Mitochondrial Complex I (NADH ubiquinone oxidoreductase) is the first of three energy transducing enzymes of the respiratory chain. Loss of Complex I activity is associated with ageing, neurodegeneration and ischaemia–reperfusion injury rendering further investigation of Complex I catalytic regulation pertinent.

The mammalian enzyme can exist in two conformationally-distinct, interconvertible forms — active (A) and dormant, de-active (D). These forms have been described in intact cells [1] and recently in various tissues in ischaemia (as further described in [2] of this issue). The high activation energy of 270 kJ/mol of the A to D transition [3] indicates the occurrence of major conformational changes in Complex I.

In this work we have developed an approach to analyse the differences in relative location of Complex I subunits in the A and the D-form. Using preparation of bovine heart submitochondrial particles (SMP), we have established the conditions in which the majority of the enzyme is present in the D-form or, alternatively, the majority is in the A-form. Several hetero- and homobifunctional crosslinkers of different length were used. Complex I was isolated using Blue Native electrophoresis and subunits were separated using 2D SDS PAGE. The crosslinking products were analysed by MS/MS and based on the presented data possible differences in the relative location of Complex I subunits are discussed.

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6P7

Paracoccus complex I as a model for the bovine enzyme: Developing methods to study proton translocation

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Complex I catalyses the two electron oxidation of NADH by quinone, to drive proton pumping across an energy transducing membrane. Much work on complex I has been done using sub-mitochondrial particles derived from the inner membrane of mitochondria, these have the active sites of the respiratory complexes facing the bulk solution to allow the direct access of substrates. An analogous system can be created using *Paracoccus denitrificans*, based on osmotic rupture of lysozyme digested cells. *P. denitrificans* is an ideal model for mammalian complex I as all the subunits can be mutated via genomic manipulation; additionally, *P. denitrificans* complex I is closely related to the eukaryotic enzyme. Monitoring NADH at 340 nm is easy, but monitoring proton pumping is much harder; available methods are either only semi-quantitative or have a response time that is too slow to follow the pre-steady state reactions that build the proton motive force. We have developed a robust system that allows small changes in pH in the bulk solution outside of the vesicles to be monitored by using pH probes. Our system allows NADH oxidation and proton translocation to be monitored alongside each other; we aim to use changes in these activities induced by mutagenesis to construct a quantitative model of complex I catalysis.

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6P8

The antidiabetic drug Metformin as an inhibitor of respiratory complex I

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Metformin is widely prescribed for the management of type 2 diabetes. It has been proposed to exert its beneficial effects by inhibiting respiratory complex I in mitochondria; the inhibition has been proposed to activate AMP kinase, triggering a cascade which increases glucose uptake, inhibits gluconeogenesis and fatty acid synthesis in the liver, and regulates insulin synthesis and secretion in pancreatic islet cells [1,2]. More recently, metformin treatment has been linked to anticancer benefits [3]. Therefore, it is crucial to determine precisely how this drug produces its observed effects.

Complex I has been proposed as the primary target of metformin due to the observed decrease in NADH-linked oxygen consumption in intact cells, isolated mitochondria and submitochondrial particles upon metformin treatment [1,4]. However, the molecular mechanism of inhibition has not yet been deduced. In this work, the effects of metformin on catalysis by isolated bovine complex I are described on a molecular level, and the projected impacts and downstream effects of the inhibition caused by therapeutically-relevant levels are discussed.

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6P9

Functional interaction between complex I and mitochondrial NOS (mtNOS)

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Mitochondrial complex I catalyses electron transfer from NADH to ubiquinone and it is the major entry point of substrates to the respiratory chain. Complex I not only produces $O_2^{\cdot-}$ through the auto-oxidation of flavin-semiquinone, but also it is sensitive to oxidants and reactive nitrogen species. NO inhibits complex I activity by S-nitrosylation or Fe-nitrosation. Mitochondrial NO production is carried out by the mtNOS, a NOS located in mitochondrial inner membrane. Persichini et al. [1] reported that mtNOS is associated to Va subunit of cytochrome oxidase. Franco et al. [2] showed that not only complex IV but also complex I proteins immunoprecipitate with mtNOS, suggesting direct physical interactions between mtNOS and complexes I and IV proteins. Therefore, the aim of this work was to characterize the functional interaction between complex I and mtNOS using phosphorylating electron transfer particles (ETPH-Mg^{2+}), i.e. inside-out vesicles, that expose NADH dehydrogenase and mtNOS to the surrounding medium. ETPH-Mg^{2+} showed a NAD^+ reductase