S15.12 Inhibition of mammalian cytochrome bc_1 complex by chromanols and related compounds

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Tocopherols (chromanols) and tocopheryl quinones occur naturally in biological membranes. Related 6-hydroxy-chromanones were observed as byproducts of industrial tocopherol processing. Because of the extensive use of some tocopherols as food supplements, the aim of the study was to assess their inhibiting properties at the mammalian cytochrome bc_1 complex as toxicological and pharmacological target. The effects of these compounds and low molecular analogues at the isolated cytochrome bc_1 complex from bovine heart were studied by dual-wavelength photometry, stopped flow photometry, and low temperature EPR spectroscopy. Our data show that α- and γ-tocopherols as well as α-tocopheryl quinone required millimolar concentrations to achieve half inhibition (IC_{50}) of the decylubiquinol: cytochrome *c* oxidoreductase activity. In contrast, γ-tocopheryl quinone and the less lipophilic 6-hydroxy-4,4,7,8-tetramethyl-chroman-2-one (TMC2O) exhibited IC_{50} values of 117±6 and 94±12 μM, respectively. Stopped flow measurements of the reduction of cytochromes in the isolated cytochrome bc_1 complex revealed that TMC2O preferably inhibited the cytochrome c_1 reduction. EPR spectra of the ascorbate-reduced Rieske iron sulfur protein at 20 K documented a shift of g_z from 2.0288±0.0005 (control) to 2.02240±0.0003 (TMC2O) indicating a specific binding of this compound. Besides toxicological implications for the effect of γ-tocopheryl quinone on the cytochrome bc_1 activity in vivo, our data suggest that synthetic modifications of TMC2O could lead to a new class of cytochrome bc_1 inhibitors.

doi:10.1016/j.bbabio.2008.05.410

S15.13 Is cytochrome *b* glutamic acid 272 a quinol binding residue in the bc_1 complex?

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On the basis of the three-dimensional structures of the *bc*1 complex in the presence of the inhibitor stigmatellin, it is assumed that the substrate quinol binding involves the cyt *b* glutamate residue E272. However, this residue is not totally conserved during evolution: in some β and γ-proteobacteria, it is replaced by valine or proline and another glutamate is conserved and corresponds to H253 in yeast. Studies of bc_1 complexes with E272/H253 mutations will be presented. Replacement of E272 with apolar residues did not abolish the *bc*1 activity, although it slowed down the kinetics of electron transfer. The *K*m value for the binding of the substrate quinol was not modified, and the EPR data showed that the quinol binding still occurred in the mutants. Binding of stigmatellin was retained, however mutations E272P,V induced resistance toward myxothiazol. The pH dependence of the *bc*1 activity was not modified in the absence of the glutamate E272. Our results suggest that residue E272 may not be involved in direct substrate binding or in its direct deprotonation. Revertants were selected with polar residues serine and threonine at position 272. The data lead us to suggest that E272 may be involved in a later step on the proton exit pathway via the interaction with a water molecule.

doi:10.1016/j.bbabio.2008.05.411

S15.14 Stochastic approach of bc_1 complex functioning

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The bc_1 complex is a central complex in the mitochondrial respiratory chain. It links the transfer of electrons from ubiquinol (QH_2) to cytochrome *c* and proton translocation across the inner mitochondrial membrane. It is widely agreed that the "Q-cycle mechanism" proposed by Mitchell correctly describes the bc_1 complex functioning. It is based on an unexpected separation of the two electrons coming from the QH_2 molecule bound at the Q_0 site of the bc_1 complex. One electron is transferred to the iron–sulfur centre (FeS) of the iron sulphur protein (ISP) and the second to the lower potential heme b_L. The electron on heme b_L moves within the cytochrome *b* to reduce the higher potential heme b_H, which in turn reduces an ubiquinone (Q) or a semiquinone (SQ) at a second ubiquinone binding site Q_i. Using a stochastic approach based on the known spatial structure of bc_1 complexes and the kinetic parameters described by Moser and Dutton, we have demonstrated the natural emergence of the Q-cycle mechanism and the quasi absence of short-circuits in the functional dimer of bc_1 complex without the necessity to invoke any additional mechanism. In this poster we studied the various parameters which influence the reaction between redox centers. The natural distances measured on the crystallographic structures appeared to minimize the short-circuits thus favouring an energetic "Q-cycle" mechanism.

doi:10.1016/j.bbabio.2008.05.412

S15.15 Remodelling bc_1 complex Q_o site in yeast to study acquired resistance to inhibitors

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Inhibitors of the mitochondrial respiratory chain enzyme cytochrome bc_1 – especially inhibitors targeting the Q_o site of the enzyme – have been developed as anti-microbial agents. They are used in agriculture to control plant pathogenic fungi and in medicine against human pathogens, such as the malaria parasite. Unfortunately, the problem of acquired resistance has rapidly emerged. Resistance is often linked to mutation within the mitochondrially-encoded cytochrome *b* that forms the Q_o site. To obtain information on the molecular basis of the resistance and of the differential evolution of the mutations, we used yeast (*Saccharomyces cerevisiae*) as a model organism. We modified the Q_o site of yeast bc_1 complex and introduced variations and resistance mutations found in pathogens. The respiratory activity, the sensitivity to inhibitors and the fitness of the yeast mutants were then analyzed. In particular, we studied the resistance mutations F129L, G137R and G143A found in many pathogenic fungi of important crops. We addressed the question of the impact of structural variations in the Q_o site and of variations in the intron/exon structure of the cytochrome *b* gene on the evolution of G143A. We also remodeled

the yeast Q_o pocket to mimic the *Plasmodium falciparum* and the human enzymes.

doi:10.1016/j.bbabio.2008.05.413

S15.16 The *P. denitrificans* cytochrome bc_1 complex: A deletion in the acidic domain of cytochrome c_1

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The cytochrome bc_1 complex from *Paracoccus denitrificans* is a three-subunit transmembrane complex involved in the bacterial aerobic respiratory chain. Its cytochrome c_1 is made up of three domains: a C-terminal transmembrane domain, a core domain which covalently binds the redox cofactor, and an N-terminal acidic domain, a unique feature in *P. denitrificans*; it has a particular amino acidic composition, which gives the subunit an overall negative charge mostly due to the abundance of Glu residues. This feature has been exploited for the purification of the complex so far on ion exchange chromatography. To analyze the role of this extra sequence, we cloned, expressed, and purified a 10×His tagged mutant complex missing the acidic domain. This complex has been characterized via SDS- and BN-PAGE, Western Blots, kinetic tests, and LILBID MS. We obtained a fully assembled complex, forming dimers (unlike the wild type which forms tetramers), showing a standard activity of about 60% the wild type. Fast kinetic experiments and further BN-PAGE analysis are conducted presently to analyze the electron transfer between c_1 and c_{552}, and the potential role of this deletion complex in the *Paracoccus* respirasome.

doi:10.1016/j.bbabio.2008.05.414

S15.17 Probing the environment of heme c_i of rieske/cytochrome *b* complexes by its phylogenetic diversity and by site directed mutagenesis

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One of the differences between cytochrome bc_1 and b_6f complexes is the presence of heme c_i in the Q_i binding site of the latter enzyme. The X-ray crystallographic structure of the b_6f complex showed that heme c_i is devoid of an axial protein ligand, leaving a vacant coordination site that might be involved in Q_i site turnover. In order to understand the function of this peculiar cofactor we investigated its presence and EPR characteristics in heliobacteria and in mutants of the b_6f complex from *Chlamydomanas reinhardtii*. EPR spectra and biochemical analyses confirmed the presence of heme c_i in the Rieske/cytochrome *b* complex from Heliobacteria. The spectra showed that a strong axial (5th) ligand to the heme iron is present and structural modelling places a Glu in a position suitable for heme c_i ligation. We compare the signature of heme c_i in heliobacteria to a mutant of the b_6f complex were a Phe in the vicinity of heme c_i iron was replaced by a Tyr and to the wild-type b_6f complex in the presence of NQNO. This inhibitor has been shown to be a ligand to heme c_i.

doi:10.1016/j.bbabio.2008.05.415

(S16) Proteomics and mitochondria symposium lecture abstracts

S16/1 Advances in defining the mitochondrial membrane proteome

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The mitochondrial proteome is a precise description of the chemical composition and functions of mitochondrial proteins. The hydrophobicity of membrane proteins causes problems with their analysis and under-representation in proteomes. Improvements in membrane protein analyses have expanded our knowledge of the mitochondrial proteome. Organic solvent combinations have been employed for selective extraction of membrane proteins and also for purification with HILIC and reverse-phase chromatography on macroporous supports. Importantly, these methods are compatible with electrospray mass spectrometry and mitochondrial membrane proteins have been characterised by molecular mass measurements and identified directly by tandem mass spectrometry of intact proteins. Molecular masses, for all thirteen hydrophobic products of the bovine mitochondrial genome, demonstrate the absence of any modifications other than *N*-formyl. These measurements have resolved uncertainties concerning the interpretation of the mitochondrial genome. Also, the chemical composition of bovine mitochondrial complex I has been finalised by the definition of the natural, stable post-translational modifications on ND proteins. Sequence data from tandem MS experiments on protein ions, have identified many components of solvent extracts including some hydrophobic proteins undetected by proteomic analyses of peptides. Two small hydrophobic proteins of unknown function, DAPIT and a 6.8 kDa proteolipid associate with ATP synthase complex in the presence of phospholipids. The functions of others are under investigation.

doi:10.1016/j.bbabio.2008.05.416

S16/2 Protein phosphorylation site analysis on different hybrid linear ion trap mass spectrometers

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Protein phosphorylation is most probably the most abundant reversible post translational modification (PTM) in the human proteome, regulated by 516 protein kinases and approx 100 protein phosphatases. Although it is an abundant PTM, it is not the easiest to study by conventional peptide mass spectrometry methods, as the combination of low stoichiometry and poor ionisation leads to a low representation of phosphopeptides in LC–MS based database searches. Utilising the selective scanning features of a 4000 Q-Trap mass spectrometer, such as precursor ion scanning and multiple reaction monitoring (MRM), protein phosphorylation site identification by LC–MS has become more routine in our laboratory. The rapid duty cycle of both precursor ion scanning and MRM permits phosphopeptide detection and sequencing by ms/ms in an LC–MS