Oxidative Phosphorylation & Mitochondrial Metabolism

3812-Pos

Coupled Electron and Proton Transfer in Complex I and Complex IV of the Respiratory Chain: Insights from Computer Simulations

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I will discuss coupled transport of electrons and protons in two key enzymes of the electron transport chain of aerobic cells: NADH dehydrogenase (Complex I) and cytochrome c oxidase (Complex IV), which are, respectively, the entry point, and the terminal enzyme in the respiratory chain.Computer simulations and theoretical modeling of ET/PT reactions in these enzymes provide important insights into the molecular mechanisms of these redox-driven proton pumps.

3813-Pos

Electrophysiology of Functional Coupling of Electron Transport Chain Complexes

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Electron transport chain in the inner mitochondrial membranes consists of four multisubunit protein complexes CI to CIV which are coupled via electron carrier molecules as well as protein-protein interactions. The coupling of the complexes is essential for the proper functioning of the chain and may be an important factor for regulation and balancing of respiration, ATP synthesis and production of reactive oxygen species. However, direct functional studies on the action of the respiratory chain in native surroundings are limited due to the poor accessibility via standard electrophysiological equipment. We performed electrophysiological analyses of electron transport chain in native inner mitochondrial membranes using the solid-supported membranes (SSM) and the SURFE^{2}R technology. The inner mitochondrial membranes were purified from pig heart mitochondria by sucrose gradient fractionation and adsorbed onto SSM sensors. The chain complexes were activated either by NADH for the studies of CI-CIII-CIV coupling, or by succinate and cytochrome *c* for the analysis of the CII-CIII function. The tested proteins were pharmacologically characterized using specific substrates and inhibitors. Serial application of different inhibitors as well as the coenzyme Q analogs decylubiquinone revealed a tight functional interplay between the complexes CI, CIII, and functional coupling to the complex CIV. The complexes CII and CIII were also functionally coupled. An excess of the coenzyme Q analog idebenone had stimulating effect on the CII-CIII activity but was reducing the CI-CIII-CIV-specific currents. In summary, the presented results demonstrate an easy and reliable approach for studying the complex functional interplay of mitochondrial transport proteins in their native environment, and can help to understand the physiology of different mitochondrial functions. Since different assay conditions can be tested on the same sensor, the technology allows highly effective comparative analysis of the different complex activities.

3814-Pos

Flash Initiated Redox Events within Cytochrome bc1 Suggest Equilibration between Hemes b: Effects of Temperature, Viscosity, Inhibitors and Substrates

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As an integral member of all major electron transfer chains, cytochrome bc_1 functions as a proton-motive pump; transferring protons across the membrane via oxidation of quinol substrates. Though a Q-cycle establishes the general consensus mechanism for the enzyme, several key questions remain. Namely, the nature of electron bifurcation at the Q_o site, and the potential for intermonomer electron equilibration and communication across the dimer. Through rapid, flash initiated oxidation of a cytochrome c surrogate, Ru_2D, we have investigated electron transfer between the iron sulfur center and cytochrome c_1; as well as the subsequent redox events of the cytochromes b via turnover at the Q_o site. Moreover, by modulating the extent of b heme reduction prior to flash initialized events, we are able to study the re-oxidation of heme b_H over an extended range of conditions. Herein, we report the effects of such variables as temperature, viscosity, inhibitors and substrates on the flash initiated redox events of cytochrome bc_1, with an emphasis on the redox events of the b hemes. Toward such ends, we show that a single turnover at the Q_o site can effectuate the oxidation of two equivalents of heme b_H, suggesting equilibration between the two low potential chains. This work was supported by NIH Grant GM20488 and RR15569.

3815-Pos

In Yarrowia Lipolityca Mitochondria the Association of NADH Dehydrogenase Type II with the Cytochrome Complexes Depends on the Growth Phase

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In Yarrowia lipolytica mitochondria, the electron transport from NADH to O2 is branched by alternative respiratory components. One external NADH dehydrogenase (NDH2e) and an alternative oxidase (AOX). Both enzymes are peripheral single-subunit oxido-reductases not implicated in proton gradient formation. Thus, if electrons pass through those two enzymes, the oxidation of NADH is not able to conserve energy. During exponential growth, this is undesirable; however, during the stationary phase this process may help to maintain a high rate of oxygen consumption. To prevent the electron flux between alternative components, either AOX may be interacting with the complex I or NDH2e with complexes III and IV. We have evaluated the participation of the alternative components on electron transport and on supramolecular structures of mitochondria from wild type and a αnubm mutant, where complex I is inactive and NDH2 was redirected to the matrix side (NDH2i)4. In order to determine whether there are specific interactions between NDH2e and other respiratory complexes, we measured oxygen consumption rates with different respiratory substrates and inhibitors. We suggested an interaction of NDH2e (but not NDH2i) with cytochrome complexes, indicating that the interaction sites are located in the intermembrane face of the cytochromic complexes. Furthermore, we identified by native PAGE, in-gel activity and mass spectrometry this interaction between NDH2e with complexes III and IV in the wild type. Also, larger supercomplexes5 and a complex V dimer were found. This association pattern seems to vary during the stationary phase as NDH2e is overproduced saturating its binding site in Cyt IV and thus appearing as the free enzyme.

3816-Pos

Halophilic Properties of Mitochondria from the Salt-Tolerant Yeast Debaryomyces hansenii

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The osmotolerant, oleaginous and metabolically versatile yeast Debaryomyces hansenii is considered a marine organism. Sea water contains 0.6 M Na+ and 10 mM K+; large quantities of these cations permeate into the cytoplasm of D. hansenii. Therefore, proteins and organelles within the cytoplasm have to adapt to high salt concentrations. Given the choice, D. hansenii accumulates K+ instead of Na+ but both cations seem to have the same effects. The effect of high concentrations of K+ or Na+ on isolated mitochondria from D. hansenii was explored. The mitochondrial respiratory chain from D. hansenii contains the canonical respiratory complexes (I, II, III and IV), plus a cyanide-insensitive alternative oxidase and an external flavone-sensitive NADH dehydrogenase type II. As in S. cerevisiae, these mitochondria undergo a phosphate-sensitive permeability transition (PT), although D. hansenii mitochondria require higher phosphate concentrations to avoid PT. In regard to K+ and Na+, and at variance with mitochondria from all other sources known, these monovalent cations promoted closure of the putative mitochondrial unspecific channel (MUC) as evidenced by the K+/Na+-promoted increase in: respiratory control, transmembrane potential and synthesis of ATP. Thus, in D. hansenii mitochondria K+ and Na+ optimize oxidative phosphorylation, providing an explanation for the higher growth efficiency exhibited by this yeast when exposed to saline environments. Thus, we propose that halophilia is conferred to cells at the subcellular level. It is becoming increasingly evident that the functions and the control mechanisms of MUCs might be different depending of the species under study.

3817-Pos

"Structure and Dynamics of the External Stalk of the FoF1-ATP Synthase"

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The FoF1-ATP synthase is the enzyme responsible for the bulk of ATP synthesized in most organisms. Although the structure and mechanism of the enzyme is generally well-understood, some important intricacies remain unclear. One of the questions still heavily discussed concerns the structure and function of the external stalk which consists of two identical or non-identical subunits b in bacteria and photosynthetic organisms.

Making use of structure prediction, de novo modeling, extensive mutagenesis, site-directed spin labeling and ESR spectroscopy, we suggested that the cytosolic, soluble parts of both the E. coli homodimeric b2 as well as

a heterodimeric bb'from a cyanobacterial ATP synthase exist as traditional left-handed coiled coils for most of their lengths. Upon binding of the soluble E. coli b2 to soluble F1, the tether region of the b-dimer disengages to form a more loosely packed arrangement that then seems to repack closely before entering the membrane-phase.
Using the completely assembled F1Fo-ATPase, we recently observed changes in the packing interactions of b2 during the formation of ATP-hydrolysis transition states. These transitions were observed through the release of spin label catalyzed by non-modified cysteine on the adjacent b-subunit. Only those spin-labels were released that were predicted by us to be close to or at the left-handed coiled coil b-dimer interface, while no release of label from positions that were predicted to be at the outer surface of the helices was observed. The results suggest that while modest conformational changes of the b2-dimer occur during catalysis, the dynamics of these changes do not appear large enough to support a left-handed to right-handed coiled coil conversion that has previously been suggested.

3818-Pos
Partial Reactions of the ATP Synthesis Reaction in the E. Coli βD380C Mutant F1Fo-ATP Synthase
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The initial stage of ATP synthesis by the *E. coli* wild type (WT) and mutant βD380C F_1F_o-ATPase was compared at saturating substrate concentrations and a proton-motive force (*pmf*) of ~315 mV. The enzymes catalyze both ATP hydrolysis (Baylis Scanlon et al., 2008, *J. Biol. Chem.* 283, 26228-26240) and ATP synthesis with similar steady-state parameters. ATP synthesis by the WT enzyme proceeded with neither burst nor lag-phase while ATP synthesis by βD380C (+DTT) showed a burst phase with a stoichiometry of ATP/F_1F_o equal to 1. The burst was simulated using a kinetic model in which the βD380C mutant has a less than 10-fold increase in Pi binding rate in combination with a less than 10-fold decrease in ATP release rate compared to WT. Resolution of the burst allows us to distinguish the partial reactions of the ATP synthesis pathway.
Comparison of the ATP release rates in ATP hydrolysis (Baylis Scanlon et al., 2007, *Biochemistry* 46, 8785-8797) and ATP synthesis suggest differences in the cooperative behavior in the two directions of the reaction. In ATP hydrolysis, positive cooperativity in catalysis is induced by nucleotide (ATP) only, while in ATP synthesis it is induced by both nucleotide (ADP) and the *pmf*. Fast steady-state ATP hydrolysis proceeds through a trisite mechanism, while ATP synthesis uses a bisite mechanism.
We further analyzed the effects of the mutation by forming a stator-rotor disulfide cross-link, βD380C-γC87 induced by DTNB. The cross-linked enzyme catalyzed absolutely no ATP synthesis in the millisecond time domain after inducing a *pmf*. This result suggests that γ subunit mobility is required for the ATP synthetic reaction to occur and is consistent with the model that high affinity Pi binding cannot be achieved without $\Delta\mu_H^+$-dependent γ-subunit rotation.

3819-Pos
Electric Field Driven Torque in ATP Synthase
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Dysfunction of mitochondrial enzymes, including ATP synthase, has been implicated in type-2 diabetes, cancer, heart disease, and neurodegenerative diseases. Our electric field driven torque (EFT) model of ATP synthase predicts a scaling law relating torque to the number of proton binding sites in the rotor (*c*-ring) and the proton motive force (pmf) across the mitochondrial inner membrane. When the F_O complex of ATP synthase is coupled to F_1, the model predicts a critical pmf to drive ATP production. In order to fully understand how the electric field resulting from the pmf drives the *c*-ring to rotate, it is also important to examine the charge distribution on the protonated *c*-ring and in the *a*-subunit, which contains the proton half-channels and acts as a stator. A self-consistent field approach is used in our calculations, based on a refinement of reported ac_{12} structural data. The calculations reveal changes in pKa for key residues on the *a*-subunit and *c*-ring, as well as titration curves and protonation state energy diagrams. Implications for the EFT model will be discussed. Support was provided by R21CA133153 from NHLBI and NCI at NIH and from NSF, and by grant E-1221 from the R. A. Welch Foundation. Additional support was provided by the State of Texas through the Texas Center for Superconductivity and the Norman Hackerman Advanced Research Program.

3820-Pos
Cardiac Pacemaker Cells Uniquely Match ATP Supply to Demand
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Sinoatrial node cells (SANC) within the heart's pacemaker govern the heart rhythm and contraction due to constitutively active, Ca^{2+}-activated adenylyl-cyclases (AC) that generate a high level of cAMP/protein kinase A (PKA) dependent, localized, submembrane-compartmentalized Ca^{2+}- and AC-cycling. This rhythmic Ca^{2+} cycling generates rhythmic action potentials (APs), which initiate Ca^{2+} release and SANC contraction. To drive the heart beat SANC must tightly regulate ATP production to supply sufficient ATP for cAMP production, Ca^{2+}-cycling and contraction. To explore how ATP supply-demand is tightly balanced in isolated SANC we measured ATP levels: in control; during PKA-inhibition by specific inhibitor peptide PKI (15μM) or by H-89 (6μM); during intracellular Ca^{2+}-buffering by BAPTA (25μM); during muscarinic-receptors (MR) activation by carbacol 1μM ± MR-inhibition by atropine (10μM). PKA-inhibition by PKI or H-89, which blocked the major ATP-consumption processes, depleted ATP by 45 ± 6% and 44 ± 5%, respectively. Thus, complete inhibition of these major ATP consumption processes in SANC significantly reduces ATP levels **even though** ATP consumption is reduced. Buffering intracellular Ca^{2+} depleted ATP by 54 ± 8%. Hence, Ca^{2+} not only has direct effects on the surface membrane potential to ignite APs, and to activate myofilament-displacement/force production, but also regulates ATP production. In contrast to SANC, in either stimulated (3Hz) or quiescent rabbit ventricular cells the same experimental perturbations had only minor (<6%) effects on ATP levels. These control mechanisms identified by pharmacological-perturbations are apparently utilized in nature, because MR-stimulation of SANC also depleted the ATP by 45 ± 10% (by blocking the ATP-consumption processes). Notably, atropine, a MR-antagonist, substantially reversed the MR-stimulation effect, resulting in only 18 ± 3% ATP depletion. These data suggest that in contrast to ventricular cells, the same signals that drive, and are derived from, the utilization of ATP in SANC also tightly couple the production of ATP to match energy demand.

3821-Pos
Analysis of Intracellular ADP Compartmentation Reveals Functional Coupling between Pyruvate Kinase and ATPases in Rat Cardiomyocytes
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Cardiomyocytes have intracellular diffusion restrictions, which spatially compartmentalize ADP and ATP. According to mathematical models, diffusion restrictions are localized in certain areas of the cell. However, the models so far have used data sets generated on rat heart permeabilized fibers, where diffusion distances may be heterogeneous. This is avoided when using isolated, permeabilized cardiomyocytes. The aim of this work was to analyze the intracellular diffusion of ATP and ADP in rat permeabilized cardiomyocytes. Intracellular energetic communication between mitochondria and ATPases was approached from several angles, where respiration rate, ATPase rate and ADP concentration in surrounding solution were determined under several conditions. The data was analyzed by mathematical models reflecting different levels of cell compartmentation. In agreement with previous studies, we found significant diffusion restriction by the outer mitochondrial membrane and confirmed a functional coupling between mitochondria and a fraction of ATPases in the cell. In addition, our experimental data shows that a considerable activity of endogenous pyruvate kinase (PK) remains in the cardiomyocytes after permeabilization. Intriguingly, a fraction of ATPases was inactive without ATP-feedback by this endogenous PK. When analyzing the data, we were able to reproduce the measurements only with the mathematical models that include a tight coupling between fraction of endogenous PK and ATPases. To our knowledge, this is the first time such a strong coupling of PK to ATPases has been demonstrated in permeabilized cardiomyocytes indicating the importance of glycolysis in energy production for cardiac function.

3822-Pos
Diffusion Restrictions in Cardiomyocytes from Low-Performance Heart
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In adult mammalian cardiomyocytes, intracellular diffusion restrictions affect metabolic regulation. Despite extensive studies on rat cardiomyocytes, their cause and role in vivo is still unclear. Intracellular membrane structures may play a role. Previous studies suggest that rainbow trout permeabilized