Summary

ATP synthase is a membrane protein, which plays an essential role in the energy metabolism of the living cell. ATP synthase is evolutionarily strongly conserved among eukaryotes and prokaryotes. In most organisms the primary function of the enzyme is to synthesize adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate. ATP synthase is an essential enzyme in the energy metabolism of the bacterium Mycobacterium tuberculosis. This bacterium grows very slowly and can survive for years without replication in the human body. The energy metabolism of the tubercle bacterium, in particular ATP synthase, may show adaptations to this environment, and may be different from other bacteria and human cells. We investigated ATP synthase in this pathogen and showed that, although ATP synthase in mycobacteria is able to produce ATP efficiently, it cannot reverse its function, in contrast to other enzymes. Possibly, this is an adaptation mechanism of the bacterium to survive in an environment with low concentrations of nutrients.

The World Health Organization (WHO) declared tuberculosis (TB) a global emergency. One third of the world population harbors TB and approximately 2 million people die every year. Many tubercle bacteria are resistant to currently used tuberculosis antibiotics, thus increasing the importance of the development of new drugs. Recently, it was shown that ATP synthase is a promising target for new anti-tuberculosis drugs. The diarylquinolines, a new family of antibiotics, bind specifically to ATP synthase of M. tuberculosis. Typical inhibitors of ATP synthase lack selectivity and inhibit ATP synthase in bacteria as well as human mitochondria. In a phase I clinical study with healthy volunteers, short-term administration of the diarylquinoline TMC207 in humans was found to be safe and well tolerated without serious adverse effects. We showed that mycobacterial ATP synthase displayed more than 20,000-fold higher sensitivity for TMC207 compared to that of human mitochondrial ATP synthase. This suggests that TMC207 may not elicit ATP synthesis-related toxicity in mammalian cells. We also investigated the mode of binding between TMC207 and mycobacterial ATP synthase. Results support a mechanism predicted by docking studies. The drug efficiently interacts with its target independent of environmental conditions such as the local pH and the proton motive force, an important property for the tubercle bacterium. A proteomic approach elucidates the metabolic response of the bacteria to the presence of the antibiotic. These results may reveal points of physiological vulnerability of the tubercle bacterium. Availability of isolated mycobacterial ATP synthase may help to investigate interaction between TMC207 and ATP synthase. Moreover, the isolated ATP synthase may be used to shed light on the unusual subunit composition of the enzyme.

The experiments and results in this dissertation are an important contribution to the fundamental understanding of energy metabolism and ATP synthase in pathogen bacteria as well as to the development of new compounds that inhibit the energy metabolism in tubercle bacteria.