High-Frequency EPR and ENDOR of Protein-Cofactor Sites Involved in Photosynthetic Electron Transfer

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The photosynthetic conversion of light into useful chemical energy involves rapid, sequential electron transfer resulting in charge separation. In photosynthetic bacteria, these light-initiated reactions occur in integral membrane proteins called reaction centers (RCs). Following photoexcitation of a bacteriochlorophyll dimer, P, the electron is transferred through one set of cofactors terminating in the electron transfer between two quinone molecules, Q_A and Q_B. Recently, we reported the first observation of time-resolved high-frequency (HF) ENDOR of the transient charge separated state $P^+Q_A^-$. These studies provide a novel approach for probing electron transfer and proton uptake pathways through the protein as well as protein reorganization events following photoinduced charge separation.[1,2] We are currently using HF ENDOR methodologies to explore the nature of low temperature $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer.

The electron transfer between Q_A and Q_B is a conformationally-gated, temperature-activated reaction that is coupled to proton movement. Interestingly, this reaction can be allosterically modulated by metal ion binding to a site on the RC surface.[3] As a result of necessary conformational changes, $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer is not observed at low temperature for RCs frozen in the dark. However, electron transfer between the quinones does occur below 40 K for RCs frozen under illumination. These observed alterations of reaction kinetics by illumination while cooling have been linked to trapping the RC in altered conformations induced by charge separation. We have initiated a detailed spectroscopic study of the heterogeneous polypeptide environments surrounding the redox cofactor site Q_B of *Rb. sphaeroides* RCs to explore the nature of low temperature $Q_A^-Q_B \rightarrow Q_AQ_B^-$ electron transfer. HF pulsed D-band (130 GHz) matrix Mims ENDOR has been applied to directly look for differences in protein environments surrounding the quinones in "active" vs. "inactive" conformations with respect to electron transfer.

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