S4.9 Complex I from *Yarrowia lipolytica* contains two different acyl carrier proteins

Krzysztof Dobrynin, Albina Abdrakhmanova, Sebastian Richers, Stefan Kerscher, Ulrich Brandt
Johann Wolfgang Goethe-Universität, Fachbereich Medizin, Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Frankfurt am Main, Germany
E-mail: Dobrynin@zbc.kgu.de

In eukaryotic complex I from different sources one mitochondrial acyl carrier protein ACPM was identified as a bona fide subunit. ACPMs that carry a phosphopantetheine linked to a conserved serine residue are homologous to the prokaryotic acyl carrier proteins involved in fatty acid synthesis. In purified complex I from *Yarrowia lipolytica* two different ACPMs were identified by mass spectrometric analysis and immunodetection of C-terminally tagged variants. The aim of this study was to analyze the role of these two acyl carrier proteins, ACPM1 and ACPM2. Both proteins were detectable in the membrane fraction but not in the soluble fraction of mitochondria. An *acpm1*Δ strain was not viable and an *acpm2*Δ strain displayed loss of complex I assembly and activity. For both ACPM genes, replacement of the conserved serine residue that binds the phosphopantetheine prosthetic group with alanine had the same effects as the respective gene deletions. Thus, while an essential function in assembly and/or stability of complex I is evident for ACPM2 and likely for ACPM1, ACPM1 appears to have an additional function essential for survival of *Y. lipolytica*. The lysophospholipid content was determined for intact mitochondria from strain *acpm2*Δ and the *acpm2*-S88A mutant strain. However no differences to the parental strain were found.

doi:10.1016/j.bbabio.2008.05.140

S4.10 NADH dehydrogenases from *Rhodothermus marinus*

Ana P. Batista, Andreia S. Fernandes, Miguel Teixeira, Manuela M. Pereira
Instituto de Tecnologia Química e Biológica, UNL, Oeiras, Portugal
E-mail: abatista@itqb.unl.pt

The NADH dehydrogenases catalyse electron transfer from NADH to quinone, being the entry point of most electrons which go through the respiratory chains. The bacterial NADH dehydrogenases can be divided into at least three groups: the H^+-translocating NADH dehydrogenase (complex I), the non proton-pumping NADH:quinone oxidoreductase (NDH-II) and the Na^+-translocating NADH dehydrogenase (Na^+-NDH). The number and type of NADH dehydrogenases present in the membranes of *Rhodothermus marinus* has been investigated. *R. marinus* is a thermohalophilic bacterium, first isolated in Iceland and lately in Azores. Its respiratory chain has been extensively studied. So far a complex I, a succinate dehydrogenase, an alternative complex III and three different oxidases were identified. The existence of a complex I in *R. marinus* has already been established and characterized. Recently we have been dedicated to the identification of the subunits using MS. In the membranes of this bacterium two other proteins having NAD(P)H:quinone oxidoreductase activity may be present. One of these is a dihydrolipoamide dehydrogenase, which is a member (as NDH-II is) of the flavoprotein disulfide reductase family. This protein is a homodimer with a molecular mass around 50 kDa per monomer, has FAD as prosthetic group and a redox-active disulfide. Its possible association with complex I was investigated. The study of the third protein with this activity is underway.

doi:10.1016/j.bbabio.2008.05.141

S4.11 Kind of blue: Ultra-short wavelength fluorescence from tryptophan in transhydrogenase dI

Karina Tveen Jensen[a], Giovanni Strambini[b], Margherita Gonnelli[b], Ben H. Hesp[c], Jaap Broos[c], J. Baz Jackson[a]
[a]*School of Biosciences, University of Birmingham, UK*
[b]*CNRS, Instituto di Biofisica, Pisa, Italy*
[c]*Department of Biophysical and Physical Chemistry, University of Groningen, The Netherlands*
E-mail: j.b.jackson@bham.ac.uk

Until now the Trp in azurin was found to emit fluorescence at a shorter wavelength than Trp residues of other proteins. Trp72 in the dI component of transhydrogenase has a slightly longer emission wavelength but we have isolated three mutants (M97A, M97L and M97V) which emit to the blue of even azurin. Indole has two low-lying excited singlet states (^1L_a and ^1L_b) with similar energies. In a vacuum ^1L_b lies below that of ^1L_a and is consequently the emission state. The permanent dipole moment of ^1L_a is greater than that of ^1L_b and the former is therefore stabilised more by interactions with a protein matrix. It was frequently suggested that azurin is the only known protein to emit from ^1L_b. However, fluorescence excitation anisotropy experiments show that azurin, and also wild-type dI, emit predominantly from ^1L_a. In contrast, the three M97 mutants of dI do emit predominantly from ^1L_b. This view is supported by comparisons of the intensities of vibrational bands in high-resolution low-temperature fluorescence spectra of wild-type and mutant dI, and azurin, with gas-phase model spectra of indole. Phosphorescence spectra and decays of dI also attest to the exceptional rigidity of its protein core.

doi:10.1016/j.bbabio.2008.05.142

S4.12 Inhibition of H^+ transfer in transhydrogenase by Zn^{2+}

Simon J. Whitehead[a], Masayo Iwaki[b], Peter R. Rich[b], J. Baz Jackson[a]
[a]*School of Biosciences, University of Birmingham, UK*
[b]*Department of Biology, University College London, UK*
E-mail: j.b.jackson@bham.ac.uk

Transhydrogenase in bacteria and mitochondria couples the reduction of $NADP^+$ by NADH to the inward translocation of H^+ across their membranes; it is driven by Δp. Earlier work (Whitehead et al, 2005) showed that transhydrogenase activity in bacterial membranes was inhibited by Zn^{2+} and Cd^{2+}. We have now purified *E.coli* transhydrogenase and removed its His tag. Low concentrations of metal ions (Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} but not Ca^{2+}, Mg^{2+}, Co^{2+}) inhibited the complete transhydrogenation reaction in purified enzyme but *stimulated* "cyclic" transhydrogenation. The latter is a partial reaction of the enzyme not accompanied by H^+ transfer. We propose that the metal ions inhibit the H^+-transfer steps. A βH91K mutant (see work of PD Bragg, J Rydstrom et al) catalyses only very low rates of complete transhydrogenation but has an active cyclic reaction with a lowered sensitivity to Zn^{2+}. An FTIR signal attributable to His was observed upon addition of Zn^{2+} to a film of wild-type transhydrogenase layered onto an ATR prism. The signal was ~45% lower in the βH91K mutant. The results support the view that βH91 participates in proton transfer through transhydrogenase, is a site of Zn^{2+} inhibition, and that part of the FTIR signal induced by Zn^{2+} can be attributed to this residue.

doi:10.1016/j.bbabio.2008.05.143