1P.1 Structural insights into complex I obtained from new subcomplex Iδ

Heike Angerer[1], Klaus Zwicker[1], Volker Zickermann[1], Lucie Sokolova[2], Heinrich Heide[1], Mirco Steger[1], Bernhard Brutschy[2], Michael Radermacher[3], Ulrich Brandt[1]
[1]*Molecular Bioenergetics Group, Medical School, Cluster of Excellence Frankfurt "Macromolecular Complexes", Center for Membrane Proteomics, Goethe University, Frankfurt am Main, Germany*
[2]*Institute of Physical and Theoretical Chemistry, Goethe University, Frankfurt am Main, Germany*
[3]*Department of Molecular Physiology and Biophysics, College of Medicine, University of Vermont, Burlington, VT, USA*
E-mail: angerer@zbc.kgu.de

Mitochondrial NADH:ubiquinone oxidoreductase (complex I) is an L-shaped membrane protein that contains at least 40 different subunits. Until a high-resolution structure of the holo-complex becomes available, the analysis of subcomplexes can provide useful information about the overall structural organisation of complex I. Exposing complex I purified from *Yarrowia lipolytica* to the chaotropic detergent N,N-dimethyldodecylamine N-oxide (LDAO) resulted in formation of a new 820 kDa subcomplex, termed Iδ. As determined by combining dSDS-electrophoresis, LILBID- and ESI-mass spectrometry, subcomplex Iδ harbours 31 subunits and comprises the hydrophilic subcomplex Iλ and the membrane-bound subcomplex Iβ. The missing subunits were ND1, ND2, ND3, ND4L, NUPM, NUXM, NB6M, NIMM and ST1. Subcomplex Iδ showed full non-physiological NADH:HAR activity and contained all EPR detectable Fe–S clusters. However, using the ubiquinone analogs DBQ or Q_1 as substrates no inhibitor-sensitive catalytic activity was detectable and could also not be recovered by addition of lipids. This indicated that subcomplex Iδ had lost its native enzymatic function. Structural characterisation of subcomplex Iδ by single particle electron microscopy revealed a structure in which the peripheral arm and a large fragment of the membrane arm appeared to be tethered by a thin connection. These findings suggest that in subcomplex Iδ the boot-shaped parental complex I had lost its "heel", i.e. a part roughly corresponding to subcomplex Iγ. Implications for the arrangement and functional roles of subcomplexes Iα, Iβ, Iλ and Iγ and the position of subunit ND6 in subcomplex Iδ will be discussed.

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1P.2 External NAD(P)H dehydrogenases in amoeba *Acanthamoeba castellanii* mitochondria

Nina Antos-Krzeminska, Wieslawa Jarmuszkiewicz
Department of Bioenergetics, Adam Mickiewicz University, Poznan, Poland
E-mail: nant@wp.pl

The mitochondrial respiratory chain of plants and fungi contains multiple NAD(P)H dehydrogenases. In addition to Complex I there are at least two rotenone-insensitive dehydrogenases located on the outer surface of the inner mitochondrial membrane (ND_{ex}), i.e. external NADH and external NADPH dehydrogenases. We have investigated protozoan *A. castellanii* mitochondria in order to find the activity of ND_{ex}. We have determined the activity of both external NADH and NADPH dehydrogenases with the maximum value at pH 6.8, likewise the cyanide-resistant alternative oxidase activity. It seems to be consistent with the putative role of these enzymes which probably cooperate with each other and likely constitute a wasteful system preventing overreduction of the electron transport components in the respiratory chain. NADH dehydrogenase is probably slightly or not sensitive to Ca ions in contrast to NADPH dehydrogenase, which is Ca-sensitive. Under enzyme optimal conditions, *A. castellanii* mitochondria reveal a higher substrate affinity for external NADPH than for external NADH. Using blue-native polyacrylamide gel electrophoresis and histochemical staining (NBT + substrate-NADH and NADPH, respectively) we have identified ND_{ex} activities in solubilized *A. castellanii* mitochondria.

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1P.3 Characterisation and crystallisation of intact complex I from *Thermus thermophilus*

Rozbeh Baradaran, Michael E. Harbour, Ian M. Fearnley, Leonid A. Sazanov
Mitochondrial Biology Unit, Medical Research Council, Cambridge CB2 0XY, UK
E-mail: sazanov@mrc-mbu.cam.ac.uk

NADH–ubiquinone oxidoreductase (complex I) is the first and the largest enzyme in the respiratory chain of mitochondria and most bacteria. Complex I catalyses the transfer of two electrons from NADH to quinone, coupled to the translocation of about four protons across the membrane. The mitochondrial enzyme contains 45 different subunits, while the bacterial enzyme consists of 13–15 different subunits. Analogues of all conserved bacterial subunits are found in the mitochondrial enzyme. Previously, we have determined the crystal structure of the hydrophilic domain of complex I from *Thermus thermophilus*. However, the high-resolution structures of the hydrophobic domain or the intact complex, as well as the coupling mechanism, remain unknown. The *T. thermophilus* complex I is studied here as a minimal model of the mitochondrial enzyme. A procedure for purification of intact complex I from *T. thermophilus* was developed. All subunits of the enzyme have been identified by peptide mass fingerprinting. Single-particle EM analysis showed that the *T. thermophilus* complex I has the typical L-shape. It shows high specific activity of electron transfer from NADH to decylubiquinone, which is sensitive to inhibitors rotenone and piericidin-A. Extensive crystallisation trials identified several different crystal forms, all containing intact complex I. The preliminary findings from crystallographic data will be discussed.

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1P.4 Electron pathways in mitochondrial complex I

Christian Bergamini[1], Marco Bortolus[2], Romana Fato[1], Anna L. Maniero[2], Giorgio Lenaz[1]
[1]*Dipartimento di Biochimica "G. Moruzzi", Università di Bologna, via Irnerio 48, 40126, Bologna, Italy*
[2]*Dipartimento Scienze Chimiche, Università degli Studi di Padova, via Marzolo 1, 35131, Padova, Italy*
E-mail: christian.bergamini2@unibo.it

Complex I plays a central role in cellular energy production, transferring two electrons from NADH through a series of iron–sulfur clusters (FeS) to ubiquinone (CoQ); the electron transfer is coupled to the translocation of protons across the membrane. The FeS center N2 is the last acceptor in the electron-transfer chain, but the mechanism through which the enzyme couples the $1e^-$ reduction of the FeS centers to the $2e^-$ reduction of ubiquinone ($Q \rightarrow SQ \rightarrow QH2$) is unclear [1]. We identify two different families of inhibitors of complex I activity (class A and B) acting on the electron-transfer with different mechanisms. EPR data are coupled to fluorescence measurements on the effect of inhibitors on reactive