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Characterization of the Quinolone-Gyrase-Interaction Using Docking, Molecular-Dynamics and Site-Directed Mutagenesis

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Fluoroquinolones are an important class of anti-bacterials, but as with many anti-infectives, drug-resistance is an increasing problem. Fluoroquinolones inhibit DNA-gyrase, an enzyme, which is able to alter the topology of DNA. However, there is only little structural information. In the literature, there are two rather different proposals for the binding mode of quinolones. Via docking calculations and MD simulations, we find both binding possibilities and consider the evidence for each. Work is now underway to test our models with site directed mutagenesis techniques.

1 Introduction

Despite modern antibiotics, infectious diseases are responsible for nearly one third of human deaths worldwide, and bacterial resistance is still an urgent problem. The class of fluoroquinolone(FQ)-antibiotics is a good example. They offer a broad spectrum of activity, good pharmakokinetic properties and are relatively cheap to produce. However, FQ resistant bacteria are wide spread.

The functional target and biochemical action of FQs is known. The drugs inhibit the A_2B_2 DNA-gyrase tetramer, an enzyme which is able to alter the topology of DNA by transient cleavage[1]. This is performed by an esterification of GyrA-Tyr_{122}-OH of the enyzme to a $5'$-phosphate of the DNA. FQs appear to inhibit the religation of the DNA by stabilizing the cleaved form, ultimately resulting in bacterial cell-death[2]. Furthermore, studies of natural and in-vitro mutants offer clues as to which residues are involved in drug-resistance[3]. Unfortunately, the exact molecular action remains largely unknown. Clearly, understanding the drug-enzyme-DNA interaction in molecular terms could be the basis for the development of new FQ-derivatives refractory to resistance. Due to the lack of complete structural information for the tetramer in complex with DNA and FQ, we have been trying to build molecular models using a combination of docking methods and molecular dynamics (MD) simulations.

2 Methods

2.1 Protein-DNA-Docking and Filtering

The mode of gyrase action can roughly be divided into three individual steps. First, the DNA approaches the protein. Secondly, a phosphate (DNA) - tyrosine (protein) ester is

formed. Finally, the DNA gap is resealed by a trans-esterification and subsequent DNA release. How the protein and the DNA find each other can be regarded as a docking problem. Hence, the first step was to generate many possible DNA-enzyme conformations using a standard protein-DNA docking tool[4]. Results are filtered according to orientations of the DNA 5′-end w.r.t. to the protein's active site Tyr_{122}, distances between GyrA-Tyr_{122}-OH and appropriate DNA cleavage sites (5′-↓GRYC-3′)[5], as well as electrostatic surface potentials.

2.2 Molecular Dynamics Simulations and Molecular Docking

Two successive sets of MD simulations were performed to find plausible and stable models of the protein-DNA complex. In the first set, DNA and protein were not linked while in the second set, a covalent bond between GyrA-Tyr_{122}-OH and the DNA 5′-end was introduced. In between, implausible results were discarded. MD simulations, including 70.000 TIP4P water molecules, were run up to 2ns using GROMACS[6].

Subsequently, remaining complexes were used for molecular docking of eleven different quinolones using AutoDock4[7]. For each of the drugs, 250 different conformations and their binding modes within a box were sampled. The box was defined to contain the potential binding pocket between Ser_{83} and Asp_{87} in the QRDR (quinolone resistance determining region) of GyrA (residues 67-106) as well as the four overhanging bases of the cleaved dsDNA.

3 Results and Discussion

The first docking session (protein-DNA) yielded $>$1000 candidates of which 24 were selected for the initial MD simulations (section 2). Of these, two had distances between GyrA-Tyr_{122}-OH and the DNA 5′-end which allowed the DNA-protein ester to be introduced.

We can distinguish these complexes by the orientation of the nucleic acid w.r.t. the α_4 helix of the DNA binding HTH motif present in the QRDR. The first complex places α_4 in the major groove of the DNA as proposed by Liddington[1], whereas the second complex supports the model of Lapogonov[8] with α_4 in the minor groove of DNA. For each drug molecule in both complexes, a conformational cluster analysis was performed which was based on positional RMSD of corresponding drug atoms.

For our Lapogonov-like complex, we found a noticeable difference between the clusters containing the highest scoring FQ conformations. Although the drug molecules were placed within the same binding cavity, they appear to be rotated by $\sim$180° as shown in fig. 1. Amongst all sampled conformations, these drug arrangements were found most frequently without showing noticeable deviations with respect to their quantities. Our model indicates that the ligands might be spatially fixed with DNA and enzyme by two main anchors. On the one side, the N-containing heterocyclic substituent at C7 could be able to interact with the phosphate backbone of guanine or cytosine residues of the DNA. However, in its protonated form, this heterocycle might also interact with backbone oxygens of Ser_{83} and Ala_{84}. On the other side, the carboxyl moeity at C3 of the FQs might form a salt-bridge with the free amino-group of guanine. Moreover, our results could also allow the

presence of Mg^{2+} ions[9], which might fill the gap (7.5-8.6 Å) between the FQ's carboxyl- and keto-group, respectively, and the phosphate backbone of the DNA.

For the Liddington-like model, the highest scoring FQ conformations did not fit into the proposed binding cavity. However, as fig. 2 shows, lower scoring dockings are found for each FQ without clear preferences for a certain conformation. Anyhow, all dockings are in agreement with the common literature since they satisfy the intercalating nature of the quinolones.

This theoretical data enabled us to identify amino acids which are currently used to test the validity of our models with site directed mutagenesis.

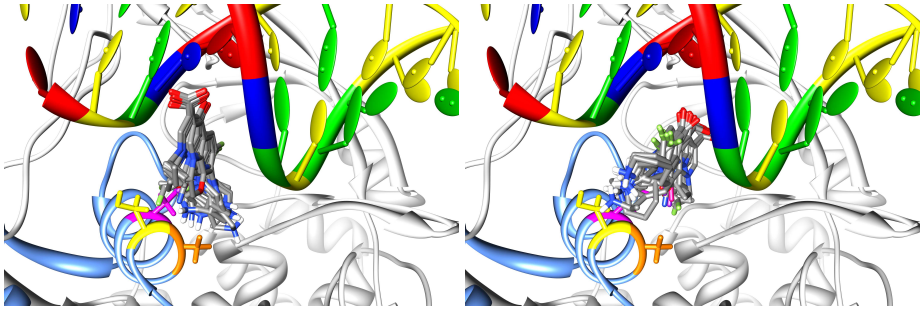

Figure 1. *Complex of DNA linked to GyrA-Tyr_{122} and docked quinolones: COOH-moeities of ligands point out of (left) and into plane (right); enzyme shown in grey, QRDR in lightblue, Ser_{83}, Ala_{84} and Asp_{87} in α_4 helix in yellow, orange and magenta, resp.; G in green, C in yellow, T in blue and A in red.*

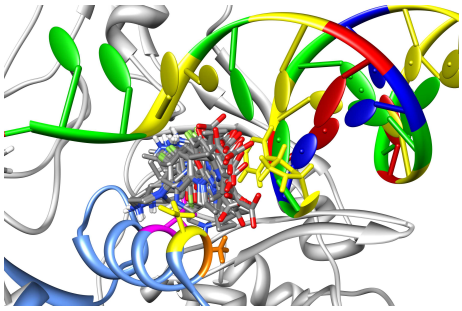

Figure 2. *Liddington-like complex of DNA linked to GyrA-Tyr_{122} and docked quinolones; color coding as in fig. 1; pictures were built using UCSF Chimera[10].*

Acknowledgments

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