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Energy metabolism has emerged as a new target-pathway for development of new anti-tubercular drugs [1], which are urgently needed to combat concomitantly emerging drug-resistant bacterial strains. A new ATP synthase inhibitor [2], the diarylquinoline TMC207, acts in a highly selective manner with strong affinity for ATP synthase from *Mycobacterium tuberculosis* and only minimal effect on the human homologue [3]. These features are prerequisite for clinical application of ATP synthase inhibitors and make TMC207 a promising drug candidate for shortening and simplifying tuberculosis chemotherapy.

We used biochemical assays and binding studies to characterize the interaction between TMC207 and ATP synthase [3]. Using inverted membrane vesicles from the non-pathogenic strain *M. bovis* BCG [4] we show that TMC207 acts independent of the proton motive force and does not compete with protons for a common binding site. The drug is active on mycobacterial ATP synthesis at neutral and acidic pH with no significant change in affinity between pH 5.25 and pH 7.5 (IC_{50} = 5-10 nM), indicating that the protonated form of TMC207 is the active drug entity. The interaction of TMC207 with ATP synthase can be explained by a one-site binding mechanism, the drug molecule thus binds to a defined binding site on ATP synthase. TMC207 affinity for its target decreases with increasing ionic strength, suggesting that electrostatic forces play a significant role in drug binding. These results will be discussed in view of existing docking studies and may provide input for structure-based design of ATP synthase inhibitors. Moreover, the high affinity of TMC207 observed at low proton motive force and low pH values may in part explain the exceptional ability of this compound to efficiently kill mycobacteria in different microenvironments.

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doi:10.1016/j.bbabio.2012.06.049

1P17

Localization and orientation of TMEM70 protein in the inner mitochondrial membrane

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Isolated ATP synthase deficiency of nuclear origin leads to a rather frequent fatal neonatal encephalo-cardiomyopathy (OMIM604273). The most common cause of the disease is c.317-2A>G mutation in *TMEM70* gene, coding for specific factor of mitochondrial ATP synthase biogenesis with unknown function [1]. Characterization of TMEM70 protein properties is thus necessary for elucidation of its biological role and better understanding of the pathogenic mechanism of the disease. In this study we analyzed in detail the biosynthesis and presumed mitochondrial localization of the protein using cells expressing GFP- or FLAG-tagged forms of TMEM70 protein as the experimental model. We have found that *TMEM70* gene encoded precursor of 29 kDa is processed into 21 kDa mature protein. The import study revealed that the TMEM70 protein is processed after transport into mitochondrial matrix, but the level of transported protein and its cellular content are very low. Immuno-cytochemical analysis and fractionation of mitochondria confirmed the localization of the TMEM70 protein in the inner mitochondrial membrane [2]. Accessibility of the tagged forms of TMEM70 protein to trypsin followed by electrophoretic or microscopic analysis demonstrated the orientation of C- and N- protein termini into mitochondrial matrix. 2D BN/SDS electrophoretic analysis of digitonin-solubilized proteins further showed, that TMEM70 protein is able either form an oligomeric structures or associate with other, yet unknown proteins.

Supported by GACR 303/11/0970, GA UK 370411, 37710 and RVO:67985823.

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doi:10.1016/j.bbabio.2012.06.050

1P18

Technical design of a module for the continuous production of biochemical energy for cell-free protein synthesis

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Introduction and aim

In order to develop a flow reaction chamber for the continuous cell free protein synthesis, one key challenge is to provide energy in form of Adenosine 5′-triphosphate (ATP) for the process. To feed the system with ATP molecules from an external source is one strategy to solve this problem. However in this case, the major drawback is that the reaction products Adenosine 5′-diphosphate (ADP) and phosphate accumulate in the reaction solution and have to be removed frequently. Another approach is the direct regeneration of ATP from ADP and the phosphate molecule within the reaction compartment. Therefore, our aim is to isolate the ATP-Synthases from *Escherichia coli* and to immobilize these proteins onto custom made carrier membranes. In the presented work, an ATP regeneration module is designed employing computational modeling to transfer a batch reaction into a continuous protein synthesis process.