S4/5 Redox-dependent nucleotide substrates and FMNH⁻/FMN binding affinities to the membrane-bound complex I

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A very potent specific inhibitor of Complex I, a derivative of NADH (NADH-OH) was used to analyse the interaction of nucleotides with oxidized and reduced enzyme in tightly coupled submitochondrial particles. Both the rate of the NADH-OH binding and its affinity to Complex I were strongly decreased in the presence of succinate. The effect of succinate was completely reversed by rotenone, Antimycin A and uncoupler. The relative affinity of ADP-ribose was also shown to be significantly affected by the enzyme reduction. Gradual inhibition of the rotenone-sensitive uncoupled NADH oxidase and the reverse electron transfer activities by NADH-OH yielded the same final titration point; however, the titration of NADH oxidase appears as a straight line whereas the titration of the reverse reaction resulted in a convex curve. The conditions for the reversible dissociation of flavin mononucleotide (FMN) from the membrane-bound Complex I were found. The catalytic activities of the enzyme declined when bovine heart submitochondrial particles were incubated with NADH in the presence of rotenone or cyanide at alkaline pH. FMN protected and fully restored the NADH-induced inactivation whereas riboflavin and flavin adenine dinucleotide did not.

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S4/6 Substrate induced conformational changes in respiratory complex I

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The reaction of the proton-pumping NADH:ubiquinone oxidoreductase, the respiratory complex I, is accompanied by major conformational changes. It is not clear whether these changes are essential elements for substrate binding, electron transfer, or proton translocation. The addition of NADH to the *E. coli* complex I induces conformational changes as revealed by electron microscopy, CD-spectroscopy, FT-IR spectroscopy, and site-directed spin-labelling. The conformational changes are partly due to the reduction of the complex and partly to the binding of NADH as deduced from the effects caused by the binding of NADPH. The addition of NADH and NADPH leads to a reduction of the Fe/S clusters of the complex. However, the enzymatic activity with NADPH is not sensitive to inhibitors of the quinone-site and not coupled with proton translocation. We conclude that NADPH is not a substrate for complex I. This is corroborated by the increased production of superoxide radicals after addition of NADPH. We propose that binding of NADPH leads to a reduction of complex I but leaves the quinone reduction site in a closed state. Binding of NADH induces additional long-range conformational changes, which are required to give access to the ubiquinone reduction site.

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S4/7 Is super-complex organization of the respiratory chain required for optimal electron transfer activity?

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The supramolecular assembly of the main respiratory chain enzymatic complexes in the form of "supercomplexes" has been proved by structural and functional experimental evidence. This evidence strongly contrasts the previously accepted Random Diffusion Model stating that the complexes are functionally connected by lateral diffusion of Coenzyme Q and cytochrome c.

This review provides an analysis of the functional consequences of the intermolecular association of the respiratory complexes pointing out the role of Coenzyme Q and of cytochrome c as channeled or as freely diffusing intermediates in the electron transfer activity of their partner enzymes. The association of Complex I with a Complex III dimer appears to be a common feature of mitochondrial respiratory chains, assuring electron channeling through bound Coenzyme Q, whereas the extent of association of Complex IV appears to depend on the mitochondrial type so that electron transfer often appears to be effected by random diffusion of cytochrome c.

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(S4) NADH dehydrogenases symposium abstracts (poster and raised abstracts)

S4.8 Optimizing and stabilizing a subcomplex of respiratory complex I for crystallization trials

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Respiratory complex I is the first enzyme in the mitochondrial electron transfer chain. It oxidises NADH, reduces ubiquinone and pumps four protons across the inner mitochondrial membrane, contributing to the proton motive force that is used to generate ATP. Currently, there is no atomic resolution structure available for the mitochondrial enzyme. Complex I from bovine heart mitochondria can be fractionated into subcomplexes to simplify it for functional and structural studies. One such subcomplex, Iα, comprises 26 subunits, has a molecular weight of 560 kDa, and encompasses the hydrophilic domain, and a portion of the hydrophobic domain. Modifications to the purification of subcomplex Iα aimed to prepare protein suitable for crystallization trials. Our criteria stipulated that the subcomplex must have a full complement of redox cofactors (eight iron–sulphur clusters and a flavin mononucleotide) and remain stable and mono-disperse over the course of a week. Electron paramagnetic resonance spectroscopy was used to probe the integrity of the iron–sulphur clusters in the subcomplex, and gel filtration chromatography was used to ascertain the effect of different detergents on the stability and dispersity of the subcomplex. Subcomplex Iα was most stable in the polyoxyethylene (C_xE_y) series of detergents, in particular, $C_{12}E_9$ and $C_{12}E_8$, though the gel filtration traces indicate that the subcomplex may not be completely homogeneous.

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