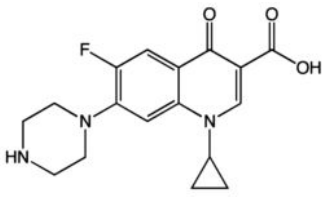
Cove Soyars

Role of hydrophobicity in Ser-83 amino acid substitutions of DNA gyrase subunit A encoding gyrA

Introduction

Antibiotic resistance is a pressing problem in both healthcare and the food industry. Approximately 25,000 people die in Europe every year from bacterial infections acquired in hospitals that are resistant to antibiotics (Freire-Moran, et. al, 2011). It has been the opinion of recent studies that overuse of antibiotics is a contributing factor to this resistance (Zaman, et. al, 2017). In the context of the food industry, there is evidence supporting the notion that giving livestock antibiotics can lead to antibiotic resistant bacteria being transferred to people that have never taken the drugs themselves (Garau, et.al, 1999).

Quinolones are a class of antibiotics that were once used only to treat simple urinary tract infections and have now, with new generations of the drugs, become very effective outside of the urinary system. Their improved utility is partially because of the addition of a fluorine group, creating the subclass of fluoroquinolones; including ciprofloxacin (see figure), a now widely-prescribed antibiotic (Correia, et al. 2017). Quinolones are now used to treat a wide range of bacterial infections, but are still susceptible to resistant strains. Unfortunately, in every bacterial species that has been treated with quinolones, resistant strains have been observed (Aldred, et. al, 2017).



Ciprofloxacin, a fluoroquinolone (Aldred et. al, 2017)

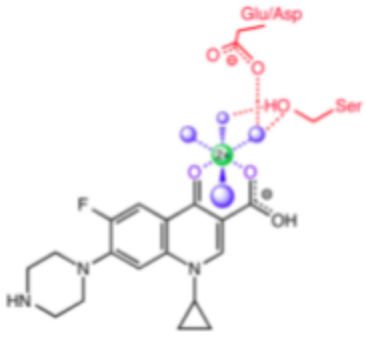
Quinolones target DNA replication by binding to the DNA/DNA gyrase complex. DNA gyrase is a bacterial type II topoisomerase. DNA gyrase, like other type II topoisomerases, acts by creating double-stranded breaks in DNA (using ATP) and then religating them later in order to relieve supercoiling tension so that DNA helicase can operate and initiate DNA replication. Quinolones insert themselves in between bases of the cleaved DNA and block religation. When the replication fork meets a gyrase that is interacting with a quinolone, gyrase is removed and the double-stranded break remains. Thus, quinolones operate by breaking apart the bacterial genome (Aldred, et. al, 2017).

There is substantial evidence that the Ser-83 position of the A subunit of DNA gyrase, coded by the gene gyrA, plays a crucial role in the binding of quinolones to DNA gyrase. First, a serine residue is highly conserved in the 83rd position of the DNA gyrase A subunit (see figure).

Alignment figure coming soon!

Second, a 2017 study (Qin, et. al) found that out of 400 quinolone resistant strains isolated, 302 (75.5%) had a mutation at the 83rd position. Third, Madurga et. al (2008) found Van der Waals attraction forces between quinolones and the Ser-83 residue using AutoDock 3.05 software.

It is believed that the Ser-83 residue of gyrA is involved in forming a water-metal ion bridge (Aldred et. al, 2017 & Correia et. al, 2017). The water-metal ion bridge, as described by Aldred et. al (2017), consists of a non-catalytic Magnesium ion surrounded by four water molecules (see figure: Water-metal ion bridge). The carbonyl oxygens of the C4 and C5 carbons of the drug (colored purple) interact with the Magnesium ion (colored green). The four water molecules (colored blue) interact with the Magnesium ion as well as form hydrogen bonds (dashed red lines) with the hydroxy group of the Ser-83 residue (colored red). This proposed mechanism of quinolone binding could account for the Van der Waals forces detected by Madurga et. al (2008). Furthermore, it would be reasonable that a loss of a hydrophilic side chain in the 83rd position of gyrA, caused by a mutation of Serine to an hydrophobic amino acid, would interrupt these forces.



Water-metal ion bridge (Aldred et. al, 2017)

Several point mutations from Serine to another amino acid have been found in quinolone resistant strains of bacteria. Mutations of Ser-83 to Alanine, Isoleucine, Leucine, Tryptophan, Tyrosine, and Valine have been found (Correia et. al 2017) in addition to Phenylalanine (Ngoi et al 2014). Using the results of Monera et. al (1995)'s determination of the hydrophobicity of amino acids using reverse-phase chromatography, the amino acids listed above are more hydrophobic than Serine. However, there are amino acids (Cysteine, Methionine, and Glycine) that according to Monera et. al (1995) are more hydrophobic than Serine) that have not been found in the 83rd position of gyrA of resistant strains (see figure: Monera et. al (1995)'s relative hydrophobicities).

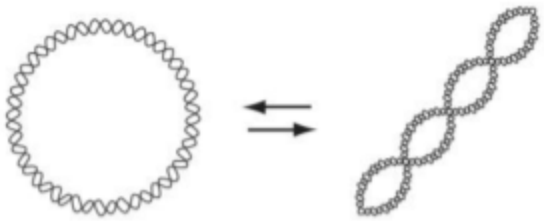
Monera et. al (1995) used reverse phase chromatography where more hydrophobic amino acids took longer to fall through a column than hydrophilic amino acids. Δt_R is the difference in the time it took for Glycine to fall out of the column and the time it took for the amino acid of interest to fall. The darkened cells indicate amino acids that have not been found in the 83rd position of gyrA in resistant strains.

Amino acid	Δt_R (min)
Serine	-0.4
Alanine	3.2
Isoleucine	7.7
Leucine	7.6
Tryptophan	7.6
Tyrosine	4.9
Valine	5.9
Phenylalanine	7.8
Cysteine	3.8
Methionine	5.8
Glycine	0

Experiment

This study seeks to find whether substitutions from Serine to Cysteine, Methionine, and Glycine in position 83 of gyrA will confer quinolone resistance. To do this, mutants of the gyrase Subunit A with the mutations S83C, S83M, and S83G will be purified and the quinolone resistance of the mutant gyrases will be measured by observing the function of gyrase at different levels of ciprofloxacin.

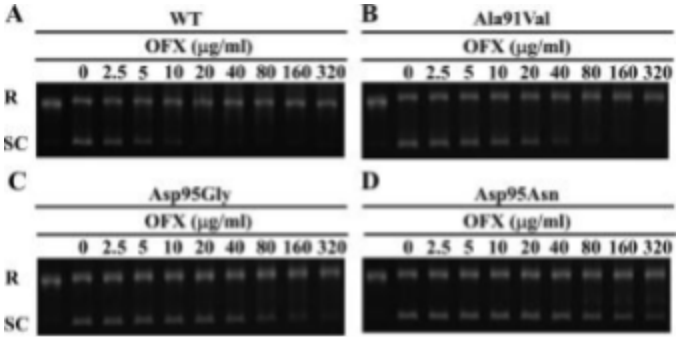
DNA supercoiling assay



Gyrase can convert relaxed DNA (left) to supercoiled DNA (right) (Nitiss et. al, 2012)

Gyrase relieves tension in DNA by relaxing positive supercoils. It also can introduce negative supercoils to relaxed DNA (Schoeffler et. al, 2008), a property which is exploited by the DNA supercoiling assay (see figure: supercoiled DNA). To test whether or not gyrase is functioning, it can be placed (both subunits) in a buffer with relaxed a relaxed DNA plasmid and ATP for a period of time and then the solution can be ran on agarose gel (Nitiss et. al, 2012). DNA that is supercoiled by a functioning gyrase will travel further on the gel because the DNA is in a more compact conformation than a relaxed plasmid (Stellwagen, et. al, 2010).

The supercoiling assay was used in the context of antibiotic resistance in Yokoyama et. al (2011). Here the researchers sought to test if homologous quinolone resistance conferring amino acid substitutions of M. tuberculosis gyrase also conferred resistance in M. Leprae gyrase. To do this, they placed both A and B subunits of M. Lepra gyrase, a relaxed DNA plasmid, and ATP in a buffer with a variable amount of the quinolone ofloxacin and incubated the solution. The solution was then run on agarose gel to give the results seen in figure: supercoiling activity in presence of ofloxacin. The two homologous mutations being tested (Asp95Gly and Asp95Asn), in squares C and D showed supercoiling bands at higher ofloxacin concentrations than the wild-type gyrase (square A).



Yokoyama et. al (2011). Supercoiling activity at various concentration of ofloxacin. The bands labelled 'SC' represent supercoiled DNA.

The present study will employ a similar experimental strategy to that of Yokoyama et. al (2011) Except with the S83C, S83M, and S83G mutations of E. coli gyrase and ciprofloxacin.

Mutant Protein Synthesis and Purification

The Polymerase Chain Reaction (PCR) will be used to create mutant gyrA plasmids from cloned wild-type gyrA in an expression vector. The primers for this reaction were designed using Agilent QuikChange Primer Design (see figure: primer design). PCR will also be used to insert 6x Histidine tags for purification, since Yokoyama et. al (2011) found that histidine tags to not interfere with the catalytic function of gyrase. gyrB will need to be cloned as well for the supercoiling assay.

Mutant	Primer
Ser83Cys	5'-gtcatagaccgcgcagtcaccatggggatggtattta-3' 5'-taaataccatccccatggtgactgcgcggtctatgac-3'
Ser83Met	5'-cgtgtcatagaccgccatgtcaccatggggatgg-3' 5'-ccatccccatggtgacatggcggtctatgacacg-3'
Ser83Gly	5'-gtgtcatagaccgccccgtcaccatggggatg-3' 5'-catccccatggtgacggggcggtctatgacac-3'

The mutated expression vector will then be transformed into E. Coli cells, which will express the mutant proteins. The expression inducer depends on the exact vector used. The cells will be sonically lysed and the protein will be collected using affinity chromatography since the produced proteins are tagged.

Discussion

It is expected that the mutant gyrases will have supercoiling activity at higher concentrations of ciprofloxacin than the wild-type gyrase. This would further support the Van der Waals forces found by Madurga et. al (2008) as well as the metal-ion bridge proposed by Aldred et. al (2017) because both of these depend on the properties hydrophilic properties of Serine, which were lost in the mutant proteins.

On the other hand, the mutants having supercoiling activity at a similar concentration of ciprofloxacin could imply several things. This result could imply that the Ser-83 substitution mutations alone are not enough to confer resistance to quinolones. It could also imply that the conclusions of Madurga et. al (2008) and Aldred et. al (2017) concerning the role of intermolecular forces between Ser-83 and the drug in the quinolone binding mechanism.

One foreseeable limitation of the study is that the supercoiling assay may not produce significantly different banding; this would lead to inconclusive results.

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