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Infection with *Mycobacterium tuberculosis* is a major world health problem leading to nearly 2 million deaths per year with about 2 billion people latently infected. Although antibiotics are available for tuberculosis (TB) treatment, multi-drug resistant strains as well as mycobacteria staying dormant within the host strongly demand development of drugs with new targets. Diarylquinolines (DARQs) are a novel class of antibiotics highly active against *M. tuberculosis*. In this study we validated ATP synthase as the antibiotic target using biochemical and binding assays. In a biochemical assay, DARQs inhibited ATP synthase at nanomolar concentrations. BIAcore studies with DARQ-coated chips revealed strong binding for purified ATP synthase, but not for the hydrophilic F1 part. Point mutations in hydrophobic subunit-c lead to decreased binding affinity. These results establish ATP synthase as the target for DARQs and suggest that inhibition of energy production is a promising approach for antibacterial drug discovery. Experiments to determine the effect of DARQs on mitochondria and to elucidate the role of ATP synthase in the physiologically dormant state of mycobacteria are under way, recent progress will be discussed.

doi:10.1016/j.bbabio.2008.05.047

S1.10 Keysteps during proton translocation by ATP synthases

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The proton motive force, consisting of the proton gradient (ΔpH) and the electrical membrane potential ($\Delta\psi$), is the driving force of ATP synthesis in H^+-translocating enzymes. The two components are thermodynamically, but not kinetically equivalent. It is therefore speculated, that ATP synthases from different environments have adapted their ATP synthase to efficiently utilize the predominant driving force (high $\Delta\psi$ and low or inverse ΔpH in bacteria and mitochondria; low $\Delta\psi$ and high ΔpH in chloroplasts). We have compared the proton transport rates through the F_0 part of the ATP synthases of *E. coli*, spinach chloroplasts and the alkaliphilic bacterium *Bacillus* TA2.A1 in dependence of the proton concentration. We demonstrate, that enzymes from different origins show unequal pH profiles, if protons are pumped from the *P*-side to the *N*-side. We further introduced critical amino acid residues of the ATP synthase from the alkaliphilic bacterium in the enzyme of *E. coli* and obtained similar pH profiles as observed in the alkaliphilic wild type. Together with additional data, we propose a multistep model for H^+-transport through the F_0 part in hydrolysis and synthesis direction, in which the membrane potential and the proton gradient play distinct roles during H^+-translocation.

doi:10.1016/j.bbabio.2008.05.048

S1.11 Mutational analysis of the function of the βDELSEED-Loop of ATP synthase

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The DELSEED-Loop in the C-terminal domain of the β subunit of ATP synthase is often regarded as the structural element that drives rotation of γ during ATP hydrolysis. However, removal of all five negative charges in the DELSEED motif itself (→AALSAAA) had no significant effect on the rotational torque (Hara et al. (2000) JBC 275, 14260). A large portion of the interaction between the loop and γ is due to the conserved hydrophobic residues βI373, βI376, and βL377 (*E. coli* numbering). A recent molecular dynamics study implied specifically residues I376 and L377 of the β_E subunit as important for driving rotation of γ (Pu and Karplus (2008) PNAS 105, 1192). We generated the single mutants βI373A, βI376A, βL377A, the double mutant βI376A/βL377A, and the triple mutant βI373A/βI376A/βI377A. Whereas the triple mutant failed to assemble, the other mutants were able to catalyze ATP synthesis and hydrolysis. Especially the double mutant showed activities that were slightly higher than wild-type. Interestingly, a βD372A/βI373A/βI376A/βL377A/βE381A quintuple mutant was assembled and showed reduced, but significant ATP synthesis and hydrolysis activity. The results indicate that more pronounced changes of the loop structure might be required to disrupt function. In our newest set of mutants, we are generating deletions of 3–4 amino acid residues.

doi:10.1016/j.bbabio.2008.05.049

S1.12 Supramolecular organization of the yeast F_1F_o-ATP synthase within the inner mitochondrial membrane and in detergent extracts

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The yeast mitochondrial F_1F_o-ATP synthase is a large complex of 600 kDa that uses the proton electrochemical gradient generated by the respiratory chain to catalyze ATP synthesis from ADP and Pi. For a large range of organisms, it has been shown that mitochondrial ATP synthase adopts oligomeric structures. Moreover, several studies have suggested that a link exists between ATP synthase and mitochondrial morphology. In order to understand the link between ATP synthase oligomerization and mitochondrial morphology, more information is needed on the supramolecular organization of this enzyme within the inner mitochondrial membrane. We have conducted an electron microscopy study on wild type yeast mitochondria at different levels of organization from spheroplast to purified ATP synthase complex. Using electron tomography, freeze fracture, negative staining and image processing we show that cristae form a network of lamellae, on which ATP synthase dimers assemble in linear and regular arrays of oligomers. Our results shed new light on the supramolecular organization of the F_1F_o-ATP synthase and its potential role in mitochondrial morphology.

doi:10.1016/j.bbabio.2008.05.050