Electron Transfer Systems

1547-Pos Effect of Q_o site Inhibitor JG144 on Electron Transfer Reactions of Cytochrome bc_1

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Board B523

Long range movement of the iron-sulfur protein (ISP) between the cytochrome b (cyt b) and cyt c_1 redox centers plays a key role in electron transfer within the cyt bc_1 complex. JG144 is a new cytochrome bc_1 Q_o site inhibitor that immobilizes the ISP in the b conformation. Electron transfer in the cyt bc_1 complex was studied using the ruthenium dimer, Ru_2D, to rapidly photooxidize cyt c_1 and initiate the reaction. Flash photolysis of a solution containing reduced wild-type *Rhodobacter sphaeroides* cyt bc_1 and Ru_2D results in photooxidation of cyt c_1 within 1 μs, followed by electron transfer from the iron-sulfur center [2Fe2S] center to cyt c_1 with a rate constant of $k_1 = 60{,}000\ s^{-1}$. JG144 binding to the Q_o site decreases k_1 to $10{,}000\ s^{-1}$ indicating that a conformational change on the surface of cyt b decreases the rate of release of the ISP from cyt b. The effects of JG144 binding on electron transfer were also studied in a series of mutants in the cyt b *ef* loop of *Rhodobacter sphaeroides* cyt bc_1 in order to examine the role of this loop in controlling the capture and release of the ISP from cyt b.

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1548-Pos Mutagenesis of Cytochrome f Y160 and R156: Effects on Redox Properties

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Board B524

Protein functional groups interacting with heme propionates have often been suggested to affect the redox properties of the heme. Structures of cytochrome f show one of the heme propionates to interact with Y160 and R156. To examine whether such interactions influence the redox properties of the cytochrome f heme, we have performed site directed mutagenesis of these sites in the lumenal domain of cytochrome f from Chlamydomonas reinhardtii. A Y160L mutant was found to shift the redox potential of cytochrome f by −20–30 mV. Such a shift could be due to either the loss of a hydrogen bond between the Y160 phenolic group and the heme propionate or due to the loss of aromaticity at this position. To answer this question, we have prepared a Y160F mutant of cytochrome f and found its redox potential to be identical to that of the wild type protein. We conclude that the loss of aromaticity at this position and not the loss of a hydrogen bond to the heme propionate is responsible for the redox potential change in the Y160L mutant. The same heme propionate is also observed to interact electrostatically with R156. A R156L mutant has also been prepared. In contrast to the other mutants in which the reduced form is stable to air, the resulting R156L mutant is highly susceptible to air oxidation, suggesting that the loss of the electrostatic interaction between R156 and the heme propionate significantly alters of the protein's redox properties.

1549-Pos Cation-Pi Interactions in Cytochrome f: Relation to Electron Transfer Between Cytochrome f and Plastocyanin

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Structures of cytochrome *f* show the presence of two cation-pi interactions which appear to be in locations of potential structural or functional importance. One is the K165/Y171 interaction which is located at the interface of the large and small domains of cytochrome f. The other interaction is the R13/Y149 interaction which appears to help position the heme binding loop relative to the rest of the large domain. At last year's meeting, results were presented indicating these interactions to be contributors to the stability and redox properties of cytochrome f. To assess whether these interactions might also influence the electron transfer properties of cytochrome *f*, we have performed of site directed mutagenesis of these sites in the water soluble lumenal domain of cytochrome *f* from Chlamydomonas reinhardtii. R13 and K165 were replaced with I, A, and G in an S106C mutant designed for covalent attachment with photoactive Ru-complexes. The mutants were labeled with Br-CH2-(bpy)Ru(bpy)2. Flash illumination results in rapid electron transfer from the excited state of the Ru-complex to the heme. When plastocyanin is included, the subsequent electron transfer from the cytochrome f to the plastocyanin copper can be observed. Only minor differences were observed among the mutants when compared to wild type suggesting that the K165/Y171 and R13/Y149 cation-pi interactions do not have a major impact on the interaction of plastocyanin with cytochrome f nor on electron transfer between the two proteins.

1550-Pos Stigmatellin Induces Reduction of Iron Sulfur Protein in the Oxidized Cytochrome bc_1 Complex

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Stigmatellin, a Q_P site inhibitor, inhibits electron flow from iron-sulfur protein (ISP) to cytochrome c_1 in the cytochrome bc_1

complex. The binding of stigmatellin to the complex raises the midpoint potential of the ISP from +290 mV to +540 mV. The binding of this inhibitor to the fully oxidized complex, oxidized by catalytic amounts of cytochrome *c* oxidase and cytochrome *c*, results in the reduction of the ISP. The amount of ISP reduced is proportional to the amount of inhibitor used and reaches to a maximum when the ratio of inhibitor to enzyme complex reaches unity. A g = 2.005 EPR peak, characteristic of an organic free radical, is also observed when stigmatellin is added to the oxidized complex and its signal intensity is linearly dependant on the amount of stigmatellin used. Addition of ferricyanide, a strong oxidant, to the oxidized complex also results in the generation of a g = 2.005 EPR peak that is oxidant concentration dependant. Oxygen radicals are generated when stigmatellin or ferricyanide is added to the oxidized complex in the absence of an exogenous substrate, ubiquinol. The amount of oxygen radicals formed is proportional to the amount of stigmatellin or ferricyanide added. Based on these results, it is proposed that ISP becomes a strong oxidant upon stigmatellin binding, and can extract electrons from an organic compound, likely an amino acid residue, resulting in the reduction of the ISP and generation of organic radicals. This organic radical then reduces molecular oxygen to form superoxide radical.

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1551-Pos Spectral Properties of Intermediates Generated during the Reactions of the *Rhodobacter sphaeroides* Cytochrome *c* Oxidase with Dioxygen

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The reaction of dioxygen with fully reduced cytochrome *c* oxidase (aa_3) from *Rhodobacter sphaeroides* was investigated at room temperature following photolysis of the CO-bound enzyme. Time-resolved optical absorption difference spectra (post-minus prephotolysis) were collected by a gated multichannel analyzer in the Soret and visible regions (300–700 nm) between 100 ns and 500 ms after photolysis. Singular value decomposition (SVD) and global exponential fitting gave six apparent lifetimes, 1.3 μs, 18 μs, 38 μs, 100 μs, 1.1 ms and 20 ms. Spectra of the intermediates involved in different electron transfer steps were extracted based on the conventional mechanism, which involves the sequential formation of the fully reduced enzyme $\mathbf{R_s}$, the dioxygen-bound compound $\mathbf{A_{R,s}}$, the so-called $\mathbf{P_{R,s}}$ intermediate, the oxyferryl state $\mathbf{F_s}$, and the oxidized enzyme $\mathbf{O_s}$. The extracted intermediate spectra were compared to analogous spectra for the bovine heart aa_3 oxidase and to bench-made model spectra of the intermediates of *R. sphaeroides* aa_3. The spectrum of the *R. sphaeroides* $\mathbf{A_{R,s}}$ intermediate is different from that of $\mathbf{A_{R,s}}$ of the bovine enzyme and the former is best modeled with a linear combination of 20% **R** and 80% **A.** The spectrum of the postulated $\mathbf{P_{R,s}}$ intermediate is also different between the two enzymes and neither equals that of the model **P** spectrum. While the spectrum of the $\mathbf{P_{R,s}}$ in the bovine enzyme is best modeled at pH 7.4 with the spectra of **A**, **P** and **F** in equal ratios, the spectrum of the $\mathbf{P_{R,s}}$ in *R. sphaeroides* aa_3 is best modeled with a mixture of 45% **A** and 55% **F**. These results suggest that the mechanism of dioxygen reduction to water in both enzymes may be more complex than the previously proposed conventional sequential mechanism.

1552-Pos Synthesis and Characterization of Cross-Linked Cu (II)-Complexes as Models of the Active Site of Cytochrome *c* Oxidase

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Board B528

The role of the post-translationally modified tyrosine in heme-copper oxidases continues to be a subject of great interest. In order to provide insight into the nature of the cross-linked tyrosine, we have synthesized tridentate cross-linked histidine-phenol ether and ester ligands and the corresponding Cu(II) complexes, which are chemical analogs of the active site of several heme-copper oxidases. The structure of the Cu-complexes was verified by single-crystal X-ray analysis. The geometry about the copper atom is a tetragonal distorted square pyramid, with three nitrogens (pyridine, imidazole, and amine) and a chloride in a square plane, and a weakly coordinated chloride ion in an axial position (Cu-Cl: 2.75 Å). Spectrophotometric titrations of the Cu-complexes showed a single pK_a value of 7.8 for the phenolic proton, which is significantly lower than that of unperturbed tyrosine (10.1). Time-resolved optical absorption studies indicated the formation of phenoxyl radical in the tridentate ligands upon UV photolysis, which was quenched in the copper complexes. FTIR double-difference spectra, generated following UV photolysis of the tridentate ether Cu-complex and its ^{13}C(6)-complex, are consistent with the formation of a phenoxyl radical. This suggests that Cu_B^{2+} and the putative cross-linked tyrosyl radical formed during dioxygen reduction may not be spin-coupled through the cross-linked Cu_B-His ligand at the enzyme active site. The cross-linked tyrosine may play a critical role in the catalytic function of cytochrome oxidase by providing an electron and a proton for the cleavage of the dioxygen bond, thus possibly preventing the generation of reactive oxygen species (ROS).

1553-Pos Probing Transient Conformational Changes in Rhodobacter sphaeroides Cytochrome c Oxidase Subunit III with a Thiol-Reactive Fluorophore

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Board B529

Cytochrome *c* oxidase (COX) uses electrons from ferrocytochrome *c* to reduce molecular O_2 to H_2O, conserving the energy of this redox reaction by vectorial proton transport across the bacterial or inner-mitochondrial membrane. The possible role of transient conformational changes in COX subunits during its catalytic cycle is not currently understood. In this work, the transient dynamic properties of *Rhodobacter sphaeroides* COX were monitored during steady-state electron transfer using the thiol-reactive fluorophore 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (IAEDANS). COX treated with IAEDANS (COX-AEDANS) exhibited labeling solely in subunit III, as shown by SDS-PAGE, and had an [AEDANS]:[COX] stoichiometry of 2.0±0.1 (n=3), indicating that on average two of the three native cysteines in subunit III were labeled. Slow enzymatic turnover of oxidized COX-AEDANS at pH 9.6 was initiated by addition of cytochrome *c* and ascorbate (TN=$2s^{-1}$), and the COX redox state was monitored kinetically by absorbance spectroscopy. A stable mixture of COX redox states was present during the 4 minutes prior to oxygen depletion when COX became fully reduced (as confirmed by dithionite addition). Fluorescence intensity of COX-AEDANS was also monitored at 471nm (an isosbestic point for COX and cytochrome *c*) under the same conditions. Upon addition of cytochrome *c*/ascorbate, a rapid decrease in fluorescence was observed until steady-state conditions were reached. This decreased fluorescence intensity (10%±0.3%, n=5) continued for the duration of steady-state turnover until anaerobsis occurred, at which point the fluorescence increased to 95%±0.2% (n=5) of the starting fluorescence intensity. COX-AEDANS inhibited by 5.5mM KCN or injected with buffer instead of electrons did not demonstrate these reversible fluorescence changes. This data indicates that the subunit III conformational states in the oxidized, reduced and actively catalyzing forms of COX are distinct.

1554-Pos Energy Transfer Dynamics Of F-Chlorosomes In An Electrochemical Cell

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Board B530

The main focus of this study is to investigate the energy transfer dynamics of the light antenna structure derived from filamentous green bacteria (F-chlorosome) at the bioelectronic chlorosome-electrode interface in an electrochemical cell. While energy transfer within the *Chloroflexus aurantiacus* bacterium, which contains the F-chlorosome as its main light harvesting component, has been extensively studied in its native cellular environment, efforts to harness its extraordinary light capturing function in an isolated artificial setting has been limited. F-chlorosomes are uniquely flattened, ellipsoidal bodies with nanoscale dimensions that utilize aggregates of bacteriochlorophyll-*c* molecules as its main light-harvesting element. Here, isolated F-chlorosomes are characterized for their electron transfer properties in solution via an apparent physical adsorption to an electrode surface and by a direct covalent immobilization to an indium tin oxide electrode surface using various electrochemical techniques. Under physical adsorption conditions, initial chronoamperometric studies show that after adsorption a repeatable photocurrent of ~1–2 nA/cm^2 from the electrode is observed. Electrochemical impedance spectroscopy confirms a change in the electron transfer properties of the electrode for immobilized systems, where interfacial resistance to electron transfer decreases by ~10 9 /cm^2 in the dark. Preliminary studies suggest that the photocurrent for covalently immobilized F-chlorosomes on indium tin oxide electrodes increases with light intensity with additional enhancement from the base electrode system. The observed photocurrents from immobilized F-chlorosome light antenna systems, which do not contain any reaction centers, suggest that given close proximity to the electrode a direct energy transfer could occur without any intermediary electron transfer components as found in nature. The results from this study will lay the foundation for the novel biophotonic devices with applications in biofuel cells, biosensors, and retinal prostheses.

1555-Pos Electrical Detection of Photosynthetic Activity in Leaves and Thylakoid Suspensions

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Board B531

We report on measurements of the fundamental (linear response) and higher harmonics (nonlinear response) generated by thylakoids and whole plants in response to low-frequency sinusoidal electric fields. Their temporal behaviors exhibit features that correlate extremely well with physiological activity, particularly light-activated photosynthetic processes. Systematic studies of the 1st, 2nd, and 3rd harmonics generated by thylakoid suspensions indicate the following.

1. When the photosynthetic electron transport chain is activated by light in the presence of a suitable electron acceptor, such as ferricyanide, temporal changes in harmonic response for fixed frequency and amplitude correlate with light activation of photosynthetic activity.
2. These features also correlate with oxygen evolution during photosynthesis.

Finally, in whole plants, the observed temporal evolution and frequency-dependence of light-activated harmonic generation suggest that the method may be able to selectively probe specific photosynthetic processes.

1556-Pos Role Of External And Matrix pH In Mitochondrial ROS Generation

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Board B532

Imbalance of cellular redox homeostasis is implicated in development of a number of pathologies such as diabetes, heart failure, neurodegenerative diseases, and chronic obstructive pulmonary disease. Mitochondria as a major source of cellular reactive oxygen species (ROS) have been a spot light in current cell biology. The aim of this work was to study the effect of pH as a factor linked directly to the mitochondrial ROS generation. ROS generation was explored on a model of isolated rat brain mitochondria by using succinate as a substrate and manipulating matrix pH by inorganic phosphate and nigericine either in distinct incubation media, or in the same medium during incubation, and using the ionophores differently affecting matrix pH. Under more physiological conditions when glutamate and malate were used as substrates the role of pH in ROS generation was shown to be qualitatively the same, as well as after complete depolarization induced by an inhibitor of complex I rotenone. The present results demonstrated that matrix pH is an essential factor, which determines ROS production by mitochondrial respiratory chain. The revealed phenomena outline the details of general ROS production mechanism; the data obtained could be used for further theoretical analysis aimed in the most detailed description of mitochondrial electron transport accompanied by ROS production.

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1557-Pos Isoflurane Produces Reactive Oxygen Species, Trigger of Anesthetic-induced Preconditioning, by Inhibition of Complex I in Rat Cardiac Mitochondria

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Board B533

Background: Pharmacological pretreatment with volatile anesthetics, such as isoflurane, protects the myocardium against ischemia/reperfusion injury. It is well known that reactive oxygen species (ROS) play an important role in anesthetic-induced preconditioning (APC). Recent evidence suggests that a small amount of ROS which are generated by mitochondria during the preconditioning phase stimulate protective pathways against cardiac injury; however, the exact origin and mechanism of ROS stimulation by isoflurane are not fully understood. Here, our aim was to elucidate mechanisms of isoflurane-induced initial ROS generation as trigger for APC.

Methods: Effects of isoflurane were tested in isolated cardiac mitochondria from the rat. The rate of mitochondrial ROS production was measured spectrofluorometrically by using the fluorescent probe Amplex red in the presence of horseradish peroxidase, and mitochondrial oxygen consumption was measured with a Clark-type oxygen electrode.

Results: The rate of ROS production was significantly increased (21.6 %) in the presence of isoflurane when electrons were delivered to complex I, using pyruvate and malate as substrates. On the other hand, the rate of ROS production was attenuated by isoflurane when electrons were delivered to complex II, using succinate as substrate. This attenuation is mimicked by complex I inhibitor rotenone, suggesting that isoflurane is inhibiting reverse electron flow through complex I. Further, isoflurane significantly inhibited states 2, 3 and 4 respiration with pyruvate and malate as substrates. However, this inhibitory effect of isoflurane on the respiration was not observed with succinate as substrate in the presence of rotenone.

Discussion: These results suggest that isoflurane generates ROS by affecting complex I of the electron transport chain in cardiac mitochondria, which may in part underlie the initial mechanism of ROS production as the trigger for APC.

1558-Pos Detection of Specific Mitochondrial Targets of Isoflurane in Isolated Cardiomyocytes by Measurement of NAD(P)H and Flavoprotein Autofluorescence

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Board B534

Background: There are controversial data about volatile anesthetic's effect on mitochondrial bioenergetics. Among others, inhibition of electron transport chain complexes or uncoupling of mitochondrial respiration have been reported. Here, we investigated the effect of isoflurane on the redox state of NADH and flavoproteins (FPs), participants in distinct electron pathways during mitochondrial respiration.

Methods: We simultaneously measured kinetics of NAD(P)H/$NAD(P)^+$ and FP changes in isolated rat cardiomyocytes by detection of the corresponding autofluorescence signal in response to different doses of the volatile anesthetic isoflurane. These effects were compared to the effects of various mitochondrial toxins with well-characterized targets within the electron transport chain. NAD(P)H and FPs were excited via two-photon laser (720 nm) and a visible laser (488 nm), respectively.

Results: DNP (100 μM) and FCCP (4 μM) as mitochondrial uncouplers both induced oxidation of FPs and NAD(P)H. On the other hand, cyanide (4 μM), an inhibitor of complex IV of the electron transport chain, blocked the electron flux downstream from NAD(P)H and FPs and therefore induced reduction of both. Inhibition of complex I by rotenone (1 μM) reduced $NAD(P)^+$ by blocking NADH oxidation on complex I while FP redox state was unaltered. TTFA (0.25 mM), a complex II blocker, induced strong oxidation of FPs, and just partial oxidation of NAD(P)H. Similar to DNP and FCCP, isoflurane induced oxidation of FPs and NAD(P)H indicating a depolarizing effect of isoflurane on mitochondria. Interestingly

however, the oxidation rate of NAD(P)H decreased with increasing isoflurane concentration. This implies a dose-dependant inhibition of complex I of the electron transport chain by isoflurane.

Conclusion: Isoflurane exhibits a dual effect on mitochondria, leading to depolarization of mitochondria and inhibition of complex I.

Oxidative Phosphorylation

1559-Pos Modeling Mitochondrial Energetics and Ion Dynamics

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During ischemia, the intracellular environment becomes more acidic and Na^+ loading occurs, affecting not only EC coupling but also the energy supplying machinery. Because ion transport determines mitochondrial pH, volume, and oxidative phosphorylation, we extended a previous single mitochondrion model to account for the dynamics of Na^+, H^+ and phosphate exchange in addition to Ca^{2+} transport, TCA cycle flux and oxidative phosphorylation. The computational model is described by 13 ordinary differential equations, in which the kinetics of each ion carrier has been studied individually to match its behavior in *in vitro* assays. Na^+ transport through the Na^+H^+ exchanger has been modeled as a six-state compulsory order kinetic mechanism. Phosphate transport occurs through a Pi:OH transporter represented by a random bireactant kinetic scheme. The behavior of the assembled mitochondrial model is analyzed and compared with experiments performed with isolated mitochondria. The level of TCA cycle intermediates and the measured flux through the cycle as reported in the literature are well reproduced by the model under steady state conditions. The range of respiratory fluxes simulated corresponds to the physiological range determined in isolated mitochondria. Under transient conditions induced by pulses of ADP or protonophoric uncouplers, the model simulates a decrease in mitochondrial membrane potential ($\Delta\Theta_m$), pyrimidine nucleotide (NADH) levels and an increase in respiratory rate. Conversely, the addition of substrate elicits reduction of the pyridine nucleotide pool and polarization of the membrane. This more comprehensive computational model of mitochondrial energetics and ion transport will improve our ability to study the dynamic changes in EC coupling and energetics during ischemia-reperfusion or metabolic acidosis in the future.

1560-Pos Mathematical Model Of Mitochondrial Energy Metabolism

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Board B536

We present a model of the energy metabolism of the isolatedmitochondrion. The model is composed of a system of ordinarydifferential equations describing the Krebs cycle, oxidativephosphorylation and membrane transport processes. Enzyme catalyzedreactions of the Krebs cycle and some carrier proteins in the model aredescribed by expressions corresponding to their kinetic schemes. Thisassures that the specific reactions adhere to physical limitations setby the overall catalytic capacity of the mitochondrial matrix enzymes.For respiratory chain enzymes an approach based on the thermodynamic properties of the reaction and the system has been taken.In addition to enzymatic reactions the model also takes into account the buffering of protons and metal ions in detail.

The resulting system is optimized to fit the data of several experiments performed on isolated mitochondria. Optimization process consists of applying genetic algorithms and gradient descent methods for finding the set of parameters best suited for reproducing experimental results. Model calculations performed with these optimum parameters are able to fit experimental data and, more importantly, are able to reproduce results from experiments not included in the optimization process.

1561-Pos Different Steady State Kinetics Of Regulation Of Mtck-dependent Respiration In Isolated Mitochondria And Permeabilized Cardiac Cells In Situ:Local Restrictions Of Atp And Adp Diffusion At Outer Mitochondrial Membrane And Enhanced Functional Coupling Of Mtck With Ant

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This work was done to understand better the reason of differences of regulation of mitochondrial respiration in vitro and in permeabilized cardiac cells in situ. It is well known that in permeabilized cells apparent Km for exogenous ADP in control of mitochondria respiration is significantly higher (usually 300 – 400 μM) than in isolated mitochondria (10 – 20 μM). We studied the role of mitochondrial outer membrane in cardiomyocytes in situ for functional coupling between mitochondrial creatine kinase (MtCK) and adenine nucleotide translocase (ANT) following up endogenous ADP fluxes and MtCK kinetics in presence of trapping system PEP-PK for free ADP. In isolated heart mitochondria more than 50% of endogenous ADP recycled by MtCK is trapped in medium by PEP-PK system with apparent Kmapp for MgATP about 0.2–0.3 mM. However, in situ in permeabilized cardiomyocytes, endogenous ADP generated in MtCK reaction is inaccessible for ADP trapping system. This gave us a unique opportunity to perform complete kinetic analysis of the coupled MtCK reaction in the cells in situ by