functional genomics project, a genetic system has been established for the homologous (over-)production and manipulation of the SdhABE complex from *W. succinogenes*. The catalytic properties of the purified enzyme were examined using various possible electron donor and acceptor substrates. Strikingly, for the SdhABE complex annotated as a 'succinate dehydrogenase', no succinate oxidation activity could be detected, neither with DCPIP, nor with methylene blue, nor with the high-potential quinone EQ-0 as electron donor. Although the complex catalyzes fumarate reduction with the menaquinol-6 analog 2,3-dimethyl-1,4-naphthoquinol ($DMNH_2$) the activities are very low. In addition to menaquinol-6, membranes of *C. jejuni* and of *W. succinogenes* contain a second quinol, 8-methylmenaquinol-6 (8-$MMKH_2$-6). Supplying an 8-$MMKH_2$-6 analog as a substrate increased specific quinol:fumarate reductase activity by about one order of magnitude. Furthermore, studies on variant enzymes demonstrated that the hydrophilic subunits of the complex are, in contrast to all other members of the superfamily, exported into the periplasm via the tat-pathway. Our studies reveal that the putative succinate dehydrogenase is in fact a novel periplasmic 8-methylmenaquinol:fumarate reductase with no detectable succinate dehydrogenase activity. These results provide an explanation for apparently puzzling previously published observations on the regulation of the *C. jejuni sdhABE* operon.

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