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.

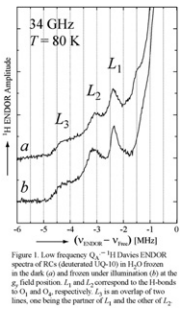

Figure 1. Low frequency $Q_A^{\bullet-}$ ^{1}H Davies ENDOR spectra of RCs (deuterated UQ-10) in H_2O frozen in the dark (*a*) and frozen under illumination (*b*) at the g_z field position. L_1 and L_2 correspond to the H-bonds to O_1 and O_4, respectively. L_3 is an overlap of two lines, one being the 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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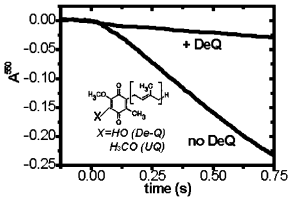
Catalytic light induced electron transfer in the photosynthetic reaction center (RC) involves reduction of the loosely bound secondary quinone Q_B. In this study we investigated the activity of demethyl ubiquionone (De-Q), the immediate precursor for the synthesis of ubiquinone (UQ), a critical component 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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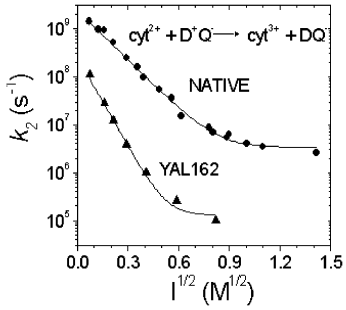
The contact between the electron transfer proteins cytochrome c_2 (cyt) and Reaction Center (RC) is centered on the hydrophobic residue Tyr L162. In the YAL162 mutant a greater ionic strength dependence of the second order electron transfer rate constant k_2 was found even though no charge changes were 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