

S10.28 Differences in H₂O₂ production, $\Delta\psi_m$, JO₂, electron transport chain enzyme activities and glutamate release in synaptosomes isolated from 6- and 18-month old rats

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Detrimental changes to mitochondrial function have been shown to occur with age. The free radical theory of aging attributes the acquired dysfunction to the stress caused by increased levels of ROS production. In this study we examined the levels of H₂O₂ production in synaptosomes and identified markers of age related damage to nerve terminal mitochondrial function, including $\Delta\psi_m$, JO₂ and electron transport chain enzyme complex activities in rats of two age groups, 6 and 18 months old. The rate of H₂O₂ production in synaptosomes was found to be higher in the 18-month old group compared to that of the 6-month old group, but were not different in isolated nonsynaptic mitochondria from the two groups. $\Delta\psi_m$ and JO₂ were found to be significantly lower in synaptosomes obtained from the brains of 18 month old rats. Measurement of the individual electron transport chain complex enzyme activities revealed reduced complex II/III and complex IV activities. In addition, Ca²⁺-independent glutamate release was found to be increased at a lower threshold level of complex I inhibition in the older synaptosomes, suggesting an increased susceptibility to low concentrations of inhibitor (rotenone). These data suggest that aging leads to increased nerve terminal reactive oxygen species production while simultaneous deleterious effects occur on the bioenergetic capabilities of *in situ* synaptosomal mitochondria.

doi:10.1016/j.bbabbio.2008.05.254

S10.29 Regulation of H₂O₂ generation by uncoupling protein UCP1 in bat and thymus mitochondria

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Teleologically, activated uncoupling proteins (UCPs) would be expected to decrease reactive oxygen species (ROS) generation by the mitochondrial respiratory chain. In our work we applied an Amplex Red/H₂O₂ assay to test the ability of UCP1 to regulate ROS generation in rat mitochondria isolated from brown adipose tissue and thymus. Our data show that inhibition of UCP1 by GDP caused a significant increase in ROS generation by brown adipose tissue (BAT) mitochondria. This effect was most apparent in mitochondria respiring on succinate under state 2 conditions (in the absence of rotenone which would allow reverse electron transport), where UCP1 inhibition by GDP caused a ~26 fold ($p < 0.01$) increase in ROS generation. In the presence of rotenone the increase was still significant but was reduced to 4.6 fold. In parallel with H₂O₂ generation measurements, membrane potential was monitored by uptake of fluorescent probe Safranin. We are currently performing equivalent experiments using BAT mitochondria isolated from UCP1^(-/-) mice and their control littermates to establish whether we can confirm our observation in rat mitochondria and thus whether UCP1 activity could influence ROS production in brown adipose tissue.

doi:10.1016/j.bbabbio.2008.05.255

(S11) Terminal oxidases symposium lecture abstracts

S11/1 The proton pumping machinery of cytochrome c oxidase

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Cytochrome c oxidase (Cyt cO) is a membrane-bound enzyme, which catalyzes the four-electron reduction of O₂ to H₂O and energetically couples this reaction to proton pumping across the membrane. When addressing the proton-pumping mechanism of Cyt cO it is particularly constructive to investigate structural variants of Cyt cO in which O₂ is reduced to water, but the catalytic reaction is uncoupled from proton pumping. There are two classes of such mutant Cyt cOs, (i) in which the catalytic turnover rate is dramatically slowed due to impaired proton uptake, and (ii) in which it is similar to that of the wild-type Cyt cO. While in case (i) the uncoupling could be conveniently explained in terms of delayed protonation of a "pump site" due to the slowed proton uptake, in the latter case the reason for uncoupling is related to changes in intrinsic thermodynamic/kinetic parameters associated with specific reaction steps within the enzyme. Rationalizing these changes at a molecular level offers mechanistic insights into the structural elements involved in the pumping machinery of Cyt cO. In my talk I will present results from studies of several class (ii) mutant Cyt cO and discuss the molecular mechanism of proton pumping.

doi:10.1016/j.bbabbio.2008.05.256

S11/2 Biogenesis of cytochrome c oxidase — bacterial approaches to study cofactor insertion into subunit I

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Biogenesis of cytochrome oxidase is a complex process involving more than 30 known accessory proteins in yeast. Here, we focus on the process of cofactor insertion into subunit I of cytochrome c oxidase, using the soil bacterium *Paracoccus denitrificans* as a model organism. CtaG, the *Paracoccus* homolog of yeast Cox11 with a presumed role in copper delivery to the Cu_B center, was purified and characterized spectroscopically. A previously unreported signal at 358 nm allows monitoring copper transfer from copper-loaded CtaG to an acceptor. To mimic a potential cotranslational insertion process, a cell-free expression system has been established, producing subunit I in good yield in the presence of detergents. With such an "open" system it will be feasible to trap and purify assembly intermediates after directly adding individual cofactors, purified assembly proteins, or *P. denitrificans* membranes. Homologous gene loci specifying Surf1 have been identified in *Paracoccus*, and located in two operons for terminal oxidases: *surf1q* is the last gene of the *qox* operon (coding for a *ba*₃-type ubiquinol oxidase), and *surf1c* is found at the end of the *cta* operon (encoding subunits of the *aa*₃-type cytochrome oxidase). Using single and double deletion strains for both *surf1* genes, we show that both copies are functional, but strictly serve their cognate oxidases only. Cytochrome c oxidase was purified from double deletion strain membranes, and the loss of heme *a*₃ in the active site indicates that Surf1c, though not indispensable for oxidase assembly, is involved in an early step of cofactor insertion into subunit I.

doi:10.1016/j.bbabbio.2008.05.257