It has been, recently, proposed that Q_A moves and rotates ~60° upon its reduction.[1] Here we investigated possible changes using ENDOR spectroscopy that provides a very sensitive (to ~0.01 Å) probe for the binding site of $Q_A^{\bullet-}$.[2] Identical RC samples were made - (*a*) one frozen in the dark (ground state) and then illuminated generating $D^{\bullet+}Q_A^{\bullet-}$ and (*b*) one frozen under illumination (excited state) which trapped $D^{\bullet+}Q_A^{\bullet-}$ in ~70% of the RCs at 80 K. Figure 1 shows the resultant ^{1}H ENDOR spectra of $Q_A^{\bullet-}$. The peaks labeled L_1, L_2 and L_3 correspond to the two H-bonds to $Q_A^{\bullet-}$.[2,3] Essentially no differences in the ENDOR spectra were observed indicating that the interactions of $Q_A^{\bullet-}$ with the protein are the same in the ground state as in the excited state. These results are irreconcilable with the proposed rotation.[1] Thus, Q_A is preset in an environment that favors its reduction.

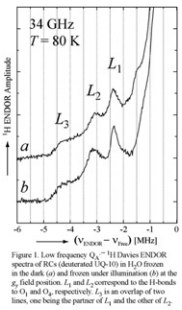

Figure 1. Low frequency $Q_A^{\bullet-}$ ^{1}H Davies ENDOR spectra of RCs (deuterated UQ-10) in H_2O frozen in the dark (*a*) and frozen under illumination (*b*) at the g_z field position. L_1 and L_2 correspond to the H-bonds to O_1 and O_4, respectively. L_3 is an overlap of two lines, one being the partner of L_1 and the other of L_2.

[1] Heinent et al. (2007), *J. Am. Chem. Soc*. **129**, 15935. [2] Flores et al. (2007), *Biophys. J*. **92**, 671. [3] Sinnecker et al. (2006), *Phys. Chem. Chem. Phys.* **8**, 5659.

1225-Pos Board B69

Demethyl Ubiquinone Inhibits Catalytic QB Activity In Reaction Centers From Rhodobacter sphaeroides*

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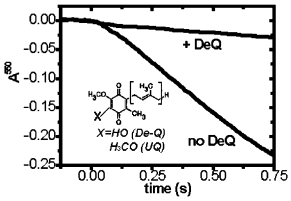
Catalytic light induced electron transfer in the photosynthetic reaction center (RC) involves reduction of the loosely bound secondary quinone Q_B. In this study we investigated the activity of demethyl ubiquionone (De-Q), the immediate precursor for the synthesis of ubiquinone (UQ), a critical component of the electron transport pathways in both prokaryotes and eukaryotes (1). Upon addition of 2 microM De-Q (K_D ~ 0.2 microM), catalytic Q_B activity was inhibited > 10-fold at pH 8 (Figure). These results show that De-Q binds more tightly than UQ thereby inhibiting its reduction. In addition, optical shifts of bacteriocholorophyll were observed consistent with an anionic De-Q; the solution pKa of De-Q was measured to be ~ 6 (not shown). We propose that De-Q near neutral pH is anionic which facilitates binding acting as a non-reducible analog of ubisemiquinone in the RC. (1) Poon *et al.* (1999) *J Biol Chem.* **274**, 21665-21672. *Supported by NIH (GM 41637).



Light induced electron turnover in the presence and absence of 2 microM DeQ (shown in inset); DeQ differs from UQ in that a titratable hydroxy group replaces a methoxy group.

1226-Pos Board B70

Kinetics and Energetics of Electron Transfer Reactions in a Photosynthetic Bacterial Reaction Center Assembled with Zinc Bacteriochlorophylls

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Electron transfer processes were studied in the reaction center (RC) of a *Rhodobacter sphaeroides* magnesium chelatase (*bchD*) mutant that assembles with six chemically identical chlorin molecules. A previous study [Jaschke & Beatty, Biochemistry, 2007] and this work show the complete absence of bacteriochlorophyll (containing Mg as the metal) and bacteriopheophytin from the *bchD* mutant RC. Instead, bacteriochlorophylls containing a Zn atom as the metal (Zn-BChl) occupy the binding sites of the special pair (P), accessory bacteriochlorophyll (B), and primary electron acceptor (H). In spite of significant differences in cofactor composition, electron transfer from excited P through B to H proceeds with high efficiency and with rates nearly identical to the wild type RC. The rate of electron transfer from H to Q_A is also the same as that observed in the wild type RC. Thus, the protein-cofactor interactions, mainly through electron sharing between the metal of the BChl and the protein, play an important role in adjusting the energies of the cofactors to form an efficient electron transfer system. The study also suggests that the overall electron transfer from P to H is more sensitive to the energy change between P and B than B and H, and can tolerate a large variation in the redox energy of H.

1227-Pos Board B71

Eseem And Hyscore Analysis Of Q_A- In Native And 15N Labeled Reaction Centers From *Rhodobacter sphaeroides*

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Reduction of the primary acceptor quinone (Q_A) in the photosynthetic reaction center *Rhodobacter sphaeroides* generates a semiquinone anion radical. This radical species has been studied using electron spin echo envelope modulation (ESEEM). Evidence has supported hydrogen bonding between the carbonyl groups of Q_A and nitrogen from His M219 and peptide nitrogen from Ala M260. In this study 3-pulse ESEEM and 2D-HYSCORE measurements on native and ^{15}N labeled reaction centers were used to directly measure the hyperfine interactions (hfi) between the semiquinone and surrounding nitrogen nuclei. 3-pulse ESEEM spectra of native reaction centers looked similar to previously reported results (Spoyalov et. al. 1996). Nuclear quadrupole coupling (nqc) produced a very complicated HYSCORE spectrum, but ^{15}N labeling eliminated the nqc and allowed for hfi to be measured. HYSCORE measurements showed the semiquinone coupled to 2 unique nitrogen nuclei. One set of cross peaks appeared far more intense in the spectrum. The difference in intensity suggests contributions to nitrogen coupling in addition to geometry.

1228-Pos Board B72

A Neutral Mutation Changes the Ionic Strength Dependence of the Rate of Electron Transfer between Cyt c2 and RCs from Rb. sphaeroides

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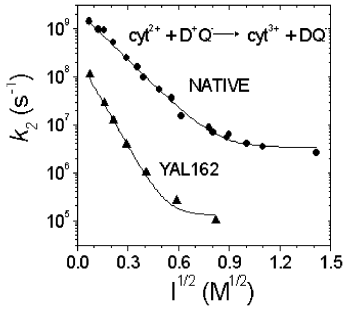
The contact between the electron transfer proteins cytochrome c_2 (cyt) and Reaction Center (RC) is centered on the hydrophobic residue Tyr L162. In the YAL162 mutant a greater ionic strength dependence of the second order electron transfer rate constant k_2 was found even though no charge changes were made. We explain this result by a transition state model (figure). For Native RC, k_2 is diffusion limited (electron transfer occurs before dissociation) and decreases with ionic strength due to increasing energy of the transition state for association. For YAL162 RCs, mutation of Tyr L162 increases the dissociation rate and decreases the electron transfer rate so that k_2 is in the fast exchange (pre-equilibration) regime (dissociation occurs before electron transfer). Here ionic strength effects are due to changes in binding energy, which are greater than changes in transition state energy (by ~2-fold) accounting for the steeper slope for the mutant. The decreased electron transfer rate due to this mutation demonstrates the importance of hydrophobic interactions in binding and electron transfer.



(1)Gong *et al.* (2003) *Biochem.* **42**, 14492.
*Supported by NIH(GM41637).

1229-Pos Board B73

Generation, Characterization And Crystallization Of A Subunit Iv Fused Mutant Cytochrome bc_1 Complex From *Rhodobacter Sphaeroides*

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The cytochrome bc_1 complex from *Rhodobacter sphaeroides* contains a three-subunit core complex and a supernumerary subunit (subunit IV). Although a 2.1 Å resolution x-ray crystallographic study of the wild-type complex has been achieved recently, the dissociation of subunit IV during crystallization has undermined structural information of subunit IV. To overcome this difficulty, we have constructed and characterized mutants with the N-terminus of subunit IV fused to the C-terminus of cyt. c_1 (c_1-IV fusion). A polyglycine (6 or 14 residues) linker was placed between the two involved proteins to ease the constraint that might result from the fusion of two subunits in the assembling process. A 6-histidine tag was placed at the C-terminus of IV (c_1-6G-IV_{His} and c_1-14G-IV_{His}) for the ease of purification. Both mutant cells grew photosynthetically

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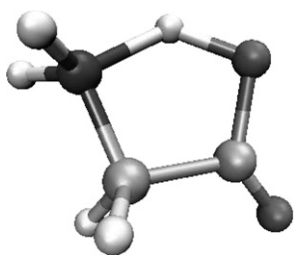
Proton transfer is important for chemistry in general and for protein function in particular. Water is often involved as a donor, a receptor, or an element in a chain of concerted transfers. Recently we have shown that the amphiprotic behavior of water can be captured by a simple model that is inspired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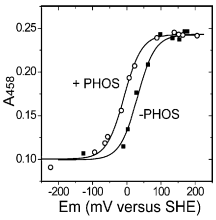
MitoNEET was recently discovered as a binding target for the anti-diabetes drug pioglitazone (1). It harbors a pH-labile 2Fe-2S cluster coordinated by three cysteines and one histidine (His87) (2). We measured a pH-dependent redox potential of +35 mV (pH 7.5) that lies intermediate between most low potential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