Abstract

DNA plays a key role in all living organisms by encoding its genetic blueprint. Due to this information being written in linear, base by base fashion it would be impractical to store it as one long extended string, as it would simply not fit into the cell. In bacteria the DNA is stored in tightly twisted form and the maintenance of this highly packed state is conducted by specialized enzymes called topoisomerases. These can break, twist and reconnect the double helix of DNA to deliver the desired packaging. Among the topoisomerases one, DNA gyrase, has the unique ability to introduce underwinding into the DNA – negative supercoiling.

This enzyme is found mainly in bacteria, but can also be found in Archaea and the eukaryotic group Apicomplexa. Bacteria include many pathogenic organisms and, as DNA gyrase is not found in humans, it constitutes a very attractive target for antibiotic drug development. Any inhibition of its action will disrupt the DNA maintenance mechanism, and may result in cell death. Many of the currently used antibiotics specifically target gyrase, however in light of ever-growing resistance against these, the study of gyrases and its inhibitors remains a very important research topic.

The aim of this thesis is to understand more about the existing nonbacterial DNA gyrases and to see how our understanding of gyrase may lead to new treatments that target them. In particular, I aimed to understand mechanism of action of a new small molecular compound specifically targeting the gyrase from Plasmodium falciparum to aid in the fight against malaria; secondly I aimed to study the structure of a archaeal gyrase to provide more knowledge of this enzyme in less studied organisms and find unique adaptations to extreme environment.

Within the first part of this thesis, in order to find new possible therapeutics, an effort was made to express and purify the complete *Plasmodium falciparum* gyrase (*Pf*Gyr). DNA gyrase consists of two subunits – GyrA and GyrB and its active as heterotetrameric complex $GyrA_2GyrB_2$. While preparation of the *Pf*GyrA subunit was not possible, *Pf*GyrB was prepared in sufficient amounts and this subunit was proven to be active in hybrid complex with *E. coli* GyrA (*Ec*GyrA*Pf*GyrB). Capitalizing on this, a library of small molecular compounds was first screened against the *Ec*GyrA*Pf*GyrB and new two most promising candidates were discovered: alizarin red S and

purpurogallin. The latter one was subsequently extensively studied using the *Ec*GyrA*Pf*GyrB in terms of its mechanism of action and proven to target specifically *Pf*GyrB. Finally, the drugs activity was shown *in vivo* giving inhibitory effect on growth of *Plasmodium falciparum*, confirming its potential use as a antimalarial.

As the expression of complete *Pf*Gyr holocomplex proved unfeasible, another nonbacterial gyrase from a hyperthermophile *Archaeoglobus sulfaticallidus* was chosen to conduct the second part section of this thesis, which aimed to uncover new information on the structure/function of less studied gyrases. Due to its habitat, characterised by elevated temperatures and salt concentrations *As*Gyr is expected to possess distinct adaptations, exaggerating some of the key features of the gyrase, thus making it a very interesting subject to study. This gyrase was purified with high yields and purity allowing for its in-depth characterisation. The enzyme was thoroughly studied biochemically, biophysically and structurally using recent advancements in cryogenic electron microscopy (cryo-EM).

One of the most striking features of the enzyme discovered was its unique GyrA C-terminal domain (CTD), a part of the gyrase responsible for DNA binding and wrapping. This domain was revealed to contain a unique highly positive charged pattern on its surface. For comparative studies a mutant - *As*GyrA_Mut - was created which CTD charge distribution mimicked one on *Ec*GyrA. Comparison of activity of three tested gyrases (*As*GyrA, *As*GyrA_Mut and *Ec*Gyr) demonstrated that the charge distribution plays a key role in stabilisation of DNA-enzyme complex, preventing DNA slippage likely to occur at elevated temperatures and high salt concentration.

Another adaptation is the absence of acidic tail at the *As*GyrA_CTD. The acidic tail is typically required for destabilisation of gyrase-DNA complex, however, for organisms living at elevated temperature such a mechanisms may not be necessary as the thermal energy is sufficient to achieve this, therefore evolved to mainly increase the stability of the complex.

The studies of DNA supercoiling activity of *As*Gyr revealed that it is very efficient in introducing negative supercoils into DNA. Additionally, during the biochemical analysis of the enzyme it was found that it has an ability to effectively relax positively supercoiled DNA in an ATP-independent manner, which was speculated to not be

possible. Surprisingly this activity was also observed in control experiments with extensively studied *E. coli* gyrase. This finding stands in stark contrast in current understanding of gyrase activity and substantiates a revisiting of current state of knowledge.

Lastly, the acquired structure is first to date cryo-EM structure of a full DNA gyrase in complex with DNA and moxifloxacin, a commonly used antibiotic. This allowed for verification of current knowledge of this drug mode of action in more native conditions.

To summarize, within this study two examples of nonbacterial gyrases were investigated: gyrase from *Plasmodium falciparum* in terms of development of a new-antimalarial, and gyrase from *Archaeoglobus sulfaticallidus* in terms of its structure and activity. For *Pf*Gyr, a new inhibitor was found specifically targeting the *Pf*Gyr*B* providing a template for creation of new generation of anti-malarial drugs. The studies of *As*Gyr revealed what adaptations of gyrase are needed for its activity in extreme temperatures and salts concentration, highlighting specificity of electrostatic interaction in gyrases, governing the DNA binding and release mechanisms. Additionally, the structure of *As*Gyr in complex with DNA and MFX allowed for verification of current state of knowledge of the mechanism of action of this drug and the biochemical characterization of this enzyme revealed that all gyrases might possess ability to relax negatively and positively supercoiled DNA in ATP-independent way, where the latter activity was not previously thought to exist.

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