an alanine residue. It was found that this substitution significantly altered the charge transfer band seen in wild-type QFR and SQR with oxaloacetate. The spectral and kinetic data are consistent with a loss of catalysis showing the importance of the H-bond to substrate in the mechanism of fumarate reduction and succinate oxidation by both SQR and QFR. The X-ray crystallography of the FrdA Thr234Ala enzyme also shows a dramatic domain rearrangement between the capping domain and flavin domain in FrdA. This movement opens a substrate channel to the active site of the enzyme by altering the capping domain position. A comparison of the location of the capping domain in the open and closed states in the mutant protein suggests that in complex II enzymes, movement of the domain may be coupled to stabilization of the transition state by the threonine side chain.

doi:10.1016/j.bbabio.2008.05.346

S13/3 Activated Q-cycle as a common mechanism for the cytochrome bc_1 and cytochrome b_6f complexes

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The aim of our studies is to understand the electron/proton coupling in the cytochrome bc_1 complex (bc_1). The functioning of bc_1 can be described by a dimeric Q-cycle scheme. The main traits of such a mechanistic scheme, which we have been developing during the last decade, are (i) the possibility of electron exchange between the monomers, (ii) the alternating ubiquinol binding in two ubiquinol-oxidizing centers *P*, (iii) the electrostatic compensation of electron transfer up to the ultimate step of the center *N*-catalyzed ubiquinol formation that is coupled with major voltage generation. Based upon own data on the kinetic correlation between the flash-induced redox changes of cytochrome *b*, voltage generation, and proton transfer in membrane vesicles of *Rhodobacter capsulatus*, we have put forward a scheme of a dimeric, activated Q-cycle. This scheme implies that under physiological conditions the bc_1 is maintained in an "activated" state, with a bound semiquinone in center *N* of one monomer and a reduced high-potential heme *b* in the other monomer, owing to continual priming by oxidation of membrane ubiquinol via center *N*. If bc_1 is pre-activated, then, in accordance with experimental observations, oxidation of each ubiquinol molecule in center *P* leads to ubiquinol formation in the one of enzyme's centers *N* and to the voltage generation. The applicability of this scheme to the plant cytochrome b_6f-complexes will be discussed.

doi:10.1016/j.bbabio.2008.05.347

S13/4 Structural and biochemical characterisation of the alternative oxidases

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In addition to the conventional cytochrome *c* oxidase, plant mitochondria contain a nonprotonmotive alternative oxidase (AOX) that couples the oxidation of ubiquinol directly to the reduction of molecular oxygen. In thermogenic plants, AOX is responsible for heat generation, whilst in non-thermogenic species, the oxidase is thought to play a more fundamental role in the regulation of energy metabolism. AOX may be involved in facilitating TCA cycle turnover, protection against oxidative stress, and preservation of plant growth homeostasis. AOX proteins are not restricted to plants, but also occur in pathogenic organisms including the blood parasite *Trypanosoma brucei* and the intestinal parasite *Cryptosporidium parvum*. Because of their absence in the mammalian host, AOX proteins are potential therapeutic targets in these systems. Although no high-resolution AOX structure is available to date the accepted structural model predicts that AOX is an integral (~32 kDa) interfacial membrane protein that interacts with a single leaflet of the lipid bilayer, and contains a non-haem diiron carboxylate active site. This model is supported by extensive site-directed mutagenesis studies and EPR spectroscopic experiments have confirmed the presence of a binuclear iron centre. This talk will focus on the recent identification of other residues and regions important for enzyme catalysis, access of oxygen to the active-site and ubiquinol-binding.

doi:10.1016/j.bbabio.2008.05.348

(S13) Electron transport chain and proton pumps symposium abstracts (poster and raised abstracts)

S13.5 An alternative site for proton entry from the cytoplasm to the quinone binding site in the *Escherichia coli* succinate dehydrogenase

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Escherichia coli succinate dehydrogenase (Sdh) belongs to the highly conserved Complex II family of enzymes which does not generate a proton motive force during catalysis. Because of its electroneutrality, the quinone reduction reaction must consume cytoplasmic protons which are released stoichiometrically from succinate oxidation. The X-ray crystal structure of *E. coli* Sdh shows that residues $SdhB^{G227}$ and $SdhC^{D95}$, as well as $SdhC^{E101}$, are located at or near the entrance of an observed water channel that has been proposed to function as a proton wire connecting the cytoplasm to the quinone binding site. However, the pig and chicken Sdh enzymes show an alternative entrance to the water channel that is greeted by the conserved $SdhD^{Q78}$ residue. In this study, these four residues were studied by site-directed mutagenesis. We show that the observed water channel in the *E. coli* structure is the functional proton wire *in vivo*, while *in vitro* results indicate alternative entrances for protons, possibly located at $SdhD^{Q78}$. *In silico* examination of the *E. coli* Sdh also reveals a possible H-bonding network leading from the cytoplasm to the quinone binding site, also via $SdhD^{Q78}$. Based on these results we propose an alternative proton pathway in *E. coli* Sdh that is functional only *in vitro*.

doi:10.1016/j.bbabio.2008.05.349

S13.6 Heme-free variants of *Escherichia coli* succinate dehydrogenase

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We sought to elucidate the role of the heme *b* in the *Escherichia coli* Complex II homolog, succinate dehydrogenase (succinate:quinone oxidoreductase, SQR). A mutation of either heme-coordinating His residues to Tyr successfully created SQR mutants which were unable to incorporate heme into their membrane anchor domains yet the mutants were assembled properly in the bacterial inner membrane. The complete loss of heme *b* in the mutant enzymes was verified by both optical and EPR spectroscopy. In the absence of heme, enzyme turnover was only minimally impacted as upwards of 50% of activity was retained in the mutants. Q-site architecture was not seriously affected by the mutations as site still stabilized a EPR-detectable semiquinone radical intermediate. Moreover, the possible role of the heme in the suppression of reactive oxygen species was examined and such a function could not be identified. Here we have shown that although the heme b is redox active in *E. coli* SQR, electron transfer through the heme is not absolutely essential for enzyme catalysis.

doi:10.1016/j.bbabio.2008.05.350

S13.7 Heterologous overexpression of eukaryotic cytochrome *c* and cytochrome *c* heme lyase to study the mechanism of cytochrome *c* maturation

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Our purpose is to study the interaction of eukaryotic cytochrome *c* heme lyase (CCHL) and cytochrome *c* during the maturation of the latter in a controlled environment. In mitochondria, CCHL assists both in the transport of the polypeptide of cytochrome *c* through the outer membrane and in the covalent assembly of heme into the cytochrome pocket via thioether linkages to the cysteines of the conserved CXXCH motive. In the *E. coli* system the latter function can be investigated separately, since the co-expression of CCHL's gene with c-type cytochromes' genes in the cytoplasm of *E. coli* generates intact cytochrome proteins. Little is known, however, about the structure of CCHL and about the mechanism of its catalytic action on cytochrome *c* and heme. We have improved a cytochrome *c* – CCHL coexpression system to a reliable, tightly controllable one to achieve a high expression yield for mutants of horse heart cytochrome *c*. This system is used in a general study of posttranslational cytochrome *c* protein modification by CCHL. Purification of the heterologously expressed CCHL is under way with the aim of investigating the cytochrome *c* maturation in a simplified and controlled *in vitro* environment as well.

doi:10.1016/j.bbabio.2008.05.351

S13.8 Tuning the electron transfer rate by the redox potential of cytochrome *c* in complex with cytochrome *c* oxidase

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Our aim is to study the electron transfer in the cytochrome *c* – cytochrome *c* oxidase (COX) complex by controlling electron transfer rates. The photoinduced redox label 8-thiouredopyrene-1,3,6-trisulfonate (TUPS) has been attached to a cysteine replacing Ala15 of horse heart cytochrome *c*. Electron transfer between TUPS and heme c was measured spectroscopically. A second Met80 → His mutation of the axial ligand resulted in a decrease of the heme redox potential. Electron transfer measurements on the A15C/M80H double mutant indicated acceleration of the reverse (10 μs → submicrosecond) and deceleration of the forward electron transfer (submicrosecond → 20 μs), due to the altered driving forces for these processes. Ascorbate cannot reduce the heme of the double mutant, but serves as an electron donor to the triplet excited state of TUPS. With ascorbate the TUPS triplet was converted to the TUPS negative radical, which reduced heme c more efficiently than the TUPS triplet. Both the A15C and the A15C/M80H mutants were complexed with bovine heart and with *Paracoccus denitrificans* COX to follow electron transfer through the putative physiological route. Various efficiencies of COX reduction were observed. An apparent electron deficiency was assigned to the spectrally silent reduction of CuA, and the reduction of heme a was detected.

doi:10.1016/j.bbabio.2008.05.352

S13.9 Protein control of [3Fe–4S] cluster redox chemistry in two *Escherichia coli* complex II paralogs

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We have used a combination of EPR spectroscopy and site-directed mutagenesis to investigate the role of the protein environment in determining the midpoint potentials (E_m values) of the [3Fe–4S] clusters that are present in two *Escherichia coli* Complex II paralogs: menaquinol:fumarate oxidoreductase (FrdABCD) and succinate:ubiquinone oxidoreductase (SdhCDAB). In both cases, a conserved Lys residue, FrdB-K228 or SdhB-K230, is hydrogen bonded to the carbonyl oxygen of one of the cluster-coordinating Cys residues. The E_m of the [3Fe–4S] cluster of FrdABCD is lowered from −70 mV in the wild-type to −180 mV (ΔE_m=−110 mV) and −170 mV (ΔE_m=−100 mV) in a FrdB-K228L mutant and a FrdB-K230E mutant, respectively. In an SdhB-K230L mutant, the [3Fe–4S] cluster E_m is lowered from +70 mV to +50 mV (ΔE_m=−20 mV). Sequence analyses reveal significant differences between conserved residues surrounding the [3Fe–4S] cluster in the FrdABCD and SdhCDAB families. The residue prior to the first cluster coordinating Cys is a Ser in FrdABCD (Ser-203), but an Arg in SdhCDAB (Arg-205). A FrdB-S203R mutant has little effect on the E_m of FR3, whereas a SdhB-R205S mutant elicits a $\Delta E_{m,7}$ of −30 mV on S3. The residue following the first [3Fe–4S] cluster coordinating Cys is a Thr in FrdABCD (Thr-205), but a His in SdhCDAB (His-207). A FrdB-T205H mutant elicits a $\Delta E_{m,7}$ of +82 mV on FR3, whereas an SdhB-H207T mutant elicits a $\Delta E_{m,7}$ of −68 on S3. Interpretation of the data in terms of the protein structures of provides important insights into the role of the protein scaffold in defining the [3Fe–4S] E_m values of the *E. coli* Complex II paralogs.

doi:10.1016/j.bbabio.2008.05.353

S13. 10 Relationship between the supramolecular organization of the respiratory chain and electrons competition

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