wild-type RBS COX; however, the α-region of the reduced spectrum for E90H(SIII) displayed a slight red shift of the absorbance maximum by 1 nm, resembling that seen for the SIII-depleted oxidase. E90H(III) and E90H/H212(III) mutant proteins were determined to contain stoichiometric amount of each subunit by SDS-PAGE gel, similar to wild-type COX. Electron transfer rates observed at pH 6.5, for E90H(III)/H212E(III) and E90H(SIII) COX, were decreased by 17 and 39% respectively, as compared to WT COX. The mutants exhibit little or no suicide inactivation when compared to the SIII-depleted RBS COX. pH dependence of electron transfer activity for E90H(III)/H212E(III) and E90H(III) were also not altered when compared to WT enzyme. Proton pumping experiments on reconstituted E90H(III) and E90H(III)/H212E(III) in liposomes will be discussed.

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An Anhydrous Proton Transfer Pathway in the Cytochrome B6F Complex

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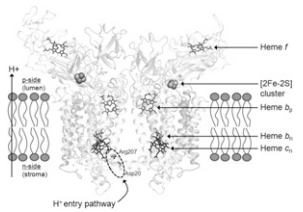
Cytochrome b6f, a hetero-oligomeric energy transducing membrane protein complex, catalyzes proton-coupled electron transfer reactions of the substrate quinone to generate as much as two-thirds of the total proton gradient used for ATP synthesis in oxygenic photosynthesis. Proton transfer pathways within b6f have remained unidentified due to limitations of crystallographic resolution1,2,3,4,5. using new crystallographic information based on a 2.70 Å crystal structure of the native complex, and structures obtained in the presence of quinone analogue inhibitors, tridecyl-stigmatellin (TDS) and 2-nonyl-4-hydroxyquinoline-N-oxide (NQNO) (resolution 3.07 Å and 3.25 Å respectively) seen in close proximity to heme cn on the electrochemically negative (n) side of the b6f complex, an anhydrous proton uptake pathway is defined that delivers protons from the n-side aqueous phase to plastoquinone bound at heme cn. A hydrated channel is identified on the electrochemically positive (p) side of the complex, that may provide a route for proton exit to the p-side aqueous phase. These n- and p-side pathways contribute to the first complete description of quinone-mediated transmembrane proton transfer.



1Kurisu et al. 2003, 2Stroebel et al. 2003, 3Yan et al. 2006, 4Yamashita et al. 2007, 5Baniulis et al. 2009

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Electrostatically Constrained Pathway of Intra-Monomer Electron Transfer in the Cytochrome B6F Complex of Oxygenic Photosynthesis

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The preferred pathway of trans-membrane electron transfer in the symmetric dimeric cytochrome b6f complex, involving four b-hemes organized as two pairs in symmetric monomeric units, was studied by simultaneous measurement of heme b redox state by absorbance measurement and heme-heme exciton interaction by excitonically split circular dichroism spectra in the Soret band. The rate of heme reduction by dithionite, in the dimeric or monomeric complex, coincides with an increase in amplitude of the split CD spectrum. The similarity of the time course of absorbance and CD changes, together with crystal structure information on inter-heme orientation and separation, imply that chemical reduction of the intra-monomer heme pair bn and bp occurs preferentially in the monomeric unit of the dimer. This preference is attributed to the negative free energy of the heme exciton interaction. The relatively small contribution of inter-monomer exciton interactions is attributed to the distance and angular dependence of the interactions. A slow rate of heme reduction is a consequence of an electrostatic energy barrier caused by injection of electrons into the apolar protein interior without compensating charge, inferred from an increased rate of heme reduction and generation of the split CD signal at acidic pH, a kinetic deuterium isotope effect, a red shift in the Q-band absorbance maximum of the integral chlorophyll a attributed to a Stark Effect associated with the internal electrical field, and a similar time course of heme reduction and generation of the Stark Effect. Thus, the Stark Effect arises from injection of electrons into the hydrophobic protein core without charge-compensation that would occur physiologically through H^+ uptake and translocation by the complex. Funding-NIH-GM038323. (SDZ and SSH share first authorship).

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Increased Superoxide Production in the Cytochrome B6F Complex: A Function for the Enigmatic Chlorophyll-A

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The structural basis for superoxide production in cytochrome bc complexes is relevant to understanding the mechanism of generation of deleterious reactive oxygen species and partition of electron transfer in the branched quinol oxidation pathway in cytochrome bc complexes. The specific rate of superoxide production, normalized to the the electron transfer rate, was determined for the yeast cytochrome bc1 complex (provided by B. L. Trumpower) and the cytochrome b6f complex from spinach thylakoid membranes and cyanobacteria. Although electron transfer rates were comparable in bc1 and b6f complexes, the specific rate of superoxide production was 10-20 fold greater in the b6f complex. Whereas antimycin A, a specific n-side quinone analogue inhibitor of the cytochrome bc1 complex, caused a large increase in the superoxide production rate of the bc1 complex, no comparable effect was found for NQNO, an n-side quinone analogue inhibitor in b6f, as defined by spectrophotometry and a crystal structure. These differences between bc1 and b6f complexes imply an increase in branching ratio for reduction by plasto-semiquinone of O2 to O2-, relative to reduction of heme bp for trans-membrane electron transfer. The change in branching ratio is ascribed to a longer semiquinone residence time in the p-side binding niche, due to steric restriction of the quinone binding site by the chlorophyll phytyl chain, as seen in a crystal structure. The presence of this phytyl chain can be seen to result in a smaller accessible volume for binding sites of a p-side quinone analogue inhibitor. The longer residence time of quinol/semiquinone would facilitate trans-membrane signaling, e.g., activation of n-side LHC kinase (NIH GM-38323).

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Investigation of Excited State Charge Redistribution of the Reduced Anionic Flavin in DNA Photolyase and Simple Solvents by Stark Spectroscopy

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The two-electron reduced anionic form of flavin adenine dinucleotide ($FADH^-$) is the catalytically active redox cofactor in DNA photolyase. This enzyme repairs UV-induced cyclobutane pyrimidine dimers (CPD) via a photo-induced electron transfer (PET) reaction, in which the $FADH^-$ is excited with blue light. The UV-visible absorption spectra of reduced anionic flavins, characterized decades ago, show a peak at ~350 nm with a broad shoulder at ~420 nm. We have shown, through earlier linear dichroism experiments, that these absorption bands arise from two distinct electronic transitions, a controversial result at the time. Here we have used Stark spectroscopy as a complementary approach to substantiate this result. The sensitivity of the amplitude and band shape of the Stark spectra unambiguously resolves the broad absorption band structure into two separate transitions; in fact, an analysis of the Stark data is not possible under the assumption of a single electronic transition. The magnitude of the difference dipole moment of the low energy $S_0 \rightarrow S_1$ transition (~420 nm) is about three times smaller than the $S_0 \rightarrow S_2$ transition (~350 nm). These experimental assignments were supported with TD-DFT calculations which help to assign the direction of charge redistribution. A picture emerges in which the xylene moiety of the isoalloxazine ring becomes electron-rich upon excitation into either absorption band. Interestingly, this part of the isoalloxazine ring is in close proximity to the bound CPD, suggesting that this is the site for PET to the CPD.

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Engineering and Tuning of Oxygen Reactivity in Heme Protein Maquettes

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There is a dearth of quantitative data related to reactive oxygen species (ROS) production, particularly superoxide, from protein systems. Understanding of ROS chemistry in proteins is necessary for avoiding damaging oxidative reactions involved in biological processes including aging and ischemic injury. These reactions are also necessary to control in order to develop robust artificial enzymes and photosystems for alternative energy production.

Here we show multi-faceted control over the oxygen reactivity of ferrous heme containing designed protein maquettes. We demonstrate control over superoxide production through both inner and outer sphere electron transfer (ET) mechanisms. Inner sphere ET occurs through oxygen binding and both oxygen