oxygen species (ROS) production and to enzymatic activity assays. Submitochondrial particles (SMP) were treated with class A or B inhibitors. NADH addition initiated the electron transfer. In our system class A inhibitors (rotenone, piericidin A) increase ROS production from complex I, whereas class B inhibitors (stigmatellin, mucidin, CoQ2) have no effect on ROS production. We measured the presence of semiquinone (SQ) at 180 K and state of reduction of the iron sulfur cluster N2 at 12 K in SMP inhibited with class A and class B inhibitors. Our data confirm a strong SQ signal reduction in the presence of rotenone while the signal intensity is less reduced in samples treated with stigmatellin [2]. N2 spectra show different reduction state in presence of rotenone and stigmatellin. In presence of stigmatellin the center is mainly oxidized. We hypothesize a two-step reduction performed by N2, possibly following a rearrangement of the site [3]. Rotenone like inhibitors, not allowing the access of the quinone to the active site, would block the enzyme in a conformation that only permits electron delivery from N2 to oxygen. In this conformation hydrophilic quinones like CoQ1 can be reduced by N2 to a semiquinone species in a non physiological site. This semiquinone can rapidly react with molecular oxygen to form anion superoxide. Stigmatellin like inhibitors would block the enzyme in a conformation allowing only the first step of quinone reduction (Q→SQ) in the physiological reduction site, but blocking any further reduction; this conformation does not allow reaction of N2 with oxygen.

References

[1] Brandt U (2006) *Ann Rev. Biochem.* **75:** 69–92.
[2] Ohnishi T. *et al.* (2005) *FEBS Lett.* **579** 500–506.
[3] Fato R *et al.* (2008) *Biochim. Biophys. Acta* **1787:** 384–3892.

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1P.5 NADH binding to complex I: Implications for the mechanism

John M. Berrisford, Leonid A. Sazanov
Medical Research Council, Mitochondrial Biology Unit, Cambridge, UK
E-mail: sazanov@mrc-mbu.cam.ac.uk

Complex I plays a central role in cellular energy production, coupling electron transfer between NADH and quinone to proton translocation. We have determined several X-ray structures of the oxidized and reduced hydrophilic domain of complex I from *Thermus thermophilus* at up to 3.1 Å resolution. The structures reveal the mode of interaction of complex I with NADH, explaining known kinetic data and providing implications for the mechanism of ROS production at the flavin site of complex I. Bound metals were identified in the channel at the interface with the frataxin-like subunit Nqo15, indicating possible iron-binding sites. Conformational changes upon reduction of the complex involve adjustments in the nucleotide binding pocket, as well as small, but significant, shifts of several α-helices at the interface with the membrane domain. These shifts are likely to be driven by the reduction of nearby Fe–S clusters N2 (the electron donor to quinone) and N6a/b. Cluster N2 is coordinated by unique motif involving two consecutive (tandem) cysteines. An unprecedented "on/off switch" (disconnection) of coordinating bonds between the tandem cysteines and this cluster was observed upon reduction. Comparison of the structures suggests a novel mechanism of coupling between electron transfer and proton translocation, combining conformational changes and protonation/de-protonation of tandem cysteines.

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1P.6 The role of the isolated [2Fe–2S] cluster adjacent to the flavin mononucleotide of mitochondrial complex I: Does it influence catalysis at the flavin site?

James A. Birrell, Judy Hirst
Medical Research Council, Mitochondrial Biology Unit, Cambridge, UK
E-mail: jb@mrc-mbu.cam.ac.uk

Complex I is the proton pumping NADH:ubiquinone oxidoreductase of the mitochondrial inner membrane. Complex I from bovine mitochondria contains eight iron–sulphur clusters (two [2Fe–2S] clusters and six [4Fe–4S] clusters). Seven of them link the NADH oxidation site to the ubiquinone reduction site. The eighth cluster, named 2Fe[24] or N1a, is ligated by the 24 kDa subunit; it is isolated from the main chain of clusters but adjacent to the flavin mononucleotide and close enough to accept electrons from it. Whether the 2Fe[24] cluster has a role in the mechanism of complex I is not known. It is possible it minimises the lifetime of the semi-reduced flavin species, decreasing the rate of superoxide production and/or preventing direct hydrogen peroxide production by the fully reduced flavin. Complex I from *Escherichia coli* contains a homologous cluster with a reduction potential 0.1 V higher than that of the bovine cluster; complex I from *E. coli* also produces hydrogen peroxide rather than superoxide. The *E. coli* cluster is probably reduced during catalytic turnover, and so may be incapable of minimising the semi-reduced flavin. In this study, complex I from *Yarrowia lipolytica* was used to establish the role of the 2Fe[24] cluster. Mutations were generated in the closely homologous NUMH subunit, to increase the reduction potential of the [2Fe–2S] cluster to that observed in *E. coli*. The effects on catalysis and superoxide production by the complex are described.

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1P.7 Mitochondrial acyl carrier proteins in *Yarrowia lipolytica*: Guilty by affiliation with complex I

Martina Ding, Ulrich Brandt
Molecular Bioenergetics Group, Medical School, Cluster of Excellence Frankfurt "Macromolecular Complexes", Center for Membrane Proteomics, Goethe University, Frankfurt am Main, Germany
E-mail: Ding@zbc.kgu.de

Mitochondrial acyl carrier proteins (ACPMs) were first discovered in the 1980s in *Neurospora crassa*. They are thought to be involved in mitochondrial fatty acid synthesis and in the production of octanoic acid via a phosphopantetheine group covalently attached to a conserved serine. Our group has previously demonstrated that *Yarrowia lipolytica* codes for two different mitochondrial acyl carrier proteins, ACPM1 and ACPM2, that both are *bona fide* subunits of complex I. Deletion of the ACPM1 gene is lethal, whereas ACPM2Δ strains are viable in a certain strain background. However, the ACPM2Δ cells showed an apparent lack of complex I, pointing towards a role in assembly/stability for the complex. In contrast, ACPM1 seems to have a function beyond complex I. The two ACPM protein sequences differ mostly in their putative mitochondrial targeting sequences. We thus created a protein consisting of the ACPM1 targeting sequence fused to the sequence of mature ACPM2. Two DNA constructs with different length of the putative ACPM1 targeting sequence were created and used for plasmid-based complementation of the ACPM1Δ strain. No viable spores were obtained, indicating that both chimeric proteins failed to take over the function of ACPM1. In the ACPM2Δ strain, both constructs led to the formation of assembled complex I, suggesting that the functional difference between the ACPM variants is mediated by the targeting sequence. Currently, various domain-swap constructs are underway