S15.8 Characterisation of the interaction between cytochrome bc_1 complex and its substrate cytchrome *c*

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In mitochondrial respiration, water soluble protein cytochrome *c* (CYC) accepts an electron from membrane bound cytochrome bc_1 complex (QCR). The interaction between QCR and CYC is transient in nature enabling turnover numbers greater than 100^{-1}s. The crystal structure for the complex was determined at 1.9-Å resolution. In the crystal structure, the homodimeric complex QCR binds only one CYC. Monovalent CYC binding is correlated with the conformational changes of the Rieske head domain and subunit QCR6p. More structured interfacial waters are associated with the QCR (cytochrome c_1 (CYC1)) than the CYC side. At the interface, the positively charged residues of CYC are more mobile than the negatively charged residues of CYC1. This pronounced hydration coupled with mobility mismatch is favorable for transient binding.

On the basis of the crystal structure, the role of interface residues in binding and electron transfer has been further probed by site-directed mutagenesis and isothermal titration calorimetry (ITC). Results from ITC show millimolar affinity interaction with endothermic enthalpy change. The QCR:CYC interface contains a cation-π interaction between F230 of CYC1 and R19 of CYC. To analyze the role of this interaction pair in electron transfer, F230L and F230W mutants were used to measure electron transfer rates by flash photolysis.

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S15.9 The alternative complex III from *Rhodothermus marinus* and its functional association with caa_3 oxygen reductase

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The bacterium *Rhodothermus marinus* has a six subunit alternative complex III. Three of these subunits are peripheral proteins (a monohemic and a tetratahemic cytochrome *c*, and a large protein containing iron sulphur centres). The other three are transmembrane proteins (two of them having quinone binding sites). In the *R. marinus* genome the gene cluster that codes for this alternative complex III is immediately followed by the gene cluster that encodes the caa_3 oxygen reductase. This observation may predict a formation of a functional association between both complexes. The aim of this study was to investigate the existence of this macromolecular organisation. By UV–Visible spectral analysis was seen that the alternative complex III is specifically reduced by menadiol since this reduction is HQNO inhibited. When reduced, the complex is directly oxidised by the caa_3 which indicates that the presence of an electron carrier between the complexes is not essential. Furthermore, by BN-PAGE was detected a band with a molecular mass consistent with the presence of both complexes together. Cytochrome *c* oxidase activity and colouring under heme-staining procedures is detected in this complex. A SDS-PAGE shows that this band comprises the subunits of both complexes. These results indicate a presence of a functional organisation between the alternative complex III and the caa_3 of *R. marinus*.

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S15.10 The role of glu272 in ubiquinol oxidation of mitochondrial cytochrome bc_1 complex

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The mitochondrial cytochrome bc_1 complex links electron transfer from ubiquinol to cytochrome *c* by a protonmotive Q cycle mechanism in which ubiquinol is oxidized at center P and ubiquinone is rereduced at center N. E272 of the conserved PEWY loop of cytochrome *b* has been suggested as ligand in the enzyme–substrate complex and as proton acceptor in parallel proton–electron transfer towards heme b_L. E272D and E272Q mutations support the importance of the residue for correct ubiquinol oxidation, showing effects such as lowered ubiquinol cytochrome *c* reductase activity, elevated bypass reactions, and altered K_M for ubiquinol. However, these effects may also be indirect and the role of E272 as direct ligand of ubiquinol is debated. Furthermore, E272 is not fully conserved. We suggested that in β- and γ-proteobacteria, in which the PEWY glutamate is substituted with valine or proline, a glutamate equivalent to yeast H253 is conserved, which could take over the proton transfer function. To challenge this hypothesis, single and double substitutions of H253 and E272 have been constructed in *Saccharomyces cerevisiae*. Eight variants were produced and the characterization of the detergent-solubilized and purified complexes will be presented.

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S15.11 Do redox properties of c_i heme depend on Q_i site occupation and structure?

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Oxidoreductases of the cytochrome bc_1/b_6f family transfer electrons from a liposoluble quinol to a hydrosoluble acceptor protein and contribute to the formation of a transmembrane electrochemical potential. In addition, cytochrome b_6f complex (Cyt b_6f) is thought to play specific roles in oxygenic photosynthesis, namely its involvement in cyclic electron transfer around photosystem I, in the regulation of 'state transitions', and in the pumping of additional protons. The crystal structure of Cyt b_6f has revealed the presence in the Q_i site of an atypical *c*-type heme, heme c_i. Unusually, the protein does not offer any axial ligand to the heme iron which becomes accessible to exogenous ligands. In this work we describe a mutagenesis approach aimed at characterising heme *c* and its interaction with the Q_i site environment, and in particular, a mutant of *Chlamydomonas reinhardtii* in which the Phe 40, from subunit IV, was substituted with a Tyrosine. Interestingly, such substitution is accompanied by a marked down-shift of its redox potential (from +100 mV to −200 mV, at pH 7). We discuss the functional consequences of this substitution in terms of quinone accessibility as well as energy landscape of the electron transfer chain.

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