at rates comparable to that of the wild-type cells. Although the bc_1 complex can be purified from both mutants, the c_1-14G-IV_{His} gave a better yield and higher activity. This purified fusion complex contains four protein subunits, has higher activity, and is more stable toward detergent treatment than the wild-type enzyme. Thus, it is suitable for the structure determination of the entire four-subunit complex. The x-ray crystallographic study of this fusion complex is in progress. This work was supported in part by a grant from NIH (GM30721).

1230-Pos Board B74

Crosstalk between Mitochondrial Malate Dehydrogenase and Cytochrome bc_1 Complex

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The cytochrome bc_1 complex (bc_1) catalyzes electron transfer from ubiquinol to cytochrome *c* with concomitantly translocating protons across the membrane to generate a proton gradient and membrane potential for driving ATP synthesis. Recently we found that mitochondrial soluble matrix proteins could increase the activity of bc_1 complex. To identify the protein(s) that is responsible for the activity enhancement, the purified, detergent dispersed bc_1 complex was incubated with soluble mitochondrial matrix proteins followed by an extensive dialysis in the absence of detergent to pull down the interacting protein(s) with bc_1 complex upon centrifugation. SDS-PAGE analysis of the precipitate showed that several proteins from matrix were in the precipitates in addition to the subunits of bc_1 complex. One of the matrix proteins with molecular weight of 35.6 kD was identified to be mitochondria malate dehydrogenase (MDH) by MALDI-TOF Mass spectrometry. The identification of MDH was further confirmed by western blot with anti-MDH antibody. Incubating purified MDH with detergent dispersed bc_1 complex increases activities of bc_1 compelx and MDH. The effect of bc_1 complex on the activities of MDH is unidirectional (oxalacetate —> malate). This novel crosstalk between citric acid cycle enzymes and electron transfer chain complexes might play a regulatory role in mitochondrial bioenergetics. This work was supported in part by a grant from NIH (GM30721).

1231-Pos Board B75

Mechanism of Internal Proton Transfer Reactions in Proteins

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Proton transfer reactions are crucial in a large array of biomolecular processes, encompassing bioenergetics, biological signaling, and enzymatic catalysis. We performed a proof of concept study regarding the mechanism of internal proton transfer reactions between buried groups in proteins. A model system, that resembles the active site structure of a PAS domain bacterial photoreceptor protein, is employed in our study. A first principles approach without adjustable parameters was used to identify the energy landscape for internal proton transfer. We will report the fundamental aspects (structure, energetics, and kinetics) of the proton transfer mechanism from our study. It is expected that this mechanism may be applied to a broad range of proton transfer systems.

1232-Pos Board B76

A Simple Model for Amphiprotic Water and Proton Transfer Reactions

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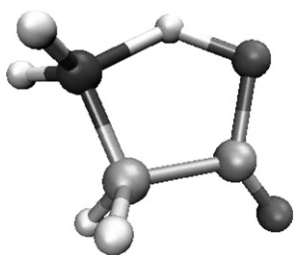
Proton transfer is important for chemistry in general and for protein function in particular. Water is often involved as a donor, a receptor, or an element in a chain of concerted transfers. Recently we have shown that the amphiprotic behavior of water can be captured by a simple model that is inspired by the traditional Lewis construct. The model comprises explicit and fully charged oxygen cores, valence electron pairs, and protons, all interacting via pair-wise pseudo-potentials that reflect Heisenberg uncertainty and Pauli exclusion. These independently mobile particles produce stable neutral, protonated and deprotonated water clusters. They also exhibit transport of protons and proton-holes through water chains. A self-consistent extension of the model to nitrogen hydrides provides a description of ammonia that forms hydrogen bonds and transports protons. Further generalization to include carbon allows us to build ''Lewis'' amino acids. In in vacuo simulations, initially zwitterionic forms of the amino acids evolve to non-ionic forms via an intramolecular proton transfer. The intermediate in this process is a 5-member ring with the migrating hydrogen bridging the amine nitrogen and a carboxyl oxygen (see figure).



1233-Pos Board B77

Substrate Dependent Mitochondrial pH Changes During Oxidative Phosphorylation

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Introduction: Several processes influence mitochondrial matrix pH such as the state of respiration (states 2,3,4), uncouplers, proton leak, flux of other ions, and substrate utilized. We compared changes in matrix pH during phosphorylation of ADP to ATP (state 3) in the presence of NADH-linked substrate pyruvate (10 mM) or $FADH_2$-linked substrate succinate (10 mM+rotenone). **Methods:** Guinea pig heart mitochondria were isolated through differential centrifugation and loaded with BCECF-AM to measure matrix pH by fluorescence spectrophotometry. Respiration, NADH, and $\Delta\psi_m$ were also measured. **Results:** Addition of either substrate caused matrix alkalinization. Addition of ADP (250 μM) to initiate state 3 respiration caused a marked decrease in matrix pH, which was larger (% max ΔpH with CCCP) and longer in succinate/rotenone ($46 \pm 1\%$, 55 ± 4 s) vs. pyruvate ($20 \pm 3\%$, 28 ± 2 s). Decreases in NADH and $\Delta\psi_m$ during state 3 were also larger and longer with succinate/rotenone than pyruvate. On conversion of all ADP to ATP (state 4), all variables returned to state 2 levels. Corresponding values for O_2 consumption (states 2,3,4 in μmol/hr/mg) for succinate/rotenone and pyruvate, respectively, were: 3.4 ± 0.1, 12.7 ± 0.4, 4.4 ± 0.2, and 0.9 ± 0.04, 12.8 ± 0.6, 1.2 ± 0.06. **Conclusion:** The degree and extent of matrix acidity is dependent on ADP phosphorylation rate, TCA turnover rate, and the number of reducing equivalents produced (proton pumping). Per electron pair, there are 10 H^+ pumped per NADH and 6 H^+ pumped per $FADH_2$. The substrate -induced differences in pH during state 3 may be due to the differences in number of protons pumped by pyruvate vs. succinate (+rotenone). A mechanistic model of mitochondrial bioenergetics and pH handling may help to characterize these differences.

1234-Pos Board B78

Redox Potential of the Outer-Mitochondrial Membrane 2Fe-2S Protein MitoNEET

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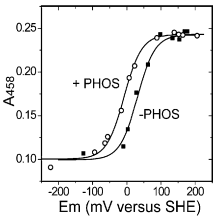
MitoNEET was recently discovered as a binding target for the anti-diabetes drug pioglitazone (1). It harbors a pH-labile 2Fe-2S cluster coordinated by three cysteines and one histidine (His87) (2). We measured a pH-dependent redox potential of +35 mV (pH 7.5) that lies intermediate between most low potential 4Cys-coordinated ferredoxin-like centers (~-300 mV) and most high potential 2Cys-2His-coordinated Rieske centers (~+300 mV) (3). In addition, its redox potential was ~40mV lower in the presence of phosphate ions. This can be explained by binding of a phosphate ion near the cluster as reported elsewhere (Homer, poster). The H87C mutant, which becomes 4 Cys coordinated, has a more negative reduction potential similar to a ferredoxin (~ -200mV). Our results show that the redox potential is sensitive to the coordination of the cluster and that mitoNEET's unique coordination geometry is likely essential for its unknown redox function.



Optical redox titration curves showing the absorbance of the 2Fe-2S cluster at 458 nm versus electrochemical potential in a cell containing 100 μM mitoNEET in pH 7.5 Tris (squares) and Phosphate (open circles).

(1) Colca et al. (2004) Am J Physiol Endocrinol Metab 286 E252-E260.
(2) Paddock et al. (2007) Proc Natl. Acad. Sci USA 104, 14342-14347.
(3) Meyer (2008) J Biol Inorg Chem 13, 157-170
Supported by NIH (GM41637, GM54038 and DK54441).

1235-Pos Board B79

Noninvasive Approach For Quantitative Analysis Of Energy Metabolism And Mitochondrial Anomalies In Living Cells

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Reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) as key metabolic cofactors in energy metabolism in eukaryotic cells. As a result, there has been recent resurgence in using these

intrinsic cofactors as natural probes for diagnostic purposes in cancer, diabetes, apoptosis, and neurodegenerative diseases. Here, we used autofluorescence dynamics assay to quantify the concentration and enzyme binding of intrinsic NADH and FAD in living cells. In these studies, cancer (Hs578T) and normal (Hs578Bst) breast cells are used as model systems to examine the sensitivity of the proposed assay to cell pathology. Our non-invasive, quantitative assay includes multiphoton microscopy and spectroscopy of NADH and FAD. Two-photon fluorescence lifetime imaging of cellular autofluorescence, in a calibrated microscope, is used to construct NADH and FAD concentration images in live cells. In addition, time-resolved associated anisotropy of cellular autofluorescence provides direct quantification of the molar fractions of free and enzyme-bound cofactors in both normal and transformed cells. Targeted inhibition of complex IV of the electron transport chain in Hs578Bst, using KCN, confirm the sensitivity of cellular autofluorescence to changes in the respiratory state activities. Comparative studies of the binding kinetics of NADH with mitochondrial malate dehydrogenase and lactate dehydrogenase in solution mimic our findings in living cells. This fluorescence dynamics assay on natural coenzymes (i.e., without the need for exogenous fluorescence dyes) is applicable to other metabolic and signaling pathways in live cells, which contrasts with conventional biochemical techniques that require cell destruction.

1236-Pos Board B80

Small but Statistically Reliable Magnetic Field Effect Observed in the Recombination of a Non-Correlated Pair of Biologically Relevant Radicals Nitric Oxide and Superoxide Anion

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Magnetic and spin effects, well studied in photo- and radiation-generated chemical systems involving spin-correlated radical pairs, are often called upon as possible mechanisms underlying magnetic effects (MFE) in highly complex biological systems. Although several biologically relevant systems do exist for which this had been indeed verified, often the radical pair mechanism is invoked solely based on the presence of radicals, such as nitric oxide, in the system. This logic has three serious problems: the complexity of the real biological systems, the lack of correlation in thus reacting radicals, and their difference from ''normal'' partners of the spin-correlated pairs. To address all these issues we created a model chemical system of nitric oxide and superoxide radical recombining to produce peroxynitrite, and studied MFE in it.

The radicals were produced as a pair via decomposition of 3-morpholinosydnonimine (SIN-1) in aqueous phosphate buffer. MFE was monitored by comparing the efficiency of peroxynitrite production in exposed and otherwise identical control samples with additional temperature controls. We used static magnets with induction 0.5T and 4.7T. No statistically significant effects were found in the field 0.5T and in temperature controls. In magnetic field 4.7T magnetic field effect of $(1.8 \pm 0.5)\%$ was obtained.

The effect is small, as expected for a non-correlated pair, but statistically reliable. It is apparently limited by extremely fast relaxation of nitric oxide in liquid due to unquenched orbital momentum in the diatomic molecule with electronically degenerate ground state, and develops in f-pairs via the Δg mechanism. Any MFE due to radical pair involving nitric oxide in biological system would probably require either rather strong magnetic field in the Tesla range, or some internal enhancer of magnetic field.

Oxidative Phosphorylation & Mitochondrial Metabolism

1237-Pos Board B81

Intracellular Diffusion Restrictions In Trout Cardiomyocytes

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Rat cardiomyocytes are compartmentalized by barriers that restrict intracellular diffusion of adenine nucleotides. The exact localization of these diffusion barriers is unknown. Some possible candidates for diffusion restriction are t-tubules, sarcoplasmic reticulum (SR) and outer mitochondrial membrane. Further, rat cardiomyocytes have several parallel rows of mitochondria and myofilaments wrapped in SR, and it is possible that peripheral mitochondria and SR restrict diffusion to more central parts of the cell. Diffusion is facilitated by the creatine kinase system. Trout cardiomyocytes lack t-tubules and have a much more sparse SR. Additionally, single cardiomyocytes have only one layer of myofilaments surrounding a central core of mitochondria. We take advantage of the structural differences between rat and trout cardiomyocytes to study intracellular diffusion restrictions further. We measured the apparent ADP-affinity of trout skinned ventricular fibres at different temperatures to cover the physiological range for rainbow trout. Measurements were performed in the absence and presence of creatine to test whether diffusion is facilitated by the creatine kinase system. Our results show that trout cardiomyocytes are characterized by a low ADP-affinity. The affinity is temperature-dependent and increases with temperature. Creatine increases affinity at all temperatures, but the affinity in the presence of creatine is also temperature-dependent. The low ADP-affinity suggests that diffusion restrictions also exist in trout cardiomyocytes despite their structural difference with much more sparse membrane structures. This makes trout cardiomyocytes a useful model to study intracellular diffusion restrictions further.

1238-Pos Board B82

Compartmentation of ATP in Cardiomyocytes and Mitochondria Kinetic Studies and Direct Measurments

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High energy demand of heart and brain cells is met by mitochondrial ATP production and energy transfer between ATP compartments mostly by creatine kinase (CK) - phosphocreatine (PCr) system. The aim of this work is to study the diffusion restrictions of ATP at the level of mitochondrial outer membrane as a basis of its compartmentation in cardiomyocytes. In a first part channelling of adenine nucleotides in mitochondria is studied via the direct transfer of the novel synthesized ATP from the adenine nucleotide translocase (ANT) to the mitochondrial creatine kinase (MtCK) by performing the complete kinetic analysis of the MtCK. A partial reconstruction of mitochondrial cytoskeletal environment was performed by incubation of heart isolated mitochondria (apparent Km for exogenous ADP $= 9 \pm 1$ μM) with 1 μM tubulin (apparent Km for exogenous ADP $= 169 \pm 52$ μM) without or with 20 mM creatine (apparent Km for exogenous ADP $= 23 \pm 6$ μM). The results showed a clear restriction in adenine nucleotide diffusion in presence of tubulin by the change of the profile of respiration kinetic linearization. This restriction was found to be overcome by the presence of creatine which can activate the MtCK reaction and increase the rate of ADP/ATP turnover due to functional coupling between MtCK and ANT. In a second part the method of fluorescence correlation spectroscopy was used to study the diffusion kinetics of ATP-Alexa 647 both in solution, isolated mitochondria and cardiomyocytes. However pronounced multicomponent diffusion kinetics was found both in isolated mitochondria and permeabilised cardiomyocytes. This diffusion time seemed to be sensitive to the ordered state of cardiomyocyte and increased when the symmetry was broken in the rounded or apoptotic cells. The fluorescent ATP was use to study ATP compartmentation.

1239-Pos Board B83

Kinetic Studies of Intracellular Compartmentalization in Permeabilized Rat Cardiomyocytes

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Cardiomyocytes are compartmentalized by intracellular barriers restricting diffusion. The aim of this work is to gain insight into diffusion restrictions in a heart muscle cell. We present a complete set of data, where mitochondrial respiration and its interaction with ATPases has been recorded using oxygraph and spectrophotometer. Although some of these data have been published in the context of different studies, we performed all our measurements in parallel in order to collect a full set of data for each batch of cardiomyocytes. These data were used as input for our new mathematical model. The model describes the dynamics of metabolites during the respiration process assuming that the system can be described as having two compartments - the extracellular and the intracellular. For that a system of ordinary differential equations has been constructed and solved numerically. The model solution was optimized to fit the experimental data. The optimization process consists of applying genetic algorithms and least square method for finding the set of parameters best suited for reproducing experimental results. One of the parameters is the diffusion restriction influencing the rates at which metabolites move between the two compartments of the system. The model gives us estimation of the overall diffusion restriction between intracellular energetic units (ICEU) and extracellular