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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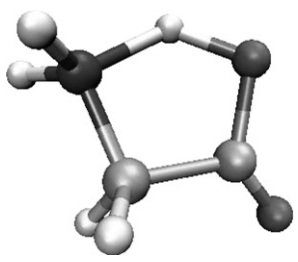
Proton transfer is important for chemistry in general and for protein function in particular. Water is often involved as a donor, a receptor, or an element in a chain of concerted transfers. Recently we have shown that the amphiprotic behavior of water can be captured by a simple model that is inspired 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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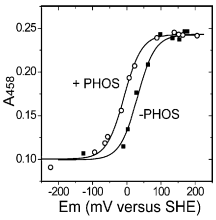
MitoNEET was recently discovered as a binding target for the anti-diabetes drug pioglitazone (1). It harbors a pH-labile 2Fe-2S cluster coordinated by three cysteines and one histidine (His87) (2). We measured a pH-dependent redox potential of +35 mV (pH 7.5) that lies intermediate between most low potential 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
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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