S4.13 A new insight into the superoxide production sites in bovine heart NADH: Ubiquinone oxidoreductase (complex I)

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Mitochondrial respiration produces superoxide as a byproduct. This could trigger oxidative stress in the organs which consume a large amount of energy. The major enzyme generating superoxide is NADH:ubiquinone oxidoreductase (complex I). Hirst's group proposed that superoxide is produced from flavin in bovine heart complex I both in the absence and presence of added decylubiquinone (DBQ) [PNAS 2006]. Using bovine heart complex I purified at Yoshikawa's laboratory, we performed similar experiments and obtained somewhat different results. We used both the acetylated-cytochrome *c* method and a spin-trap method to assay superoxide. In the absence of DBQ, our complex I produced superoxide from flavin. However, in the presence of DBQ, the site was not flavin, but iron–sulfur cluster N2. The NADH oxidation activity of our enzyme was 9–10 μmole mg^{-1} min^{-1} at 32 °C. Another complex I, prepared by the same Hirst's group but was reported in a different journal in the same year [Biochemisty 2006], had similar activities (8–9 μmole mg^{-1} min^{-1}). Strangely, the activity of the complex I used in their PNAS paper (2006) was very low (1.8 μmole mg^{-1} min^{-1} at 32 °C; only 20% of their other preparation). Was the latter preparation partially altered?

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S4.14 Characterization of the antiporter-like subunits Nuo L, M and N from respiratory chain complex I

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Complex I (NADH: quinone oxidoreductase) is the least understood enzyme in the respiratory chain. It catalyzes the oxidation of NADH and the reduction of quinone in the membrane. The electron transfer through the enzyme is coupled to proton pumping across the membrane, by a hitherto unknown mechanism. Sequence comparisons show that the Complex I membrane bound subunits NuoL, M and N are related to one particular class of Na^+ or K^+/H^+ antiporters, encoded by the gene cluster *mrp/sha/pha/mnh* in different bacteria. Therefore those subunits are prime candidates for harboring channels for proton translocation. The aim of this work is to investigate the function of the antiporter-like subunits in vivo and in vitro. Previously, the *mrpA* and *mrpD* were deleted from the *Bacillus subtilis* chromosome, resulting in a Na^+ and pH sensitive growth phenotype, that could be partially restored by expressing NuoL or NuoM from *Rhodobacter capsulatus*. In this work, we have constructed fusion proteins where the C-terminal end of the antiporter-protein is joined to cytochrome *c*. The fusion proteins were subsequently expressed in the ΔMrpA and ΔMrpD strains. This enabled quantification of the amount of Nuo proteins expressed, compared to the phenotype observed. In addition the cytochrome tag facilitates purification of the antiporter-like subunits for further studies.

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S4.15 The peripheral arm of complex I from *Yarrowia lipolytica*

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We have investigated the subunit architecture of the peripheral arm of complex I and the spatial relationship of iron sulphur cluster N2 with respect to the membrane. Electron transfer from NADH to ubiquinone in complex I is catalysed by the N and Q module. 3D reconstructions of single complex I particles from *Yarrowia lipolytica* revealed a six domain substructure of the peripheral arm and the use of monoclonal antibodies allowed the localization of two subunits of the Q module. A subcomplex lacking specifically the flavoprotein part of the N module was analysed by single particle electron microscopy. The 51 and 24 kDa subunits clearly corresponded to domain 1 located at the distal end of the peripheral arm. The X-ray structure of the hydrophilic arm fragment of *Th. thermophilus* complex I determined by Sazanov and Hinchliffe was modelled into the EM structure of the eucaryotic holo complex. The minimal distance of the iron sulphur centre N2 to the membrane phase was determined to be 35 Å. Functional implications are discussed.

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S4.16 Crystallization and structural characterization of Fab co-complexes of mitochondrial complex I

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Mitochondrial complex I is one of the most complicated and least understood macromolecular machineries. Fab and Fv fragments of immunoglobulins have been successfully employed for crystallization and structural analysis of membrane proteins. We have raised antibodies against complex I from *Yarrowia lipolytica* for structural studies and identified their binding sites by single particle electron microscopy. Here we report co-crystallization attempts of complex I with Fab fragments originating from monoclonal antibodies recognizing different epitopes within complex I. Intactness of the purified proteolytic antibody fragments was verified by gel filtration and by Western blotting. The influence on crystallization of complex I by Fab fragments depended on the binding epitope. Complex I/Fab co-complexes crystallized under conditions similar to those of the native protein. An antibody binding to the NESM subunit embedded in the membrane arm of complex I was selected for further structural studies. Current work is focused on the optimization of conditions for crystallization and X-ray data collection.

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