DOI: 10.1002/cbic.200800041

Mechanism of Binding of Fluoroquinolones to the Quinolone Resistance-Determining Region of DNA Gyrase: Towards an Understanding of the Molecular Basis of Quinolone Resistance

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We have studied the bacterial resistance to fluoroquinolones that arises as a result of mutations in the DNA gyrase target protein. Although it is known that DNA gyrase is a target of quinolone antibacterial agents, the molecular details of the quinolone–gyrase interaction remain unclear. The mode of binding of ciprofloxacin, levofloxacin, and moxifloxacin to DNA gyrase was analyzed by means of docking calculations over the surface of the QRDR of GyrA. The analysis of these binding models allows study of the resistance mechanism associated with gyrA mutations more commonly found in E. coli *fluoroquinolone-resistant strains at the atomic level. Asp87 was found to be critical in the binding of these fluoroquinolones because it interacts with the positively charged nitrogens in these bactericidal drugs. The role of the other most common mutations at amino acid codon Ser83 can be explained through the contacts that the side chain of this residue establishes with fluoroquinolone molecules. Finally, our results strongly suggest that, although Arg121 has never been found to be associated with fluoroquinolone resistance, this residue makes a pivotal contribution to the binding of the antibiotic to GyrA and to defining its position in the QRDR of the enzyme.*

Introduction

Bacterial resistance to antibiotics is the inevitable consequence of the use of antimicrobial agents. This resistance can be mediated by genes located either on the chromosome or on genetic elements of extraneous origins such as R (resistance) plasmids, transposons, and integrons.[1] These elements provide an efficient mechanism for rapid horizontal and vertical dissemination of antibiotic resistance determinants among bacterial species.[2–4]

Quinolones and fluoroquinolones are among the most extensively used drugs for the treatment of bacterial infections both in human and veterinary medicine. Such wide use has led to rapidly increasing bacterial resistance to this kind of antibiotics. The therapeutic use of quinolones began in 1962, with the introduction of nalidixic acid for the treatment of urinary tract infections in humans.[5] In the 1970s, fluoridation of the quinolone molecule at the C-6 position yielded norfloxacin, the first fluoroquinolone.[6] Ciprofloxacin, perhaps the most important and most widely used fluoroquinolone, was introduced onto the clinical market in 1987. Since then, structural revisions of quinolone molecules have provided numerous new agents for the treatment of a variety of bacterial infections. Older quinolones are active mostly against Gram-negative bacteria, whereas newer ones have broad spectra of activity including Gram-positive pathogens and anaerobes.[5,7,8] However, the future utility of these drugs is threatened by the increasing rate of emergence of quinolone-resistant bacteria.[9–13]

The emergence of quinolone resistance is accounted for by several mechanisms.[14–18] Resistance to fluoroquinolones typically arises as a result of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and changes in drug entry and efflux. Target alterations most frequently occur in GyrA, particularly within a short limited region called the "quinolone resistance-determining region" (QRDR).[19] The most common mutations include Ser83Leu and Asp87Asn of the *gyrA* gene of *E. coli*.[19,20] The level of drug resistance varies depending on the mutations and the bacterial species. Quinolone inhibition of DNA gyrase occurs through the formation of a stable ternary complex between DNA gyrase, DNA, and the quinolone molecule that blocks the progression of DNA replication.[21,22] DNA gyrase is a target of quinolone antibacterial agents; however, the molecular details of the quinolone–gyrase interaction are not clear. Quinolone resistance mutations frequently occur at residues Ser83 and Asp87 of the GyrA subunit, so it is feasi-

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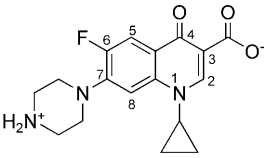
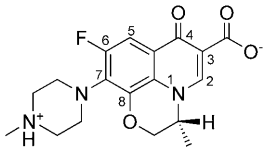
ble that these residues are involved in drug binding. A binding model of quinolone action proposed by Maxwell and co-workers[23–27] postulated that both DNA gyrase and DNA are required to bind quinolones in a stable form. This model is based mainly on the observation that alterations in DNA gyrase that confer quinolone resistance reside in the QRDR (between residues 67 and 106 of GyrA in *E. coli*) and correlate with reduced binding of quinolones to the resistant mutant enzyme–DNA complex.

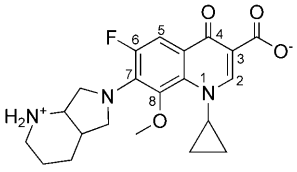
Here we have determined the binding position of quinolones to DNA gyrase in order to identify the molecular traits of their mode of action and to explain further how point mutations contribute to bacterial resistance to fluoroquinolones. Although several docking studies have been done either with the ATP binding site of the GyrB subunit[28,29] or outside the QRDR region of GyrA,[30] this is the first docking study with fluoroquinolones binding to the QRDR region of GyrA. Quinolones interact with the complex of DNA and DNA gyrase rather than with the enzyme alone, so it is necessary to modify the docking procedure in order to examine the effect of DNA on the binding of fluoroquinolones to DNA gyrase. We chose three representative fluoroquinolones—ciprofloxacin, levofloxacin, and moxifloxacin—to perform this study.



ciprofloxacin fluoroquinolone



levofloxacin fluoroquinolone



moxifloxacin fluoroquinolone

Results

Fluoroquinolone structures

Ab initio calculations were performed with ciprofloxacin, levofloxacin, and moxifloxacin. Optimized structures of ciprofloxacin and levofloxacin molecules had the attached piperazine ring in the equatorial conformation (Figure 1). This conformation preference is also observed experimentally for the *N*-methylpiperidine molecule.[31–34]

Ciprofloxacin, levofloxacin, and moxifloxacin can each exist as three chemical species (cationic, zwitterionic, and anionic) depending on the pH of the aqueous solution. For ciprofloxacin, the experimentally measured pK_{a1} and pK_{a2} values are 5.9 and 8.2.[35] For ofloxacin, which is a racemic mixture of the active (levofloxacin) and inactive enantiomer, the experimentally measured pK_{a1} and pK_{a2} values are 6.1 and 8.1.[30] Thus, at physiological pH the major contribution is from the zwitterionic state. We therefore used the zwitterionic states of ciprofloxacin, levofloxacin, and moxifloxacin in docking calculations.

Docking procedure

Before docking calculations directed towards a selected target were performed, it was necessary to define a region of the target in which the best mode of binding of the ligand would be searched. In this study, the available information on the residues most relevant to achieving greatest bacterial resistance to fluoroquinolones (Ser83 and Asp87) was used to select the region of DNA gyrase in which grid maps were calculated. In this docking procedure, the potential maps are defined in such a way that they contain or are very near these two DNA gyrase residues. From the crystal structure of a related system, which consists of the ternary complex between a human Top1 construct covalently attached to a DNA duplex with bound topotecan,[36] information about the relative disposition of the topotecan inhibitor molecule with respect to the DNA strand can be extracted. In this case, part of the ring plane of the topotecan molecule is intercalated into the duplex DNA. In addition, by solving the structure of a DNA duplex with covalently linked nalidixic acid by NMR and restrained torsion angle molecular dynamics, Siegmunt et al. found that nalidixic acid adopts a stacked conformation with nucleotide base pairs.[37] To force a similar orientational effect in the disposition of fluoroquinolones with respect to DNA, docking calculations were performed by a strategy that divides the surface of the DNA

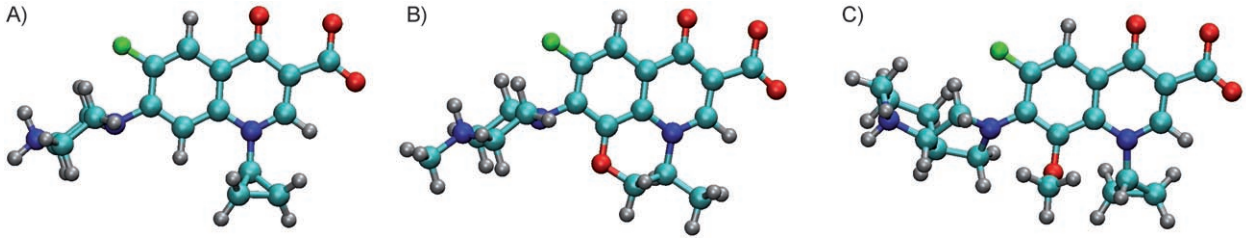


Figure 1. Structures of A) ciprofloxacin, B) levofloxacin, and C) moxifloxacin fluoroquinolones used in docking calculations.

gyrase into several narrow regions. In this model, four overlapping narrow grids (Figure 2) were designed, to obtain fluoroquinolone structures oriented perpendicularly to the expected direction of the axes of the DNA. Docked structures of fluoroquinolones showed an orientation that made it possible to establish a stacked conformation with base pairs of the DNA attached to DNA gyrase. These docked fluoroquinolones could thus participate in a ternary complex with DNA gyrase and DNA. Each box had a width of 7.5 Å and was displaced by 3.75 Å with respect to the adjacent box, so the limit of each box coincided with the center of the neighboring box. The boxes were oriented in such a way that the longest dimension of each box was perpendicular to the expected direction of the DNA axes.

For each small narrow box (Figure 2), a docking calculation with ciprofloxacin, levofloxacin, and moxifloxacin was carried out. Figure 3 shows the best docking solution for each fluoroquinolone out of the four boxes. The best solutions for all three molecules were obtained for the same box (box C), with similar patterns of binding to DNA gyrase. In each of the three fluoroquinolones the carboxylate group established a salt bridge with the guanidinium group of Arg121, whereas the positively charged N atom of the fluoroquinolone interacted with the carboxylate group of Asp87. For ciprofloxacin and levofloxacin, the carboxylate moiety also interacted with the Gly81 backbone HN proton, whereas for moxifloxacin this interaction was established to be at a large distance (4.1 Å).

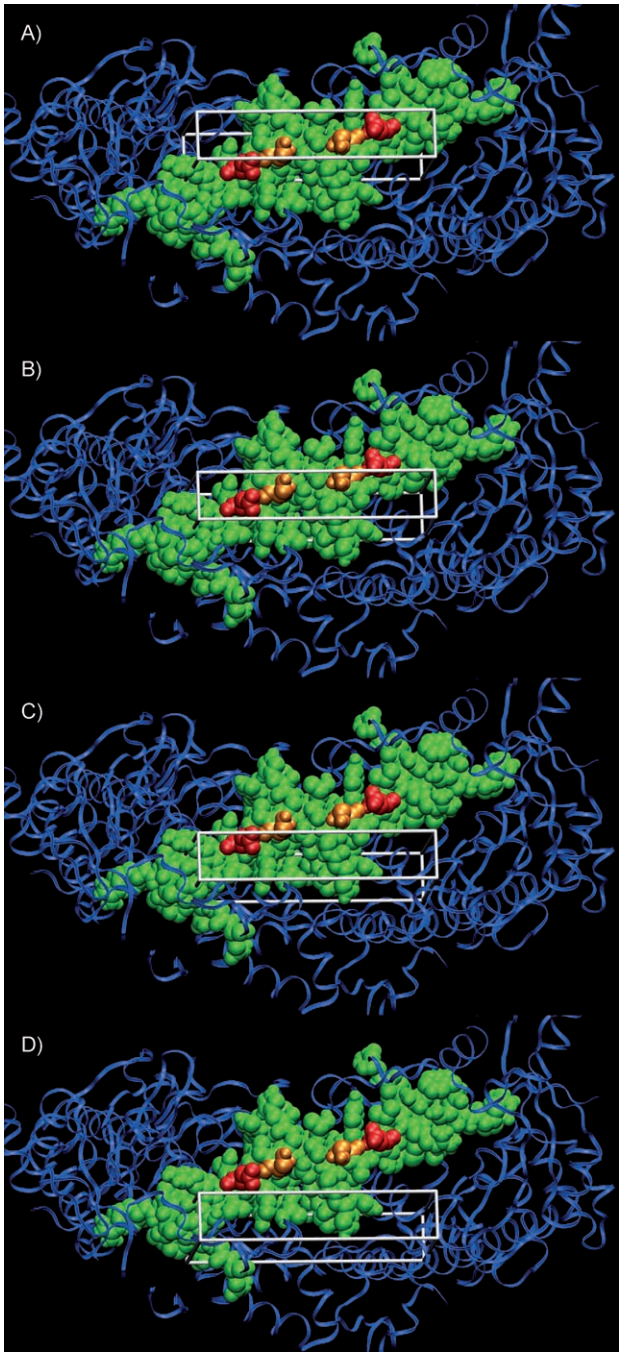


Figure 2. The four boxes (A–D) used in the calculations of docking of fluoroquinolones to DNA gyrase. The QRDR of DNA gyrase (from residues 67 to 106) is represented in CPK model. Ser83 and Asp87 are shown in orange and red, respectively. Non-QRDR DNA gyrase residues are drawn in ribbon representation.

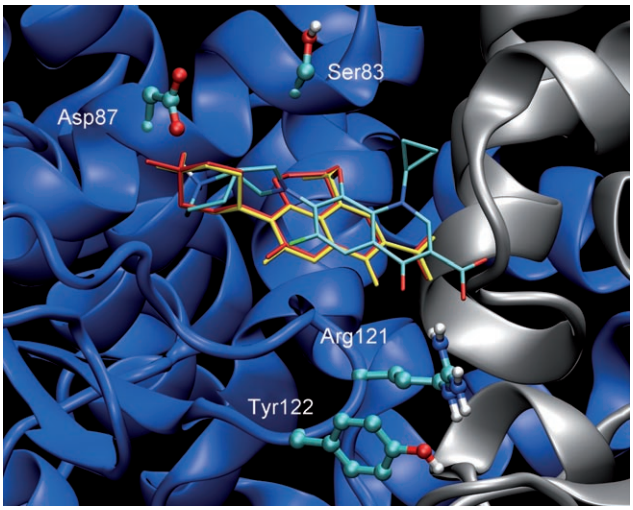


Figure 3. The best docked structures for ciprofloxacin (yellow), levofloxacin (red), and moxifloxacin (cyan) were obtained in Box C. Ser83, Asp87, Arg121, and Tyr122 are also displayed in ball and stick representation. The two subunits of the DNA gyrase are in blue and grey in ribbon representation.

Van der Waals contacts were observed between the Ser83 residue and the three compounds. Distances from the α carbon of Ser83 to the N1 and C8 atoms of ciprofloxacin, levofloxacin, and moxifloxacin structures are shown in Table 1. All distances were below 6 Å, except for the distance to N1 in the case of moxifloxacin (about 7 Å). These values indicate that the pattern of moxifloxacin binding differs slightly. The docked structure of this fluoroquinolone was slightly displaced in relation to the structures of the other two drugs (Figure 3). Consequently, for ciprofloxacin, the cyclopropyl substituent interacted with Ser83, but for moxifloxacin the interaction of the same substituent with Ser83 was less significant.

With regard to the number of intermolecular atom contacts for a selected group of fluoroquinolone atoms with DNA gyrase protein atoms, we identified two patterns of binding

Table 1. Distances from the α carbon of Ser83 to the N1 [*d*(Cα–N1)] and C8 atoms [*d*(Cα–C8)] of ciprofloxacin, levofloxacin, and moxifloxacin structures obtained in the oriented models.

	d(Cα–N1) [Å]	*d*(Cα–C8) [Å]
ciprofloxacin	5.3	5.6
levofloxacin	5.1	5.4
moxifloxacin	6.8	5.6

(Table 2). On the one hand, ciprofloxacin and levofloxacin showed a greater number of atom contacts for the N1 and C8 substituents of their fluoroquinolone rings than for their C7 substituents. On the other hand, the C7 substituent of moxifloxacin established a greater number of atom contacts with DNA gyrase than the N1 and C8 substituents.

Table 2. The number of intermolecular atom contacts for the group of N1 and C8 atoms and their substituents and for the group of C7 atom and their substituents of ciprofloxacin, levofloxacin, and moxifloxacin with DNA gyrase calculated with the LigPlot[38] program.

	N1 and C8	C7
ciprofloxacin	14	3
levofloxacin	14	3
moxifloxacin	2	12

Docking of fluoroquinolones to structures taken from molecular dynamic calculations

To study the stabilities of the binding modes of the three fluoroquinolones, new docking calculations with distinct conformations of the DNA gyrase were performed by use of the four narrow boxes procedure. Ten structures taken from a 500 ps molecular dynamics procedure were used as a target. For each protein conformation, docking calculations were carried out for each narrow box, and the minimal-energy solution was selected. The greatest number of more stable docking structures was obtained for box C (Table 3). Consideration of distinct rotamer conformations of DNA gyrase thus did not alter the pattern of binding found with the crystal DNA gyrase structure. The best structures of the three fluoroquinolones were in the same cavity of DNA gyrase, and the binding modes were very similar to those obtained with the narrow box procedure using the crystal structure of DNA gyrase.

Table 3. Distribution of the best docking solutions out of the four narrow boxes for a total of ten distinct DNA gyrase conformations.

	Box A	Box B	Box C	Box D
ciprofloxacin	0	1	9	0
levofloxacin	0	0	7	3
moxifloxacin	0	0	9	1

Discussion

Using ciprofloxacin, levofloxacin, and moxifloxacin, here we have applied a docking model in which solutions are restricted to a determined orientation over the surface of the QRDR of GyrA. We consider this model suitable for study of this system.

Docking results showed very similar patterns of binding for ciprofloxacin and for levofloxacin. It is known that mutation at the amino acid codons for Ser83 and/or Asp87 confers a bacterial resistance mechanism against these two fluoroquinolones. Our results allow us to explain these experimental observations, because Asp87 has been found to be critical in the binding of these drugs. This residue is crucial because it interacts with the positively charged nitrogen in the fluoroquinolones. In addition, we have found that Ser83 shows contacts with the N1 substituents of these two antimicrobial agents. These contacts can explain the resistance to these fluoroquinolones displayed when Ser83 is mutated with a residue possessing a side chain, which can establish steric hindrance.

For moxifloxacin, the interaction with Asp87 is also critical. However, the Ser83 mutation is tolerated. Our docking results suggest that this tolerance develops because this drug can establish better binding to DNA gyrase. The greater separation between positive and negative atoms in moxifloxacin appears to favor binding with the negative Asp87 and the positive Arg121 charge of DNA gyrase. The distances between the carboxylate C atoms and the positively charged N atoms of the fluoroquinolones were 10.6, 10.7, and 11.3 Å for ciprofloxacin, levofloxacin, and moxifloxacin, respectively. In addition, Ser83 showed less contact with moxifloxacin than with ciprofloxacin and levofloxacin. Thus, mutation of Ser83 with another residue bearing a larger side chain can produce less steric hindrance with moxifloxacin than with the other two fluoroquinolones.

Notably, the docking results suggest that the role of Asp87 in the quinolone resistance mechanism is more relevant than that of Ser83. However, mutation of Ser83 is experimentally more frequently observed, thereby indicating the contribution of this mutation to the resistance mechanism. Mutations that change Ser83 either into leucine or into tryptophan confer high levels of quinolone resistance (about tenfold increases), whereas mutations that change Ser83 to alanine result in lower levels of resistance (about fivefold increases).[39] To explain the effect of each of the experimentally observed Ser83 mutations fully, a study with the ternary complex of quinolone, DNA, and DNA gyrase would be required.

These data are consistent with previous studies that showed that a mutation in the amino acid codon Thr86 (equivalent to Ser83 in *E. coli*) of *gyrA* of *Campylobacter jejuni* produces a slight increase in the minimum inhibitory concentration of moxifloxacin but high increases in resistance to ciprofloxacin and levofloxacin. In addition, our results are in agreement with previous reports that a double mutation in the amino acid codons Thr86 and Asp90 (equivalent to Asp87 in *E. coli*) is required to generate a high level of resistance to moxifloxacin.[40]

For the three fluoroquinolones, Arg121 was another relevant point of binding. However, this residue has never been found to be mutated to achieve fluoroquinolone resistance. It is

worth noting that Arg121 is located next to the active-site tyrosine. On the basis of these observations, we propose that this residue contributes to DNA binding or cleavage and that mutations in the amino acid codon Arg121 may be detrimental to the activity of the DNA gyrase, and that mutation in this residue could consequently be lethal for the microorganism. This hypothesis would explain why this possible escape mechanism suggested by our docking procedures has never been found in nature.

Conclusions

Our study provides a structural hypothesis for the binding modes of three representative members of the fluoroquinolone antibiotics family to the QRDR of GyrA. Furthermore, analysis of these binding models allows us to study at the atomic level the resistance mechanism associated with the *gyrA* mutations most commonly found in fluoroquinolone-resistant *E. coli* strains. Finally, our results strongly suggest that, although Arg121 has never been found to be associated with fluoroquinolone resistance, this residue plays a key role in the binding of the antibiotic to GyrA and determines its position in the QRDR of the enzyme.

Computational Methods

Fluoroquinolone ab initio calculations: At the ab initio level, the density functional method was used to calculate the structures of the three fluoroquinolones. The DFT calculations were made by use of the hybrid exchange-correlation functional B3LYP and the 6–31+G** basis set. The B3LYP method is a good compromise between reliability and computational cost, as demonstrated by many examples.[41–43] The geometries of all systems were optimized in vacuum by use of the GAMESS[44] package. Convergence problems of the zwitterionic species were found in the ab initio calculations and can probably be attributed to the instabilities of these species in the gas phase. To avoid this problem, ab initio gas-phase optimizations were performed with the cationic forms of the fluoroquinolones. For docking calculations, the carboxylic proton was removed in each case, in order to use the zwitterionic form that is present in solution.

Docking calculations: The calculations were performed with the software package AutoDock3.05.[45] Kollman united-atom partial charges were assigned to protein and ligand molecules, and atomic fragmental volumes of the protein atoms were assigned by use of the Addsol utility of AutoDock3. With the aid of the AutoDock Tools, two box sizes were defined to calculate the potential grid maps. In one set of calculations, a grid map of $80\times100\times50$ points covering a large region of the DNA gyrase was used. For a second set of calculations, four narrow grids of $20\times100\times50$ points were defined. In all cases, the 0.375 Å grid-point spacing were used. The potentials grid maps were calculated by use of AutoGrid, version 3.0. The Autotors utility was used to define the rotatable bonds in the ligand. Ten dockings were performed with the Lamarckian Genetic Algorithm with use of a population size of 50 individuals with a total of 10^8 energy evaluations.

Molecular dynamics simulations: Molecular dynamics simulations of two gyrase subunits were carried out by use of Gromacs[46] with the 43a1 force field.[47] The X-ray crystal structure of DNA gyrase (N-terminal portion of *E. coli gyrA*) was used to prepare the starting coordinates (1ab4 pdb code). DNA gyrase was neutralized by addition of 24 sodium ions and was then immersed in a rectangular box containing 32415 SPC[48] water molecules. 1000 energy minimization cycles were done to remove repulsive van der Waals contacts. Equilibration dynamics of the entire system were performed at 300 K for 50 ps. Following the equilibration procedure, 500 ps MD simulations were carried out with a periodic boundary condition in the NPT ensemble at 300 K by use of Berendsen temperature coupling[49] and constant pressure (1 atm) with use of the isotropic Parrinello–Rahman procedure.[50] The LINCS algorithm[51] was applied to fix bond lengths. A time step of 2.0 fs and a nonbond-interaction cut-off radius of 14 Å were used. Electrostatic interactions were calculated with the particle-mesh Ewald (PME) method.[52] The trajectory was sampled every 2 ps for analyses.

Acknowledgements

This work was supported by the Ministry of Health, Spain (FIS 05/0068), MCYT-FEDER (BIO2005/00295 and PETRI 1995-0957-OP), and the Generalitat de Catalunya (XeRBa, 2005SGR-00444 and 2005SER-00663). The simulations were done on the MareNostrum supercomputer at the Barcelona Supercomputing Center-Centro Nacional de Supercomputación (The Spanish National Supercomputing Center).

Keywords: antibiotics · DNA gyrase · docking · fluoroquinolones · molecular modeling

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Received: January 22, 2008
Published online on August 1, 2008

