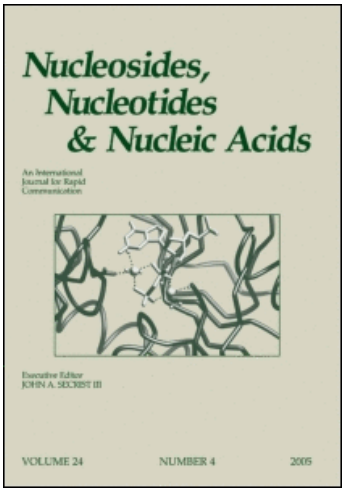

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THE INTERACTION OF DRUGS WITH DNA GYRASE: A MODEL FOR THE MOLECULAR BASIS OF QUINOLONE ACTION

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ABSTRACT: DNA gyrase supercoils DNA in bacteria. The fact that it is essential in all bacteria and absent from eukaryotes makes it an ideal drug target. We discuss the action of coumarin and quinolone drugs on gyrase. In the case of coumarins, the drugs are known to be competitive inhibitors of the gyrase ATPase reaction. From a combination of structural and biochemical studies, the molecular details of the gyrase-coumarin complex are well established. In the case of quinolones, the drugs are thought to act by stabilising a cleavage complex between gyrase and DNA that arrests polymerases *in vivo*. The exact nature of the gyrase-quinolone-DNA complex is not known; we propose a model for this complex based on structural and biochemical data.

INTRODUCTION

DNA gyrase is the bacterial enzyme that supercoils DNA[1,2]. It is a member of the topoisomerases, a group of enzymes involved in the control of DNA topology in cells[3,4]. There are two types: type I enzymes catalyse reactions involving transient single-stranded breaks in DNA, type II catalyse reactions involving transient double-stranded breaks. All topoisomerases are able to catalyse DNA relaxation, that is, to remove supercoils from DNA; gyrase is unique in that it is the only enzyme able to catalyse the introduction of negative supercoils. This reaction is ATP-dependent and is thought to be the principle function of gyrase in the bacterial cell.

DNA gyrase consists of two subunits, GyrA and GyrB, with the active enzyme being an A_2B_2 complex. The basic properties of DNA gyrase from *Escherichia coli* are given in TABLE I. The gyrase subunits are known to comprise distinct domains whose function in the supercoiling reaction has been established (FIG. 1). The mechanism of

TABLE I: Properties of *Escherichia coli* DNA gyrase

Subunit:	GyrA	GyrB
Gene	*gyr*A (2625 bp, formerly *nalA*)	*gyr*B (2412 bp, formerly *cou*)
MW (Da)	96,756 (874 amino acids)	89,762 (803 amino acids)
Major role	Breakage and reunion of DNA	ATPase activity
Inhibitor interactions	Quinolones	Coumarins, cyclothialidines, quinolones?

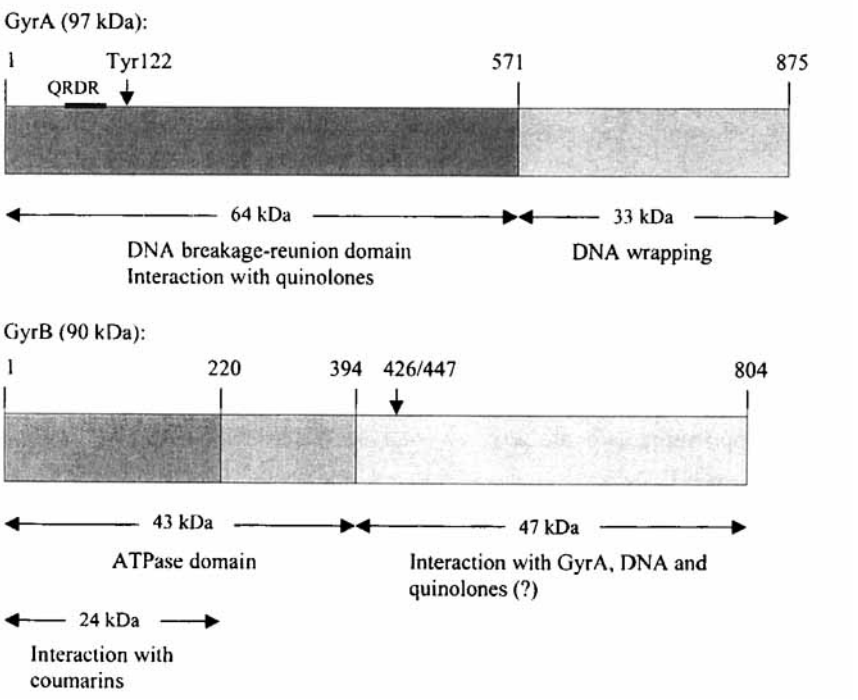

FIG. 1 Domain structure of *E. coli* DNA gyrase. Domains are shown in different shades of gray. Numbers indicate amino acids; the QRDR (amino acids 67-106) is shown as a black bar.

type II topoisomerases involves the transport of one double-stranded segment of DNA (the T segment) through a double-stranded break in another segment of DNA (the G segment)[5,6]. Although DNA gyrase conforms to this basic mechanism, it has a number of unique features that enable it to catalyse the DNA supercoiling reaction. In outline, the enzyme-DNA complex consists of ~130 bp of DNA wrapped around a protein core (A_2B_2). This wrapped DNA is cleaved by a pair of tyrosines from the N-terminal domain of GyrA (Tyr122), creating a double-stranded break with a 4-base stagger, stabilised by covalent bonds between the active-site tyrosines and the 5'-phosphates at the break site. This enzyme-stabilised DNA break occurs at a dimer interface between the GyrA subunits known as a protein 'gate'. The N-terminal domains of GyrB capture another part of the wrapped DNA segment and deliver the captured DNA (the T segment) to the double-stranded break. Strand passage involves the movement of the T segment through the G segment, which is released from the enzyme via a further protein gate. Enzyme turnover requires the hydrolysis of ATP by the N-terminal domains of GyrB. The principles of this type of strand-passage mechanism for type II topoisomerases were established through experiments on yeast topoisomerase II[5,6] and have been corroborated for DNA gyrase through structural studies on the N-terminal domains of GyrB and GyrA[7,8], and recent mechanistic work[9,10].

DNA GYRASE AS A DRUG TARGET

DNA topoisomerases are found in all cell types and are essential for cell viability. The prokaryotic enzymes (such as DNA gyrase) have become targets for antibacterial agents, and the eukaryotic enzymes are targets for anti-tumour drugs[11,12]. As gyrase is essential in bacteria and absent from eukaryotes it is an ideal drug target. Examples of agents that target gyrase are quinolones (such as oxolinic acid and ciprofloxacin) and coumarins (such as novobiocin and coumermycin A_1), whose structures are given in FIG. 2. Although a number of gyrase-specific compounds are known, studies have been largely centred on coumarins and quinolones.

THE MOLECULAR BASIS OF COUMARIN ACTION

Coumarin antibiotics, such as novobiocin and coumermycin A_1, are natural products from *Streptomyces* species. They can be shown *in vitro* to be very effective inhibitors of

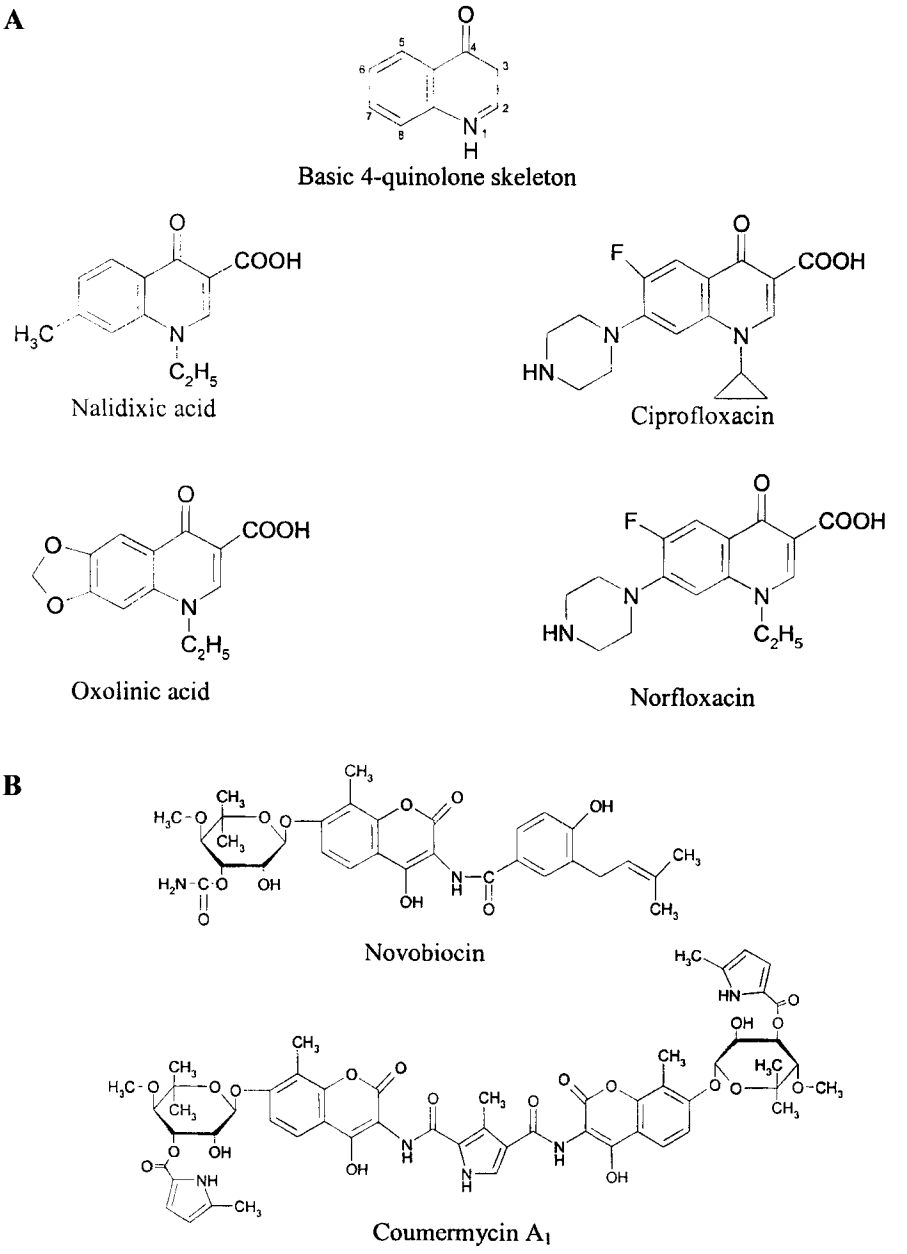

FIG. 2 Structures of compounds active against DNA gyrase. A: The quinolone drugs. Acidic quinolones are shown on the left, amphoteric fluoroquinolones on the right. **B:** The coumarins.

DNA gyrase, although they are less effective as antibacterial agents on account of their poor solubility, toxicity and low activity against Gram-negative bacteria[11]. However, through a series of structural and biochemical studies we now have a near complete understanding of how these drugs work at the molecular level. Early work had suggested that these compounds are competitive inhibitors of the gyrase ATPase reaction[13], despite their lack of structural similarity with ATP. Drug resistance mutations were found to map to the N-terminal domain of GyrB[14,15] and, when the structure of the 43 kDa GyrB fragment was solved[7], it became clear that the coumarin-binding site was likely to lie close to the ATP-binding site. Although no co-crystals of the GyrB 43 kDa domain and coumarins drugs were obtained, the cloning of a 24 kDa sub-domain of GyrB (FIG. 1)[16] has led to 3 structures with coumarins bound to this fragment[17-19]. These structures confirm that the coumarin-binding site is located in the N-terminal domain of GyrB, adjacent to the ATP-binding site, such that binding of the coumarin would prevent binding of ATP. Specifically the sugar ring of the drug (novobiose in the case of novobiocin) overlaps the binding site for the adenine ring of ATP (FIG. 3). Biochemical studies using radiolabelled drug and nucleotide confirmed that the binding of the two ligands is mutually exclusive[20]. A range of binding studies have shown that the coumarins bind gyrase with affinities (K_ds) of ~10^{-8} M[20,21].

As a consequence of these studies, we now have a very clear idea of how coumarins interact with gyrase at the molecular level. It is hoped that this information can be utilised to design more effective antibacterial agents based on the coumarin structures.

QUINOLONE DRUGS

Quinolones are synthetic antibacterial drugs, based on a 4-quinolone skeleton, that can be divided into two categories: the older acidic quinolones such as oxolinic acid, and newer, amphoteric fluoroquinolones, such as ciprofloxacin[22] (FIG. 2). This year heralds the tenth anniversary of the introduction of ciprofloxacin for use against bacterial infections. In that time there have been an increasing number of reports of resistance to fluoroquinolones amongst clinical isolates[23]. Resistance is mainly due to mutations in DNA gyrase and topoisomerase IV. In *E. coli* the primary target is DNA gyrase[24,25], but this preference is reversed in other species of bacteria[26-28].

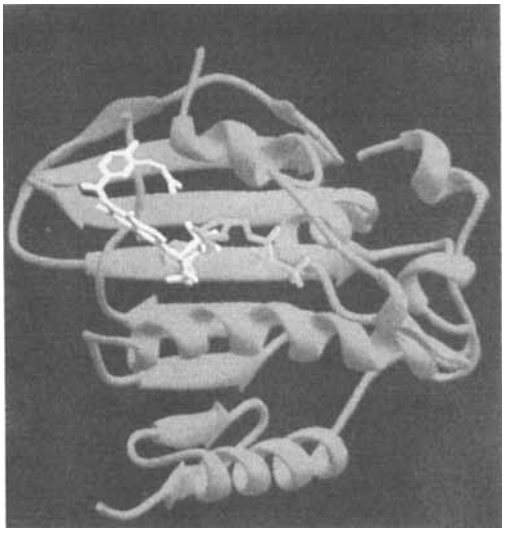

FIG. 3 The interaction of novobiocin with the N-terminal sub-domain of GyrB. The interaction of novobiocin with the 24 kDa N-terminal sub-domain of GyrB. ATP is shown in dark gray, novobiocin in white and the protein backbone shown in light gray[17] (reproduced in modified form from ref. 17, by permission of Oxford University Press).

Mode of action in bacteria

From early experiments using nalidixic acid, quinolones were shown to be potent inhibitors of DNA replication[29,30]. Quinolone-resistance mutations, which map to the *gyrA* gene, first implicated DNA gyrase as one of the principal cellular targets for these drugs[24,25]. However, it seemed unlikely that the effects mediated by quinolones were simply due to the inhibition of gyrase activity. The concentration of drug causing complete inhibition of DNA replication was significantly lower than that causing complete inhibition of the supercoiling activity of the protein[24,31,32]. This idea was further supported by the observation that bacteriophage T7 growth is inhibited by nalidixic acid, even though T7 replicates as a linear double-stranded molecule and therefore does not require gyrase activity in order to replicate its DNA[33].

The above observations led to the proposal of the 'poison' hypothesis[33], which suggests that inhibition of DNA replication is due to the formation of a gyrase-drug

complex on the DNA, which acts as a lesion, blocking passage of the polymerase (FIG. 4). This hypothesis can be explained by considering the consequences of replicating a covalently-closed-circular DNA molecule. Progression of a replication fork along a circular DNA molecule results in the build up of positive supercoils ahead of the fork. If these positive supercoils are not removed, replication will eventually cease due to topological constraint of the DNA. DNA gyrase is thought to operate ahead of the replication fork and carry out the removal of positive supercoils. Therefore, trapping of gyrase in a topoisomerase-quinolone-DNA complex would halt the progression of the DNA fork and inhibit replication. *In vitro* studies have shown that such a complex can block the progression of RNA polymerase[34] and more recently that it is also able to block the passage of DNA polymerase (LMW and AM, manuscript in preparation).

Although DNA gyrase is thought to be the principal enzyme acting ahead of the replication fork, topoisomerase IV has also been shown to be a target for the quinolone drugs[35]. It is unclear whether topo IV has any role ahead of the replication fork, although blocking of DNA polymerase by a topoisomerase IV-drug-DNA complex has been demonstrated[36]. Recent work has shown that a topo IV-norfloxacin complex on DNA can inhibit the translocation of helicases and that this may be sufficient to arrest replication fork progression[37]. These observations are consistent with earlier work which showed that a complex between bacteriophage T4 topoisomerase II and the anti-tumor drug *m*-AMSA could be disrupted by the action of a DNA helicase converting the complex into a non-reversible DNA break[38].

Although quinolone drugs lead to the formation of cleaved complexes and the inhibition of DNA synthesis and cell growth, all of these events are reversible. It is unclear what causes these cleaved complexes to become irreversible. The lethal event is thought to be the release of double-strand DNA breaks[39]. Collision of the replication fork with the topoisomerase-drug-DNA complex is not thought to be sufficient to cause the release of these DNA breaks and is has been proposed that additional factors must be involved in processing this complex. The identity of these factors is currently unknown. It is possible that there is more than one pathway by which these complexes are processed. A yeast enzyme that can process covalent complexes between eukaryotic topoisomerase I and DNA has recently been discovered and characterised[40,41]. It is possible that a corresponding activity exists in bacteria that can process gyrase-DNA covalent complexes.

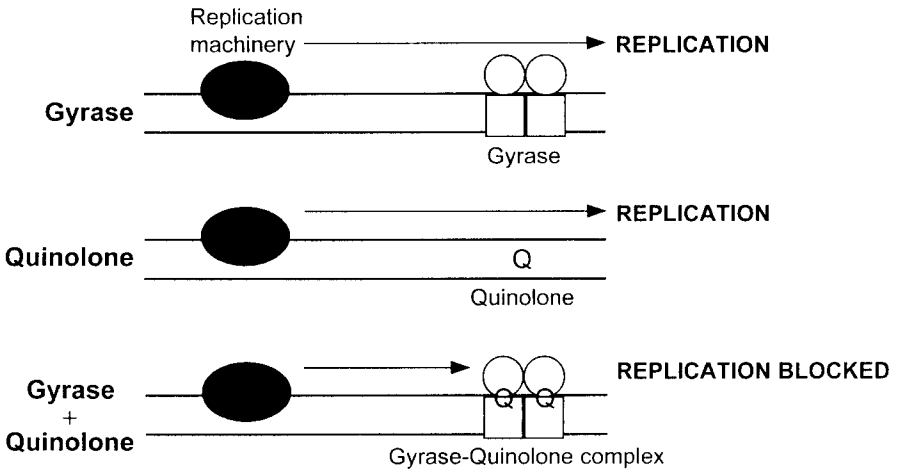

FIG. 4 Blocking of DNA replication by the quinolone-gyrase complex on DNA. The replication proteins are represented as a black ovoid, the A and B subunits of gyrase are shown as gray squares and striped circles respectively, quinolones are represented by the letter Q. Progression of the replication machinery is only blocked when both gyrase and quinolone are bound on the DNA.

Binding of quinolones to the gyrase-DNA complex

Quinolone drugs have been shown to bind to the gyrase-DNA complex[42-44]. Binding is followed by a slow DNA cleavage event in which the two strands of DNA are cleaved consecutively[45]. It is the enzyme-DNA complex, in which the DNA has both strands cleaved and covalently attached to the enzyme, that is stabilised by quinolones. This complex is thought to be bactericidal upon release of the cleaved DNA[39].

The most commonly mutated residues in resistant isolates are located within a region of the GyrA subunit between amino acids 67-106, known as the 'Quinolone Resistance Determining Region' (QRDR[46]; FIG. 1). Resistance mutations in the QRDR greatly reduce the affinity of quinolone drugs for the enzyme-DNA complex[43,44] and it is thought that the lethal complex is one in which the quinolone is bound in a pocket consisting of the residues of the QRDR and DNA. Certain residues within the QRDR are likely to have direct involvement in drug-DNA contacts. The most important are Ser83 and Asp87 as these are often the primary sites of resistance mutation[23]. Both are

solvent-exposed on the α4 helix, in the centre of the QRDR, and the catalytic tyrosine (Tyr122) is close by[8] (FIG. 5).* Most commonly mutated in instances of quinolone resistance is Ser83 in which bulky substitutions to Leu and Trp are frequently observed. Also of importance is Asp87, conserved amongst bacteria as a negatively charged residue, which appears to tolerate a number of mutations with Asn and Val being examples[23].

The QRDR may not contain all the residues involved in drug binding as it has been shown that certain GyrB mutations also induce resistance to quinolones[47]. These occur at positions Asp426 and Lys447 in GyrB, with changes to Asn and Glu respectively. Intriguingly, the Lys447 to Glu mutation confers resistance to acidic quinolones but hypersensitivity to amphoteric quinolones, while the Asp426 to Asn mutation shows a more straightforward resistance pattern to all classes of quinolones [48]. A model for a 'quinolone pocket' that involves both the GyrA QRDR residues and GyrB residues has been suggested[49]. The amphoteric quinolones (such as ciprofloxacin) are proposed to interact with Asp426 through C7 basic piperazinyl and aminopyrrolidinyl groups, an interaction that would be lost upon the substitution of Asp for Asn in the mutant. When an additional negative charge is introduced through the mutation of Lys447 to Glu it is possible to imagine the amphoteric quinolones binding more tightly to the pocket, explaining the observed hypersensitivity[49].

Although it is clear that quinolones interact with the gyrase subunits, there is also ample evidence that they can bind to DNA[50]. Direct binding to DNA has been shown by a number of groups[51-54]. These data have led to models for the interaction of quinolones with DNA alone, which involve Mg^{2+} ions bridging the contacts between the drugs and DNA[55,56]. However, other data supports complexes involving both DNA and protein[42-44] and the consensus seems to be that interactions with both gyrase and DNA are likely to be important.

The exact details of DNA-drug-enzyme interaction are not known and it is not currently possible to conclude from existing data which parts of the quinolone molecule are in direct contact with the enzyme and DNA. Determination of the crystal structure of the N-terminal domain of GyrA[8] has enabled localisation of the QRDR residues. These residues are located near the active site tyrosine (Tyr122) either side of the dimer

* See color insert for Figure 5

interface known as the DNA gate (FIG. 5). It is tempting to propose that the two symmetry-related QRDRs (one from each GyrA subunit) form two drug-binding pockets with which the quinolone molecule can interact both with the DNA (perhaps by base stacking) and with the protein (perhaps via interaction with Ser83 and Asp87). Indeed measurement of the distance between the proposed quinolone pockets (in the vicinity of Ser83 and Asp87) suggests that drug molecules bound at these locations would be able to interact with the DNA at sites 4 bases apart. In addition, the stoichiometry of two quinolones per GyrA59 dimer is consistent with data from drug-binding experiments[57].

The structure of a 92 kDa fragment of yeast topoisomerase II has also been solved[58]. This fragment shows sequence similarity to the C-terminal domain of GyrB and the N-terminal domain of GyrA (FIG. 1). The structure of the 'GyrA-like' part of this fragment is very similar to that of GyrA59 but appears to be in a different conformation, suggesting that the two structures represent two conformations of the protein during the catalytic cycle (DNA gate open and DNA gate closed). The yeast structure includes the region analogous to the part of GyrB where Asp426 and Lys447 are located. These residues would seem to be too far from the QRDR to be involved in direct drug-DNA contacts. However, a more recent crystal structure of the yeast fragment[59] shows an alternative structure in which the 'GyrB' residues are much closer to the QRDR. Therefore, it is possible that these residues are brought closer to the QRDR by large changes in enzyme conformation that may occur during part of the catalytic cycle, and that the GyrA and GyrB quinolone-resistance regions may indeed form part of a drug-binding pocket.

Based on the structural and biochemical information it is possible to propose a model for the interaction of quinolones with gyrase and DNA (FIG. 6). In this model, gyrase binds to DNA either by passing it through the ATP-operated clamp (I) or by assembly of the GyrA and GyrB dimers directly onto the DNA (II). The evidence for II is that gyrase is still able to bind a G segment even when the clamp is locked in the closed position by a nucleotide analog (N.L. Williams, A.J. Howells and AM, manuscript in preparation); under these conditions, DNA binding is somewhat reduced, suggesting that I may be the preferred route. Complex III is formed upon DNA binding across the DNA gate of GyrA. The two arms either side of the G segment wrap around the enzyme such that they can be presented to the ATP-operated clamp. Only one arm is shown in FIG. 6 for clarity.

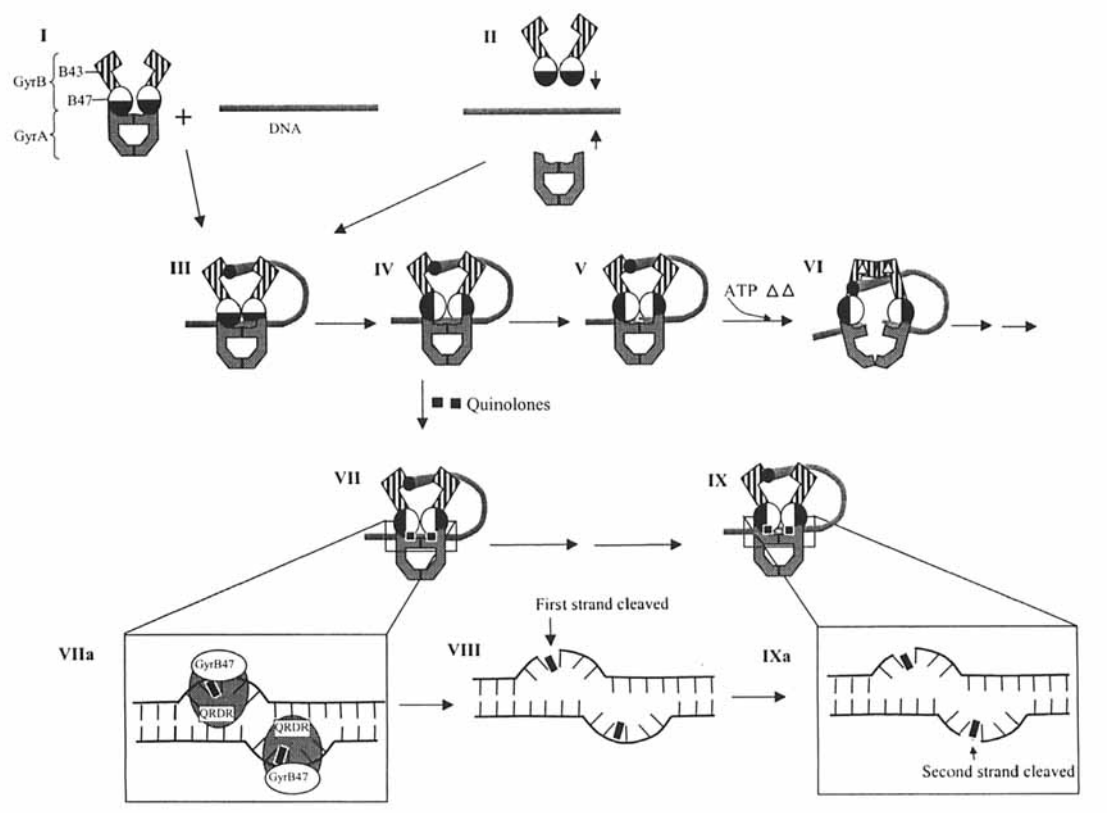

FIG. 6 Model for the molecular basis of quinolone action. The 43 and 47 kDa domains of GyrB are represented as hatched areas and the black and white circles, respectively. GyrA is represented in gray; the 33 kDa domain is omitted for clarity. In this model, gyrase binds to DNA (light gray rod) directly (I) or assembles onto it (II) to form a gyrase-DNA complex (III). Complex III undergoes a conformational change (IV) in order to cleave the DNA (V) and pull the strands apart (VI) to pass the T segment through the gap. Binding of quinolones (black squares and rectangles) is cooperative and stabilises the enzyme-DNA complex in which the conformation of GyrB is changed (IV) to give a DNA-quinolone-gyrase complex (VII). Quinolones interact with the DNA (possibly by base stacking) and are able to interact with a number of residues in the QRDR of GyrA and form contacts with GyrB (VIIa). In the presence of drug, DNA cleavage is slow and proceeds one strand at a time (VIII) before the final stabilised double-strand cleaved complex is achieved (IX and IXa). The details in VIII and IXa are shown with gyrase removed for clarity.

It is envisaged that complex III undergoes a conformational change involving the 47 kDa region of GyrB, forming complex IV in which the active site for DNA cleavage is assembled. Residues in the region of yeast topo II equivalent to the 47 kDa domain of GyrB are implicated in DNA cleavage[60]. The position of these residues in the 92 kDa yeast topo II crystal structure[58] suggest that the 47 kDa domain must undergo a large conformational change in order to bring them close to the cleavage site; these regions are indeed capable of large conformational changes[59]. During a normal reaction cycle, gyrase cleaves the G-segment across both strands to form a cleaved complex in which the DNA gate is still shut but the DNA is cleaved (V). This has been demonstrated in experiments in which cleavage of the G segment is still able to occur even when the DNA gate itself is locked in the closed position by protein cross-linking[10]. Two ATP molecules bind to the 43 kDa domains of GyrB, causing the clamp to close, capturing a piece of DNA (the T segment). This stimulates the opening of the DNA gate to form complex VI and a round of supercoiling will then proceed, involving passage of the T segment through the DNA gate, DNA religation and opening of the bottom gate of GyrA to release the T segment[9], and the hydrolysis of ATP. Quinolones are thought to bind to complex IV, as it has been shown that DNA cleavage is not a pre-requisite for drug binding[57]. Proteolysis studies have suggested that quinolone binding stabilises a complex which differs from that in which no drug is present, in that the 47 kDa region of GyrB is protected[61], i.e. suggesting that quinolones stabilise this altered conformation of the enzyme.

The interaction of quinolones with gyrase and DNA is shown in detail in VIIa. Evidence from binding experiments, using radiolabelled ciprofloxacin, suggests that two quinolone molecules bind to each complex[57]. As outlined earlier in this paper, the presence of drug-resistance mutations in the QRDR of GyrA and the 47 kDa region of GyrB suggest that they are involved in forming a quinolone-binding pocket with DNA. The crystal structure of the 59 kDa region of GyrA[8], which includes the QRDR, suggests that the DNA would have to be distorted in order to be cleaved; a representation of this distortion is given in the figure. From experiments with T4 topo II, the interaction of inhibitors such as quinolones with DNA is thought to be via stacking with the bases at the enzyme active site[62], an interaction which is presumably made easier by the distortion of the DNA. Quinolones are likely to interact at the position where the DNA is

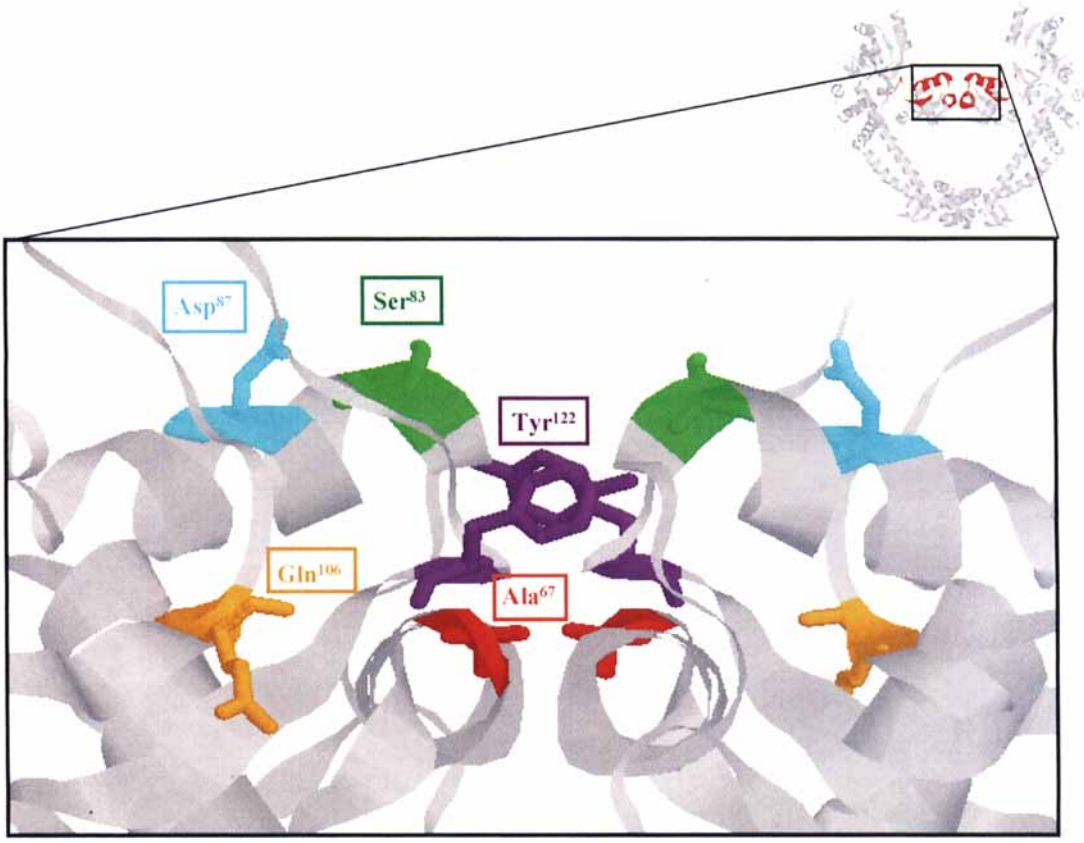

FIG. 5 Quinolone-binding pocket in GyrA. Ribbon representation of part of the α-carbon backbone of the GyrA dimer interface (the DNA gate), featuring the important quinolone-resistance determining residues of the QRDR; the catalytic tyrosine (Tyr122) is also shown. Inset: view of the GyrA59 dimer with the QRDR (residues 67-106) highlighted in red[8].

cleaved, i.e. 4 base pairs apart. Evidence to support this hypothesis comes from experiments in T4 topo II in which the binding site of the drug *m*-AMSA, which stabilises an enzyme-DNA cleaved complex, was found to be at the site of DNA cleavage[63]. This proposal is consistent with the suggested location of the quinolone-binding pocket in the gyrase structure (see above). DNA cleavage in a quinolone-stabilised complex is slow and occurs consecutively in the two strands (VIII and IXa)[45]. It is complex IX in which both strands are cleaved that is the final stabilised cleavage complex.

CONCLUSIONS

It is clear that DNA gyrase is a well-validated drug target with a number of compounds acting on this enzyme. For some of these (e.g. coumarins and quinolones) we understand their mode of action in some detail. For others (e.g. microcin B17, clerocidin), relatively little information is available. New agents are emerging from screening programs and these may have entirely novel mechanisms. Continued study of gyrase at the molecular level is necessary to understand the action of existing drugs and to provide the knowledge base to enable the understanding of the action of emerging compounds.

ACKNOWLEDGEMENT

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