the optical trap was slightly less than that for Myo5-6IQ (25 nm) but much greater than for Myo5-2IQ (10 nm). Myo5-2IQ-SAH moves processively along actin at physiological ATP concentrations with similar stride length to Myo5-6IQ in TIRF microscopy assays, and the average run length was also similar. Stopped-flow fluorescence experiments indicated that unlike WT Myo5-6IQ the rear head did not mechanically gate the rate of ADP release from the lead head of the chimera and the rate of ADP dissociation was the same from both heads. These data show that the SAH domain can form part of a functional lever in myosins although its bending stiffness might be lower. We conclude that SAH domains can act as mechanically-competent structural extensions in physiological conditions and that gated dissociation of ADP from the lead head of myosin 5 is not required for processive movement.
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Electron & Proton Transfer

2907-Pos

Functional and Protein Processing Insights from a Truncation Mutation in Subunit III of Cytochrome *c* Oxidase from *Rhodobacter sphaeroides*

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A human mitochondrial DNA mutation in the Cytochrome c Oxidase (COX) subunit III (SIII) gene causes a truncation after its three n-terminal transmembrane helices and results phenotypically in severe lactic acidosis episodes. We created the equivalent mutation at position I115 in SIII of *R. sphaeroides* COX (I115stop). Truncated SIII enzyme was expressed and purified, and SDS-PAGE showed an absence of full length SIII and a doublet band of lower molecular weight which was immunoreactive to a SIII site-specific antibody. MALDI-TOF determined these peptide masses to be 12919 m/z and 11462 m/z, results consistent with a I115 truncation in SIII (12915 m/z) and subsequent proteolytic processing after F101 (11461 m/z). Wildtype COX subunit II is known to undergo protease processing *in vivo*, yielding different forms of the subunit (IIA, IIC). SDS-PAGE and MALDI-TOF showed higher levels of the less-processed IIC form in I115stop mutant preparations as compared to wildtype, which had higher levels of the IIA form. Functional assays show the I115stop mutant has a maximal electron transfer activity that is approximately 30% of wildtype (480 ± 90 e-/s*mol versus 1670 ± 180 e-/s*mol) and exhibits suicide inactivation similar to a form of the enzyme lacking SIII altogether (I/II_{OX}). The first three helices of SIII putatively contain conserved lipid binding sites, so the electron assays were then conducted in the presence of exogenous lipids. The I115stop mutant exhibited a greater stimulation of activity due to lipid than I/II_{OX} (23% versus 5%). Additionally, protection from suicide inactivation by lipid was 2.4 fold greater in the I115stop mutant than I/II_{OX}. Taken together, the results indicate that the truncation mutation alters native subunit II c-terminal processing, and they support the hypothesis that SIII is involved in functional lipid binding.

2908-Pos

Detection of a Proton-Dependent Electron Transfer from Cu_A to Heme a of Cytochrome C Oxidase Mutant S44e Using Ruthenium Photoexcitation

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Crystal structures, sequences, and homology models of mammalian, yeast, wheat, and *Thermus thermophilus* cytochrome c oxidases show a conserved glycine hydrogen bonded to a heme a histidine ligand, while the bacterial oxidases from *Pd* and *Rs* offer the hydroxyl group from a serine (S44) for hydrogen-bonding to the H102. In order to study the effects on electron transfer due to mutation of this position to a glutamate, a photoactivatable Ru probe was attached to cytochrome c, the natural redox partner of oxidase. A laser flash of less than 0.5 us reduced cytochrome c and allowed the measurement of individual steps of electron transfer from cytochrome c to Cu_A to heme a. The mutant exhibited two phases in the rate of electron transfer from Cu_A to heme a. Both phases had amplitudes and rates that were highly dependent upon pH indicating a protonation-deprotonation event of the glutamic acid residue. In combination with previous data obtained from other S44 mutants, including the S44D mutant, these results indicate that the heme a redox potential can be dramatically altered by a nearby carboxyl and its protonation leads to a proton-coupled electron transfer process. This work was supported by grants GM26916, GM20488 and NCRR COBRE 1 P20 RR15569.

2909-Pos

Exciton Interactions Between Hemes b_n and b_p in the Cytochrome b_6f Complex

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Circular dichroism spectra have been previously utilized (1) to analyze heme-heme interactions of the two *b*-hemes in the mitochondrial bc_1 complex that were predicted to bridge the 'B' and 'D' trans-membrane helices on the *n*- and *p*-sides of the cytochrome *bc* complexes (2). It was of interest in the context of the 3.0 Å structure of the b_6f complex (3-6) and its unique bound chromophoric prosthetic groups, Chl *a* that is 12 Å from heme b_p, and heme c_n that shares electrons with heme b_n, to analyze CD spectra of the heme Soret bands in crystallization-quality b_6f complex. Sources of the cytochrome b_6f complex were the cyanobacteria, *M. laminosus* and *Nostoc* PCC sp. 7120, and spinach thylakoids. In the crystal structures, the oxidized *b* hemes are separated by 20-21Å center-center and 7-8Å, edge to edge, and rotated relative to each other by approximately 55° about an axis almost normal to the membrane plane. A bi-lobed dithionite minus ascorbate-reduced CD difference spectrum, qualitatively similar to that seen in the mitochondrial bc_1 complex, was obtained from all three sources. Positive and negative bands on the blue and red sides of a 431 nm node, the peak of the absorbance difference spectrum, are diagnostic of excitonic heme-heme interactions. There is no significant contribution to these difference spectra from the Chl *a*, heme c_n, or the heme of cytochrome *f*. *deceased; 1, Palmer and Degli-Esposti, 1994; 2, Widger *et al*. 1984; 3, Kurisu *et al*. 2003; 4, Stroebel *et al*. 2003; 5, Yamashita *et al*. 2007; 6, Baniulis *et al*. 2009.

2910-Pos

Do Tyrosine Phenolic Groups Contribute to the Alkaline Transition in the Redox Potential of Cytochrome F?

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Cytochrome f, a c-type cytochrome involved in the photosynthetic electron transport chain, has a significantly higher redox potential than most other c-type cytochromes, ranging +370 to +380 mV. Like cytochrome c, cytochrome f also exhibits an alkaline transition in which the redox potential becomes pH dependent at high pH. In the case of cytochrome c, this has been attributed to replacement of the methionine sulfur serving as the sixth iron ligand by a deprotonated amino group. This cannot be the cause for the alkaline transition in cytochrome f as there is no methionine ligand to the iron, the sixth position being occupied by the N-terminal amino group. Three tyrosine phenolic groups (Y1, Y9, and Y160) are found in close proximity to the heme in the cytochrome f structure. To explore the possibility that the ionization of one or more of these tyrosines might be responsible for the alkaline transition, we have performed site directed mutagenesis, replacing each with a phenylalanine residue which lacks an ionizible group. Each of these mutants was found to have a redox potential of 375-380 mV at pH 7.0 which became pH dependent above pH 9.0 (apparent pKa 9.3). It thus seems unlikely that any of these tyrosine residues contributes to the alkaline transition of the redox potentials in cytochrome f. Redox properties of Y160L and R156L mutants will also be described.

2911-Pos

Characterization of the Secondary Quinone (Q_B) Binding Pocket in Photosynthetic Reaction Centers Using Pulsed EPR Spectroscopy

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3-pulse ESEEM and HYSCORE pulse sequences have been used to analyze the secondary electron acceptor semiquinone anion radical (Q_B^-). Photosynthetic reaction centers from *Rhodobacter sphaeroides* have identical ubiquinone molecules functioning as primary and secondary electron acceptors. The primary quinone radical (Q_A^-) has been extensively studied, and hydrogen bonds have been characterized at both carbonyls. The structure around Q_B^- has received less attention. The O_4 carbonyl has been suggested to be hydrogen bonded to the N_δ from a histidine at residue L190. The O_1 carbonyl also possesses a hydrogen bond that is weaker than that at O_4. OH from serine at L223 is important in the hydrogen bond structure at this carbonyl. However, contributions from surrounding peptide nitrogens are suggested by x-ray structures but have not yet been investigated by EPR methods. Pulsed EPR studies of the Q_B^- radical confirm one strongly coupled nitrogen with NQI frequencies consistent with a histidine N_δ. 3-pulse ESEEM and HYSCORE spectra also contain peaks from a second nitrogen nucleus. *A priori* knowledge of the origins of these peaks is less clear, but could include contributions from a backbone nitrogen. Additionally, NQI modulation from ^{14}N is sufficiently shallow to observe