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.

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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.

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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.

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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.

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

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