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

Katalin Tenger[a], Petro Khoroshyy[a], Gábor Rákhely[a,b], László Zimányi[a]
[a]*Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary*
[b]*Department of Biotechnology, University of Szeged, Szeged, Hungary*
E-mail: tenger@brc.hu

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

Petro Khoroshyy, Katalin Tenger, László Zimányi
Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
E-mail: petro@brc.hu

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

Richard A. Rothery, Quang Tran, Nassrein Hussein, Elena Maklashina, Gary Cecchini, Joel H. Weiner
Membrane Protein Research Group, Department of Biochemistry, 474 Medical Sciences Building, University of Alberta, Edmonton, Alberta, Canada T6G 2H7
Molecular Biology Division, Veterans Affairs Medical Center, San Francisco, California 94121, USA

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

Arnaud Mourier, Anne Devin, Michel Rigoulet
I.B.G.C, CNRS-UMR 5095 1, rue Camille Saint Saëns 33077 BORDEAUX cedex, France
E-mail: Arnaud.mourier@ibgc.u-bordeaux2.fr

The yeast *Saccharomyces cerevisiae* mitochondria are slightly peculiar since they do not exhibit a complex I but have a number of external and internal dehydrogenases in the inner mitochondrial membrane. These dehydrogenases give their electrons to the quinone pool. On the outer side of the inner membrane, two dehydrogenases activities are localized: the NADH dehydrogenase and the glycerol-3-phosphate dehydrogenase. On the matrix side of the inner membrane, one can find NADH dehydrogenase and succinodehydrogenase. We have previously shown that in mitochondria isolated from *S. cerevisiae*, electrons coming from certain dehydrogenases have the right of way on electrons coming from others. Typically, electrons coming from the external NADH dehydrogenases, have the right of way on other dehydrogenases. In order to understand the possible relationship between this process and the supramolecular organization of the respiratory chain, we undertook a functional and structural study of yeast mutants that do or do not exhibit a supramolecular organization. We have thus studied the electrons competition process in a cardiolipin delta mutant known to possess an altered respiratory chain supramolecular organization. We also studied the organisation of the respiratory chain by BN-PAGE method in a mutant that exhibit an altered electron competition process. Results pertaining to this study will be presented.

doi:10.1016/j.bbabio.2008.05.354

S13.11 Towards crystallizing co-complexes of newly identified inhibitors with the QFR from *Wolinella succinogenes* and with other members of the superfamily of succinate:quinone oxidoreductases

Florian Müller[a], Gregor Madej[a], Hamid R. Nasiri[b], Harald Schwalbe[b], Hanno D. Juhnke[a], Tina Stumpp[a], C. Roy D. Lancaster[c]
[a]*Cluster of Excellence "Macromolecular Complexes", Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Frankfurt am Main, Germany*
[b]*Cluster of Excellence "Macromolecular Complexes", Institut für Organische Chemie und Chemische Biologie, Center for Biomolecular Magnetic Resonance, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany*
[c]*Chair of Structural Biology, Faculty of Medicine, Saarland University, Homburg (Saar), Germany*
E-mail: Roy.Lancaster@structural-biology.eu

The members of the superfamily of succinate:quinone oxidoreductases exhibit a high degree of variability with respect to the sensitivity of their quinone sites to various inhibitors. The goal of the project is the identification of potent inhibitors of the diheme-containing membrane protein complex quinol:fumarate reductase (QFR) from *Wolinella succinogenes* and a comparison of these newly identified inhibitors to those of other members of the superfamily, e.g. 2-heptylquinoline-*N*-oxide (HQNO). We shall present first results which are a prerequisite for the co-crystallization of these inhibitors with the respective membrane protein complexes.

doi:10.1016/j.bbabio.2008.05.355

S13.12 Hybrid protein-semiconductor photonic structures using bacteriorhodopsin and glucose oxidase

László Zimányi[a,b], Marta Martin[c], Gabriela Palestino[c], Thierry Cloitre[c], Frédéric J.G. Cuisinier[b], Csilla Gergely[c]
[a]*Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary*
[b]*Faculté d'Odontologie, Université Montpellier I, France*
[c]*GES, Université Montpellier II, France*
E-mail: zimanyi@brc.hu

The aim of this work is to characterize porous silicon (PSi) photonic structures impregnated with glucose oxidase (GOX) or solubilized bacteriorhodopsin (BR) as a first step in developing hybrid matrices for future biophotonic applications. PSi Bragg mirrors and microcavities are multilayered structures of periodic refractive index with layer thicknesses in the order of visible light wavelength. The pore size of PSi can be tuned to accommodate biomacromolecules, and the silicon surface can be functionalized for covalent protein attachment. Both proteins have previously been shown to possess nonlinear optical properties. We have used atomic force microscopy and multi-photon microscopy to characterise the surface and in depth, respectively, the GOX or BR impregnated PSi structures. Two photon fluorescence emission and second harmonic generation of the BR-PSi and the GOX-PSi systems were observed at some particular pores of PSi and subsequent enhancement of the signal arising from the proteins adsorbed within the pores was detected. The results constitute the first steps in an innovative biomimetic approach for the future design and development of protein based integrated optical devices.

doi:10.1016/j.bbabio.2008.05.356

S13.13 Multiheme periplasmic cytochromes of *Geobacter sulfurreducens*: Optimized cellular devices to face extracellular electron acceptors?

Leonor Morgado[a], Marta Bruix[b], Yuri Y. Londer[c], P. Raj Pokkuluri[c], Marianne Schiffer[c], Carlos A. Salgueiro[a]
[a]*Requimte-CQFB, FCT-UNL, Caparica, Portugal*
[b]*Departamento de Espectroscopía y Estructura Molecular, IQFR-CSIC, Madrid, Spain,*
[c]*Biosciences Division, Argonne National Laboratory, Argonne, USA*
E-mail: mlmorgado@dq.fct.unl.pt

Multiheme cytochromes are key proteins in the reduction of extracellular metal ions as Fe(III) and U(VI) in *Geobacter sulfurreducens* (Gs). In contrast with cytoplasmic acceptors, theoretical studies showed that reduction of extracellular acceptors leaded to the dissipation of the membrane potential due to cytoplasm acidification. To counteract this, additional energy transduction steps are needed to generate energy. PpcA, a small periplasmic triheme cytochrome, was proposed to contribute to the energy transduction cycle that leads to ATP synthesis in Gs. Four homologs of PpcA were identified in Gs genome, being PpcB the most closely related, with 77% sequence identity. In this study the redox centers of PpcB were characterized using NMR and visible spectroscopy techniques. Despite being sequence and structurally homologous, the functional redox properties of PpcB and PpcA are quite distinct. This correlates with the results of phenotypic studies that showed that knock-out of PpcA gene disrupts electron transfer to extracellular Fe(III), while the effect of PpcB gene deletion is notorious on the U(VI) reduction activity. This suggests that each protein uniquely modulates the properties of their co-factors to assure effectiveness in the metabolic pathways they participate.

doi:10.1016/j.bbabio.2008.05.357

S13.14 Spectroscopic and structural studies of the alternative oxidase

Anthony L. Moore[a], Peter Heathcote[b], Kiyoshi Kita[c], Mary S. Albury[a]
[a]*Biochemistry and Biomedical Sciences, University of Sussex, Falmer, Brighton, UK*
[b]*School of Biological and Chemical Sciences, Queen Mary, University of London, London, UK*
[c]*Department of Biomedical Chemistry, University of Tokyo, Tokyo, Japan*
E-mail: a.l.moore@sussex.ac.uk