14P.9 Antiporter activity of the individual complex I subunits NuoL, NuoM and NuoN from *Escherichia coli* analyzed in an *in vivo* model system

Brijesh Kumar[1], Vamsi K. Moparthi[1], Cecilie Mathiesen[2], Cecilia Hägerhäll[1]
[1]*Department of Biochemistry and Structural Biology, Center for Molecular Protein Science, Lund University, PO Box 124, S-221 00 Lund, Sweden*
[2]*Present address: The Norwegian University of Life Sciences, UMB, Postbox 5003, 1432 Aas, Norway*
E-mail: brijeshnkumar@gmail.com

The complex I (NADH:quinone oxidoreductase) membrane spanning subunits NuoL, M and N are homologous to one particular class of Na^+ or K^+/H^+ antiporters, encoded by the gene cluster denoted *mrp/sha/pha/mnh* in different bacteria. These subunits are prime candidates for harboring important parts of the proton pumping machinery of complex I. In *Bacillus subtilis* deletion of *mrpA* or *mrpD* from the chromosome resulted in a Na^+ and pH sensitive growth phenotype [1]. In this work the antiporter-like complex I subunits were expressed and their functions were compared in vivo using *B. subtilis* as model system. Expression of MrpA in a *B. subtilis* Δ*mrpD* strain and *vice versa* did not result in any growth improvement under any condition tested. The expressed NuoL could rescue Δ*mrpA* to wild-type growth properties at pH 7.4, but enhanced the growth of Δ*mrpD* only to a lesser extent at this pH. The expressed NuoN could fully restore the wild type properties of Δ*mrpD* in the pH range from pH 6.5 to 7.5. In the Δ*mrpA* strain, expression of NuoN did not improve growth at pH 7.5 but resulted in some growth improvement at pH 6.5. Cells expressing NuoM did not reach wild type growth levels in either deletion strain, but showed some growth improvement under some of the tested conditions. At pH 8.5 no strain could be rescued by any complex I subunit. Taken together, this demonstrates that (i) the antiporter-like Nuo proteins can functionally replace real antiporters and (ii) each of the three complex I antiporter-like subunits has unique functional specializations and operates at different pH. Such pH dependent regulation has previously been described for the Pha antiporter [3], further corroborating that NuoL M and N retain much of their primordial functional mechanism. The implications for the complex I functional mechanism are discussed.

References

[1] Ito M, Guffanti AA, Oudega B, Krulwich T (1999) *J. Bacteriol.* **181:** 2394-2402.
[2] Yamaguchi T, Tsutsumi F, Putnoky P, Fukuhara M, Nakamura T (2009) *Microbiol.* **155:** 2750-2756.

doi:10.1016/j.bbabio.2010.04.344

14P.10 Structure and function of *Aquifex aeolicus* sulfide: Quinone oxidoreductase

Marco Marcia, Julian Langer, Ulrich Ermler, Guohong Peng, Hartmut Michel
Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Frankfurt am Main, Germany
E-mail: hartmut.michel@biophys.mpg.de

Sulfide:quinone oxidoreductases (SQRs) are ubiquitous membrane-bound flavoprotein disulfide reductases (FDRs) that transfer electrons to the membrane quinone pool, thus being essential for sulfide-dependent respiration and photosynthesis. They are also involved in sulfide detoxification, heavy metal tolerance (in yeast) and possibly sulfide signalling (in higher eukaryotes, including humans) [1]. We determined the first complete structure of an SQR at 2.0 Å resolution. We isolated the protein from the native membranes of *Aquifex aeolicus* and could crystallize it in three forms, "as-purified", bound to the substrate quinone and bound to the inhibitor aurachin C [2]. *A. aeolicus* SQR is trimeric and binds the lipid bilayer as an integral monotopic membrane protein, an optimal topology for catalysing the reaction between a soluble and a hydrophobic substrate. The quinone-binding site is located in the membrane-binding domain on the *si*-side of FAD. The quinone ring interacts with conserved F385 and I346 and is protonated upon reduction via G318, K382 and/or neighboring solvent molecules. Sulfide polymerization occurs on the *re*-side of FAD, where the invariant C156 and C347 surprisingly bind the product of the reaction, a polysulfur chain possibly forming an S_8 ring in its mature form. Finally, the structure shows that FAD is covalently connected to the protein in an unprecedented way, via a putative disulfide bridge with C124. Based on our structural observations, we concluded that the SQR reaction needs to be significantly different from that of FDRs and we proposed two alternative reaction schemes. Unexpectedly, there is functional divergence also among SQRs themselves, since the structure of *Acidianus ambivalens* SQR, which also became available recently [3], shows significant difference in the active site in respect to *A. aeolicus* SQR. The structural comparison led us to define new structure-based sequence fingerprints for SQRs and to put the basis for further studies [4]. In particular, the characterization of eukaryotic SQRs is of high priority, because these proteins probably regulate the homeostasis of sulfide, a mediator of sympathetic neurotransmission and a key metabolite in neurodegenerative diseases [5].

References

[1] Griesbeck C *et al.* (2002) *Biochemistry* **41:** 11552-11565.
[2] Marcia M *et al.* (2009) *Proc. Natl. Acad. Sci. USA* **106:** 9625-9630.
[3] Brito J *et al.* (2009) *Biochemistry* **48:** 5613-5622.
[4] Marcia M *et al.* (2010) *Proteins* **78:** 1073-1083.
[5] Wang R (2002) *FASEB J* **16:** 1792-1798.

doi:10.1016/j.bbabio.2010.04.345

14P.11 Energy conservation by *Rhodothermus marinus* respiratory complex I

Ana P. Batista, Manuela M. Pereira
Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República (EAN), 2781-901 Oeiras, Portugal
E-mail: mpereira@itqb.unl.pt

Complex I is the largest and the least understood respiratory chain complex being the electron transfer from NADH to quinone couple to the charge translocation across the membrane. The mechanism of quinone reduction and its coupling to charge translocation is not known. *Rhodothermus marinus* complex I, our model system, is a NADH: menaquinone oxidoreductase and has been extensively characterized. We have made an exhaustive study in order to identify all the subunits present in the complex. We have addressed the charge translocation of complex I using inside-out *R. marinus* membrane vesicles and we observed a NADH-driven sodium ion efflux, together with a proton influx and an inside-positive $\Delta\Psi$. The sodium ion extrusion from the membrane vesicles was due to the activity of complex I, since it was sensitive to its inhibitor rotenone, and it was still observed when the complex I segment of the respiratory chain was isolated by the simultaneous presence of cyanide and external quinones. Using the same approach revealed that H^+ is the electrogenic ion in *R. marinus* complex I. Our results thus show that complex I translocates sodium ions to the direction opposite to that of the establishment of $\Delta\Psi$ by proton translocation, what constitutes the first description of such a process. Moreover, studying the sodium influences of the NADH

oxidation and of proton and sodium transport activities allowed us to propose a model for the mechanism of complex I in which two different ion translocation sites are coupled to electron transfer. Studies performed in the presence of inhibitors corroborate the proposed model. Furthermore, the results obtained for other bacterial complex I open new perspectives on the versatility of this respiratory complex.

doi:10.1016/j.bbabio.2010.04.346

14P.12 The alternative complex III: A different architecture using known building modules

Patrícia N. Refojo, Filipa L. Sousa, Miguel Teixeira, Manuela M. Pereira
Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal
E-mail: refojo@itqb.unl.pt

Until recently cytochrome bc_1 complexes were the only known enzymes able to transfer electrons from reduced quinones to cytochrome *c*. However, a complex with the same activity and with a unique subunit composition was purified from the thermohalophilic bacterium *Rhodothermus marinus* membranes and biochemically, spectroscopically and genetically characterized. This complex was named alternative complex III (ACIII). Later, it was observed that the presence of ACIII is not exclusive of *R. marinus* being the genes coding for this novel complex widespread in the Bacteria Domain. Furthermore, ACIII has been shown to be related to the complex iron–sulfur molybdoenzyme (CISM) family. In this work, the relation of ACIII with members of this family was further investigated by analyzing all the available completely sequenced genomes and a comprehensive description of the state of the art of ACIII is presented. In summary, it was observed that ACIII is a different complex but composed by already known modules, and is thus another example of how nature uses the same structural modules in different contexts according to the metabolic needs.

doi:10.1016/j.bbabio.2010.04.347

14P.13 Fine-tuned cooperative redox networks of multiheme periplasmic cytochromes in *Geobacter sulfurreducens*: Optimal bioenergetic adaptation to environmental changes

Leonor Morgado[1], Marta Bruix[2], Yuri Y. Londer[3], Carlos A. Salgueiro[1]
[1]*Requimte-CQFB, Departamento Química, Faculdade Ciências Tecnologia, Universidade Nova de Lisboa, Portugal*
[2]*Departamento Espectroscopía Estructura Molecular, Instituto Química-Física "Rosacolano", CSIC, Madrid, Spain*
[3]*Biosciences Division, Argonne National Laboratory, Argonne, IL 60439,USA*
E-mail: csalgueiro@dq.fct.unl.pt

A family of five periplasmic triheme cytochromes (PpcA-E) was identified in the bacterium *Geobacter sulfurreducens* (*Gs*), where they play a crucial role by driving electron transfer from cytoplasm to cell exterior, and assisting the reduction of extracellular acceptors [1]. This work reports the thermodynamic characterization of PpcA, PpcB, PpcD and PpcE using NMR and visible spectroscopies. The heme reduction potentials of these proteins are strongly modulated by heme–heme redox and redox-Bohr (heme-protonated groups) interactions, establishing specific cooperative networks. These networks can be further modulated by the periplasmic pH towards an optimal cellular bioenergetic response to environmental changes. The different functional mechanisms involved suggest that they interact with particular physiological redox partners in the cell. PpcA and PpcD appear to be optimized to interact with redox partners involving e^-/H^+ transfer though via distinct mechanisms. Although no evidence of preferential electron transfer pathway or e^-/H^+ coupling was found for PpcB and PpcE, their working potential ranges suggest that they might also have specific redox partners. The mechanistic properties described for the four *Gs* triheme cytochromes correlate with proteomics and knock-out mutant studies on *Gs* [2,3]. This work constitutes the first step in unraveling the organization of the complex network of redox proteins found in the periplasmic space of the bacterium *G. sulfurreducens*. This functional diversity provides an excellent example as to how structurally related proteins from the same microorganism can interact with particular physiological partners, establishing a rationalization for the co-existence of five homologous periplasmic triheme cytochromes in *Gs*.

This work was supported by grant PTDC/QUI/70182/2006 from Fundação para a Ciência e a Tecnologia (Portugal).

References

[1] Pokkuluri PR *et al.* (2004) *Biochemistry* **43:** 849-859.
[2] Shelobolina ES *et al* (2007) *BMC Microbiol.* **7:** 16.
[3] Ding YH *et al* (2008) *Biochim. Biophys. Acta* **1784:** 1935-1941.

doi:10.1016/j.bbabio.2010.04.348

14P.14 Characterization of the supramolecular structure of *Bacillus subtilis* aerobic respiratory chain

Sara T.N. Silva[1], Pedro M.F. Sousa[1,2], Fátima Vaz[3], Patrícia Gomes-Alves[3], Deborah Penque[3], Ana M.P. Melo[1]
[1]*Instituto de Investigação Científica Tropical, ECO-BIO, Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal*
[3]*Instituto Nacional de Saúde Dr. Ricardo Jorge, Laboratório de Proteómica, Departamento de Genética, Lisboa, Portugal*
E-mail: tnssara@gmail.com

Bacillus subtilis, a Gram-positive soil bacterium, possesses a branched respiratory chain, and is capable of using oxygen or nitrate as terminal electron acceptor. When grown in aerobic conditions, its respiratory chain comprises a type-II NADH: and a succinate:menaquinone oxidoreductase, a bc_1-like menaquinol: cytochrome *c* oxidoreductase and three, possibly four terminal oxygen reductases: a caa_3 cytochrome *c*:oxygen oxidoreductase, and an aa_3 and one or two *bd*-type menaquinol:oxygen reductases [1]. Supramolecular associations between complexes of the electron transfer chain have been demonstrated both in eukaryotes and prokaryotes, enhancing the electron transfer efficiency, and in some cases promoting stabilisation of complex I [2]. Several years ago, a supercomplex composed of a quinol:cytochrome *c* reductase and a cytochrome *c* oxidase was identified in the thermophilic *Bacillus* PS3, showing for the first time the presence of supramolecular associations of respiratory chain complexes in the *Bacillus* genus [3]. We have carried out the aerobic growth of *B. subtilis* 168, promoted cell disruption by means of a French press and isolated the membranes for further studies. Characterization of the expressed complexes was performed by UV–visible spectrophotometry and substrate:oxygen polarographic measurements of the respiratory chain enzymatic activities using specific inhibitors. To investigate supramolecular associations between these complexes, we have performed BN-PAGE and detected in gel activity of the different respiratory enzymes. Our preliminary results suggest that also the aerobic respiratory chain of the mesophilic *B. subtilis* is organized in supercomplexes.