

S4.25 Exploring the quinone binding cavity of complex I from *Yarrowia lipolytica*

Maja A. Tocilescu, Uta Fendel, Klaus Zwicker, Stefan Kerscher, Ulrich Brandt

Johann Wolfgang Goethe-Universität, Fachbereich Medizin, Zentrum der Biologischen Chemie, Molekulare Bioenergetik, Frankfurt am Main, Germany

E-mail: toci@zbc.kgu.de

The aim of this study was to explore the quinone and inhibitor binding cavity of respiratory chain complex I. In our structure based site-directed mutagenesis study we used the crystal structure of the hydrophilic part of complex I from *Thermus thermophilus* as a guide and took advantage of our model organism *Yarrowia lipolytica*. We introduced more than 90 point mutations targeting the residues lining the quinone binding cavity at the interface between the PSST and the 49-kDa subunit. In this mutant collection, we checked for complex I assembly and ubiquinone reductase activity and determined apparent K_m values for the ubiquinone analogue decylubiquinone. We measured I_{50} values for the complex I inhibitors DQA, rotenone and $C_{12}E_8$ as representatives for class A, B and C inhibitors, respectively. Our results indicate which parts of the wide cavity are critical for complex I activity and which are not. Tyrosine 144 of the 49-kDa subunit that is located next to iron-sulfur cluster N2 was identified as a key residue for quinone reduction. In addition, a possible quinone entry or exit path was identified. Our inhibition kinetics studies support the concept that class A, B and C inhibitors bind to distinct but partially overlapping sites close to the ubiquinone binding site of complex I.

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S4.26 Evidence for the presence of a Na^+/H^+ antiporter subunit in *Rhodothermus marinus* complex I

Ana M.P. Melo^{a,b}, Sara T.N. Silva^{a,b}, Lúcia M. Saraiva^b, Miguel Teixeira^b

^aFaculdade de Engenharia e Ciências Naturais, Universidade Lusófona de Humanidades e Tecnologias, Av. do Campo Grande, 376, 1749-024 Lisboa, Portugal

^bInstituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

E-mail: ana.melo@iict.pt

The rotenone sensitive NADH:quinone oxidoreductase (complex I) is the first segment of most aerobic respiratory chains, and catalyses the transfer of electrons from NADH to quinone, coupled to ion translocation across membranes. The bacterial protein is generally composed of 14 subunits, the so-called minimal functional unit. Recently, Sazanov and co-workers found an additional subunit in the peripheral arm of *Thermus thermophilus* complex I. The genomic organization of complex I encoding genes from the thermohalophilic bacterium *Rhodothermus marinus*, corroborated by reverse transcriptase PCR, suggests that this enzyme may harbor two additional subunits to the minimal functional unit, one in the peripheral arm, a pterin carbinolamine dehydratase, and another in the membrane arm, a Na^+/H^+ antiporter, also shown to be able to confer Na^+ resistance to an *Escherichia coli* strain devoid of Na^+/H^+ antiporters. Here we provide further evidence that localize the antiporter subunit in the complex I of this organism by means of immunodecoration of *R. marinus* proteins with peptide antibodies produced against the *R. marinus* Na^+/H^+ antiporter.

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S4.27 Identification of the inhibitor binding SITE(S) in bovine complex-I by photoaffinity labeling

Masatoshi Murai^a, Naoya Ichimaru^a, Takao Yagi^b, Hideto Miyoshi^a

^aDivision of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

^bDivision of Biochemistry, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037, USA

E-mail: miyoshi@kais.kyoto-u.ac.jp

The inhibitor-binding domain in complex I is thought to be closely associated with the functions of the membrane arm such as ubiquinone reduction and proton translocation. Therefore detailed elucidation of the action mechanism of diverse inhibitors including the identification of their binding sites is necessary for understanding the functional and structural features of the membrane arm. We recently developed a new-type of inhibitor of bovine complex-I, i.e. piperazine-type inhibitors, whose manner of inhibitory action differs from that of traditional inhibitors such as rotenone and piericidin A. To identify the binding site of the piperazines, we carried out photoaffinity labeling using a synthetic photoaffinity probe ($[^{125}I]$ AFP). Our work revealed that the piperazine derivative binds to the 49 kDa subunit. This work along with earlier photoaffinity labeling studies suggests that the binding domain of complex-I inhibitors which comprised of multiple subunits (49 kDa, PSST, ND1 and ND5) is located at the interface of the peripheral and membrane arms. The apparent competitive behavior of inhibitors that seem to bind to different sites may be due to structural changes at the binding site, rather than occupying the same site.

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S4.28 Crystal structure of the NADH-oxidizing FAD domain from the Na^+ -translocating NADH:quinone oxidoreductase (Na^+ -NQR)

Minli Tao^a, Marco Casutt^a, Joachim Diez^b, Günter Fritz^c, Julia Steuber^a

^aBiochemisches Institut, Universität Zürich, 8057 Zürich, Switzerland

^bSwiss Light Source at Paul Scherrer Institut, 5232 Villigen PSI, Switzerland

^cFachbereich Biologie, Universität Konstanz, 78457 Konstanz, Germany
E-mail: mltao@bioc.uzh.ch

The Na^+ -translocating NADH:quinone oxidoreductase (Na^+ -NQR) from *Vibrio cholerae* is a respiratory complex that couples the exergonic oxidation of NADH with quinone to the transport of Na^+ across the membrane. It is a membrane-bound complex comprised of six subunits NqrA-F which contains four flavins and one Fe-S center. Subunit NqrF catalyzes the initial oxidation of NADH. We expressed and purified the FAD containing domain of NqrF which exhibited high rates of NADH oxidation. Crystals of the FAD domain diffracted to 1.8 Å and belonged to space group $P2_12_12_1$. Phases were determined by MAD of a crystal soaked with $K_2Pt(NO_2)_4$ for 3 days. The FAD domain is related to ferredoxin:NADP⁺ oxidoreductase (FNR) catalyzing hydride transfer from NADPH to flavin. In FNR, the dissociation of NADP⁺ is promoted by the stacking of an aromatic residue close to the isoalloxazine moiety of the flavin cofactor. We observed a similar interaction of the Phe282 with the FAD in the FAD domain, and propose that the orientation of Phe282 determines the catalytic efficiency of NqrF.

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