

1P35**A novel TB drug targets the ATP synthase rotor ring of mycobacteria**

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The increasing number of tuberculosis (TB) infections with multi-drug resistant (MDR) *Mycobacterium tuberculosis* strains causes a severe worldwide health problem, necessitating the development of new antibiotics. A novel drug, the diarylquinoline TMC207, was found to be highly effective in the treatment of MDR-TB. It was reported that the drug targets the rotor ring of the energy converting enzyme F₁F₀-ATP synthase, which is essential for the survival of mycobacteria. However, structural and biochemical information about the precise binding site of TMC207 and its mechanism of action are not known at the level of the isolated ATP synthase or its rotor ring complex. We performed ATP synthesis experiments on a mycobacterial ATP synthase, including inhibition studies using two different antibiotics, TMC207 and mefloquine. TMC207 inhibited 50% of ATP synthesis at 0.03 μM. In contrast, mefloquine was less active, but nevertheless still an efficient inhibitor of the mycobacterial ATP synthase at 15 μM (IC₅₀). We then purified a mycobacterial ATP synthase rotor (c-) ring and established a drug competition assay using the well known ATP synthesis inhibitor dicyclohexylcarbodiimide (DCCD). The results show that TMC207 competes with DCCD at the proton binding site of the c-ring. This is direct biochemical proof that the c-ring's ion binding site is the actual target of the drug, to which it binds with high affinity and specificity. Similar results were found for mefloquine, although we detected lower affinities for the proton binding site. To further explore the highly specific drug-target interaction of TMC207 with the mycobacterial c-ring, we aim to solve its structure in complex with TMC207. X-ray diffracting 3D crystals of the rotor ring-drug complex are available. This work provides insights into the specific interaction of the isolated mycobacterial ATP synthase rotor ring with a novel and highly potent drug, in the perspective to treat infections in patients with MDR *M. tuberculosis* strains. Based on these results, an optimisation of TMC207 derivatives and further development of drugs, which target the ATP synthase to treat TB becomes conceivable.

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1P36**The effect of mutations γM23K and βL249Q on ADP-inhibition of H⁺-F₀F₁-ATP-synthase in *Escherichia coli***

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H⁺-F₀F₁-ATP-synthase catalyzes generation of ATP from ADP and inorganic phosphate using the energy of transmembrane electrochemical potential difference of protons. This enzyme can function in opposite direction as ATP-driven proton pump. ADP-inhibition is known to reduce this ATPase activity: ADP without phosphate binds

empty catalytic site and this binding induces transition of enzyme to ADP-inhibited state.

We studied ADP-inhibition on inverted membranes from *Escherichia coli*. ATP hydrolysis is accompanied by stoichiometric proton release at pH>7.2. This acidification was measured by pH indicator phenol red absorption changes.

We studied wild-type enzyme at room temperature (24 °C) and 37 °C. At room temperature ADP-inhibition was weaker than at 37 °C. Also we studied the influence of inorganic phosphate on the ADP-inhibition of the enzyme. In the presence of phosphate ADP-inhibition increased in the wild-type enzyme.

It was shown previously [1] that mutation Met23Lys in the γ subunit of the enzyme enhanced ADP-inhibition compared to the wild-type enzyme in purple bacteria *Rhodobacter capsulatus*. Our results indicate that mutation showed the same effect on membranes from *E.coli* at 37 °C, but at room temperature this effect was reversed as in the wild type. Inorganic phosphate enhanced ADP-inhibition of the enzyme carrying this mutation.

We also found that enzyme with mutation βLeu249Gln situated on the interface between α and β subunits had weak ADP-inhibition at 24 °C, but at 37 °C the inhibition was stronger. In contrast to γMet23Lys and wild-type, addition of phosphate reduced ADP-inhibition of the βL249Q mutant. Our results allow us to suppose that this position is important for modulation of ADP-inhibition by phosphate.

Reference

- [1] B.A. Feniouk, A. Rebecchi, D. Giovannini, S. Anefors, A.Y. Mulkidjanian, W. Junge, P. Turina, B.A. Melandri, *Biochim. Biophys. Acta* 1767 (2007) 1319–1330.

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1P37**Cholesterol-dependent translocation of the F₁F₀-ATP synthase inhibitor factor 1 (IF₁) to cell surface**

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The mitochondrial F₁F₀-ATP synthase is one of the most ubiquitously studied enzymes. In physiological conditions it carries out ATP synthesis, utilizing the H⁺ gradient generated by the Electron Respiratory Chain. When mitochondrial function is compromised, the enzyme acts as ATP consumer depleting cells from ATP which is inhibited by IF₁-the natural endogenous inhibitor of ATP synthase [1]. Recent reports, including ours [2], have suggested the presence of subunits or the whole complex on the cell surface of various cell types [3].

Both by immunofluorescent and western blotting analyses, we found that IF₁ co-localizes with the ATP synthase (β-subunit) and flotillin, a lipid raft marker, on the cell surface of HeLa cells. The OSCP, a subunit of the ATP synthase assembled at very late stage, was also found to pair the β-subunit and IF₁ in non-permeabilized cells, suggesting the presence of a small functional mitochondria-like organization. Notably, cholesterol loading, performed at different time intervals, reduced the mitochondrial content of IF₁, with a parallel increase of the ectopic expression.

In conclusion, our data report that IF₁ (re)localizes in the lipid rafts of plasma membranes in association with the F₁F₀-ATPase depending