S4 Complex I

S4.1 Characterisation of the active/de-active transition of mitochondrial complex I

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NADH:ubiquinone oxidoreductase or complex I is a major contributor to ATP generation in mitochondria. It is also a source of detrimental free radicals as well as a target for oxidative damage by them. An unusual feature of complex I from several species is the active/de-active (A/D) transition [1,2]. The A-form catalyses the rapid physiological reaction of NADH oxidation and spontaneously converts to the dormant D-form within minutes if the enzyme is idle (e.g. during ischaemia). The D-form can undergo re-activation during slow catalytic turnover(s). Cys-39 of the mitochondrially-encoded subunit ND3 becomes exposed upon de-activation [2]. The A/D transition plays an important role in tissue response to hypoxia, therefore deactivation of the enzyme is one of the key mitochondrial events during ischaemia. The mechanism of deactivation is currently unknown, but we identified two new subunits involved in a conformational change during the A/D transition: ND1 and 39 kDa (NDUFA9) [2]. Most likely, de-activation is a result of concerted structural rearrangement of several subunits located in the region of the quinone binding site which leads to disruption of the terminal electron transfer. We suggested a physiological role of maintaining the enzyme in the D-form during the ischaemic period. Accumulation of the D-form could decrease the initial burst of respiration followed by ROS formation after reoxygenation. Thus, the A/D transition could be an intrinsic mechanism for decreasing oxidative damage during an early phase of reoxygenation. However, Cys-39 of the ND3 subunit, exposed in the D-form, is susceptible to modification by ROS and nitric oxide metabolites. Modification arrests re-activation of the D-form and inhibits the enzyme. The nature of thiol modification defines reversibility, re-activation dynamics, effect on mitochondrial ATP-production and therefore, outcome following ischaemia–reoxygenation injury. Modulation of the A/D equilibrium of complex I in situ could ameliorate ischaemia/reperfusion damage. Progress towards further characterisation will be accessed and regulatory implications of the A/D transition in situ discussed.

References


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S4.2 Modeling pathogenic mtDNA mutations in bacteria for probing their functional consequences in complex I with special reference to proton pumping efficiency

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Mitochondrial Complex I is a target of pathogenic mutations because seven of its subunits are encoded in mtDNA. Modeling of mtDNA mutations in bacteria offers a reasonably stable platform for experimentation. Therefore we tested mutations by means of modeling them in Escherichia coli NDH-1, the bacterial counterpart of mitochondrial Complex I. A knockout E. coli NUO subunit strain was produced and complemented in trans with an expression plasmid containing the wild-type or mutated gene. Deamino-NADH oxidation and membrane energization were monitored simultaneously by spectrophotometry using Neutral Red as a probe in inverted membrane vesicles. This allowed the determination of proton pump stoichiometry and membrane leak parameters by means of curve fitting with numerical integration of a model equation throughout the d-NADH oxidation pulse. Mutation in E24 of the human ND1 subunit causes an LHON/MELAS overlap syndrome. The E. coli counterpart of ND1-E24 is NUOH-E36. Its elimination caused a 95% inhibition of d-NADH oxidation and zeroed the proton pumping. An E36D replacement approximately halved the d-NADH oxidation rate and the proton pumping stoichiometry. Elimination of NUOH-E157 in a transmembrane helix halved the d-NADH oxidation capacity but it did not affect proton pumping, although the T. thermophilus homolog NQO8-E163 has been suggested to be involved in proton translocation. ND6 is a hotspot of mtDNA mutations. M64, Y59 and A72 are sites of pathogenic mutations and were studied in E. coli NUO for effects on proton pumping. Mutations in all three sites resulted in marked decrease in d-NADH oxidation in E. coli. The most effective mutations affecting proton pumping were the Y59F mutation and the elimination of M72, which actually is a counterpart of the human A72. The ND4L/NUOK subunit contains two highly conserved glutamates in the middle of transmembrane helices. Elimination of these carboxylic moieties killed the enzyme. Putting the twin carboxyls to a single helix by replacing E72 with Q72 and I39 with D39 halved...