Topoisomerase IV Catalysis and the Mechanism of Quinolone Action*

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Type II topoisomerases alter the topological state of the genetic material by passing an intact double helix through a transient double-stranded break that they generate in a separate DNA segment (1–6). Eubacteria contain two distinct members of this enzyme class, DNA gyrase and topoisomerase IV (6). The most well characterized of the two, DNA gyrase, was discovered over 2 decades ago (7). It is unique among type II enzymes in that it is the only known topoisomerase (prokaryotic or eukaryotic) that is capable of introducing negative superhelical twists into relaxed DNA molecules (7). DNA gyrase plays critical roles in DNA replication, recombination, and transcription, as well as in the maintenance of genomic superhelical density (1, 2, 5, 6, 8, 9).

The second prokaryotic type II enzyme, topoisomerase IV, is comprised of the products of the parC and parE genes (10). Although it was long known that ParC and ParE both were required for proper chromosome segregation in Escherichia coli (11, 12), it was not realized until 1990 that these two polypeptides together constitute a novel type II topoisomerase (10). Consistent with its critical role in chromosome segregation, topoisomerase IV displays a prejudice for catalyzing intermolecular DNA strand passage events (i.e. catenation/decatenation reactions) as opposed to intramolecular events (i.e. DNA relaxation reactions) (13–16). This prejudice notwithstanding, topoisomerase IV appears to relax DNA in vivo, and recent evidence suggests that this relaxation activity plays an important role in maintaining levels of DNA supercoiling in E. coli (17).

Beyond their essential physiological functions, prokaryotic type II topoisomerases are the cytotoxic targets for quinolone-based antimicrobial agents (18–25). One member of this drug class, ciprofloxacin, is the most active and broad spectrum antibacterial currently in clinical use (23, 26, 27). Quinolones act in a perversian fashion. First, they dramatically increase levels of covalent enzyme-cleaved DNA complexes that are requisite but transitory intermediates in the catalytic double-stranded DNA passage reaction of type II enzymes (2, 8, 21–23, 25). As a result of this action, quinolones “poison” prokaryotic type II topoisomerases and convert them to potent cellular toxins that create double-stranded breaks in the genome of treated cells (2, 22, 27). Second, quinolones also inhibit the overall catalytic activity of the type II enzymes (20, 21, 28, 29). In so doing, they further damage bacterial cells by depriving them of the critical functions performed by these ubiquitous enzymes.

Despite the importance of topoisomerase IV to the survival of prokaryotic cells and to the treatment of bacterial infections, relatively little is known about the details of its catalytic mechanism. Furthermore, the basis by which quinolones stimulate DNA cleavage or inhibit the overall catalytic activity of any bacterial type II topoisomerase remains an enigma. Therefore, a series of experiments that analyzed individual steps of the topoisomerase IV catalytic cycle was undertaken to address these critical mechanistic issues. Results provide insight into the underlying basis for the high levels of DNA cleavage generated by topoisomerase IV and the preference of the enzyme for intermolecular DNA substrates. In addition, they provide a framework for interpreting the actions of quinolones against prokaryotic type II topoisomerases.

**EXPERIMENTAL PROCEDURES**

Topoisomerase IV was purified from E. coli by the procedure of Peng and Marians (21). Human topoisomerase IIa was expressed in Saccharomyces cerevisiae (30) and purified by the protocol of Kingma et al. (31). Drosophila melanogaster topoisomerase II was isolated from embryonic Kc cells as described by Shelton et al. (32). Ciprofloxacin was obtained from Sigma, and all other quinolones were synthesized at Pfizer Central Research by the procedure of Gilligan et al. (33). Quinolones were stored as 40 mM stock solutions in 0.1 N NaOH and then diluted to 8 mM with Tris-HCl, pH 7.9, immediately prior to use. Tris, ethidium bromide, and...
APP(NH)P were obtained from Sigma; SDS and proteinase K were from Merck; ATP was from Amersham Pharmacia Biotech; histone H1 was from Boehringer Mannheim; and γ-[32P]ATP was from Amersham Pharmacia Biotech. All other chemicals were analytical reagent grade.

Preparation of DNA Substrates—Negatively supercoiled pBR322 DNA was prepared by partially decatenating E. coli DNA kDNA as described (35). Monomeric (i.e. decatenated) kDNA was prepared by incubating 5 nM kDNA with 2 mM topoisomerase IV, 1 mM ATP, and 10 mM DTT in 500 mM Tris-Cl buffer (40 mM Tris-Cl, pH 7.9, 50 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 5 mM MgCl₂, and 2.5% glycerol) at 37 °C for 60 min. The reaction was stopped by the addition of 2 μl of stop solution (0.77% SDS, 77 mM EDTA, and 2.5% glycerol) and the type II enzyme was digested with proteinase K (final concentration 80 μg/ml) at 45 °C for 30 min. Samples were extracted with phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with ethanol, and resuspended in 15 μl of water. As monitored by electrophoresis in agarose gels, ≥95% of the DNA product was catenated.

Catenated DNA (kDNA) was isolated from the kinetoplasts of Crithidia fasciculata as described (35). Monomeric (i.e. decatenated) kDNA was prepared by incubating 5 nM kDNA with 2 mM topoisomerase IV, 1 mM ATP, and 10 mM DTT in 500 mM Tris-Cl buffer (40 mM Tris-Cl, pH 7.9, 50 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 5 mM MgCl₂, and 2.5% glycerol) at 37 °C for 60 min. The reaction was stopped by the addition of 2 μl of stop solution (0.77% SDS, 77 mM EDTA, and 2.5% glycerol) and the type II enzyme was digested with proteinase K (final concentration 80 μg/ml) at 45 °C for 30 min. Samples were extracted with phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with ethanol, and resuspended in 15 μl of water. As monitored by electrophoresis in agarose gels, ≥95% of the DNA product was catenated.

DNA Cleavage—DNA cleavage assays in the absence or presence of quinolones were performed as described by Corbett et al. (36). Briefly, to monitor levels of pre-strand passage DNA cleavage, 5 nM negatively supercoiled pBR322 DNA was incubated with 7.5 nM topoisomerase IV and 10 mM DTT in 20 μl of top IV assay buffer (40 mM Tris-Cl, pH 7.9, 3 mM MgCl₂, and 2.5% glycerol) at 37 °C for 8 min. Alternatively, assays contained either 75 mM Drosophila topoisomerase II (in top II assay buffer) or 150 mM human topoisomerase IIα (in 50 mM Tris-Cl, pH 7.9, 100 mM KCl, 10 mM MgCl₂, 5 mM β-mercaptoethanol, 0.5 mM EDTA, and 2.5% glycerol) and were incubated at 30 °C for 6 min or 37 °C for 10 min, respectively. Assays that monitored post-strand passage DNA cleavage also contained 1 mM APP(NH)P. In all cases, DNA cleavage reactions were stopped by the addition of SDS (1% final concentration) followed by EDTA (15 mM final concentration). Samples were digested with proteinase K as described above. Following the addition of 2 μl of loading buffer (30% sucrose, 0.5% bromophenol blue, and 0.5% xylene cyanole FF in 10 mM Tris-Cl, pH 7.9), DNA products were resolved by electrophoresis in 1% agarose gels in 40 mM Tris-acetate, pH 8.3, 2 mM EDTA, and 0.5 μg/ml ethidium bromide. DNA bands were visualized by UV light, photographed through Kodak 23A 12 filters with Polaroid type 665 film, and quantitated by scanning negatives with an E-C apparatus model EC910 densitometer in conjunction with Hoefer GS-370 Data System software. The intensity of bands in the negative was proportional to the amount of DNA present. Double-stranded DNA breaks were monitored by the conversion of negatively supercoiled plasmid to linear molecules.

DNA Religation—Reactions were carried out by the procedure of Robinson and Osheroff (37). DNA cleavage/religation equilibria were established as described in the preceding section in the presence or absence of 20 μM ciprofloxacin. Pre- or post-strand passage DNA religation assays were carried out in the absence of a nucleoside triphosphate and in the presence of 1 mM APP(NH)P, respectively. Religation was initiated by shifting the temperature from 37 to 75 °C and stopped at various times up to 150 s by the addition of SDS (1% final concentration). Samples were processed and analyzed by agarose gel electrophoresis as described in the preceding section. The apparent first order rate of religation was determined by quantifying the loss of linear DNA.

ATP Hydrolysis—Reactions were performed at 37 °C as described by Corbett et al. (36) in 20 μl of top IV assay buffer in the presence of 7.5 nM topoisomerase IV, 50 mM NaCl, 150 mM KCl (monocatenated or catenated pBR322 plasmid, or catenated or monomeric kDNA), 1 mM ATP, ~10 μCi of [γ-32P]ATP, 10 mM DTT, and 0 to 20 μM ciprofloxacin. Samples (3 μl) were removed at various times up to 10 min and spotted onto thin layer cellulose plates impregnated with polyethyleneimine (J. T. Baker Inc.). Following chromatography in freshly made 400 mM NH₄HCO₃, levels of ATP were monitored by quantifying the release of inorganic phosphate using a Molecular Dynamics PhosphoImager.

Decatenation Reactions—Assays contained 5 mM kDNA, 2 mM topoisomerase IV, 1 mM ATP, 10 mM DTT, and 0 to 50 μM ciprofloxacin and were incubated in 20 μl of top IV assay buffer at 37 °C for 15 min. Reactions were terminated by the addition of 3 μl of stop solution and digested with proteinase K as above. DNA products were resolved by electrophoresis in 1% agarose gels in 100 mM Tris borate, pH 8.3, 2 mM EDTA, stained with 1 μg/ml ethidium bromide, and visualized as described above. The percent decatenation was quantified by determining the appearance of monomeric circular DNA molecules.

Results

Topoisomerase IV Catalysis

The DNA Cleavage/Religation Reaction Mediated by Topoisomerase IV—The double-stranded DNA cleavage/religation cycle is central to the catalytic functions of all type II topoisomerases (1, 2, 38, 39). During the cleavage step of this cycle, these enzymes form a covalent intermediate with the newly created 5'-DNA termini generated by scission (1, 2, 38–40). The DNA cleavage/religation equilibrium established by type II topoisomerases can be monitored by quantitating levels of this covalent cleavage complex (39, 41).

During their catalytic cycles, type II topoisomerases undergo two separate DNA cleavage/religation reactions (1, 37, 38, 42). The first of the two occurs prior to (and is requisite for) the DNA strand passage reaction step. This “pre-strand passage” reaction can be examined by monitoring DNA cleavage/religation in the presence of ATP (binding of which triggers strand passage) (43). The second reaction occurs subsequent to the DNA strand passage event. This “post-strand passage” reaction can be examined by monitoring DNA cleavage/religation in the presence of a nonhydrolyzable ATP analog such as APP(NH)P, which triggers strand passage but does not allow enzyme recycling (42, 43).

Reflecting the fact that the type II topoisomerases need to religate cleaved DNA substrates in order to complete their catalytic cycles, the DNA cleavage/religation equilibria of these enzymes generally lie heavily toward religation (1, 38, 44). Levels of DNA cleavage tend to increase somewhat (~2–4-fold) following strand passage, due primarily to a decrease in the apparent first order rate of post-strand passage DNA religation (at least for the eukaryotic type II enzymes) (37, 38, 42).

Although the DNA cleavage/religation reactions of topoisomerase IV are essential to its physiological functions, relatively little is known about these enzyme-mediated events. Therefore, three approaches were utilized to describe further these critical reaction steps. In the first, equilibrium levels of pre-strand passage DNA scission generated by E. coli topoisomerase II were determined. In the absence of ATP, the predominance of religation was far less pronounced for topoisomerase IV than it was for previously characterized type II enzymes. As seen in Fig. 1, equilibrium levels of DNA scission for the bacterial enzyme were dramatically higher (~200- and ~40-fold, respectively) than those observed for two other non-gyrase type II topoisomerases, human topoisomerase IIα and Drosophila topoisomerase II. In fact, at equilibrium, ~8% of topoisomerase IV appears to be in a covalent DNA cleavage complex as compared with significantly less than 1% for other type II enzymes examined.

In the second approach, equilibrium levels of post-strand passage DNA cleavage were determined. As seen in Fig. 2, levels of scission were (for the most part) equal to or less than those observed in the absence of a nucleoside triphosphate when either APP(NH)P or ATP (which yields an average of pre- and post-strand passage equilibria) were included in reactions. This result is in marked contrast to previous findings with other non-gyrase type II topoisomerases (37, 38, 42).

In the last approach, DNA religation reactions mediated by topoisomerase IV were characterized. As seen in Fig. 3, prior to DNA strand passage, the apparent first order rate of religation mediated by topoisomerase IV was nearly an order of magni-
which the two DNA helices are on separate molecules (i.e., incubated in the absence of a nucleoside triphosphate cofactor (closed Assays containing 0–30 nM topoisomerase IV were DNA cleavage.

Reactions contained 5 nM pBR322 plasmid DNA and 150 n M human topoisomerase IIa (Hum α), 75 nM Drosophila topoisomerase II (D. mel), or 7.5 nM topoisomerase IV (Topo IV). Double-stranded DNA cleavage converts negatively supercoiled plasmid (form I, F1I) to linear molecules (form III, F1II). The position of nicked circular DNA (form II, F1II) is shown for reference. The bar graph quantitates the relative cleavage per mol of enzyme for an average of three independent experiments. Standard deviations averaged less than 2%. The relative cleavage of topoisomerase IV was set to 100%

FIG. 1. Topoisomerase IV displays higher levels of equilibrium DNA scission than other type II topoisomerases. The inset depicts an agarose gel showing products of enzyme-mediated DNA cleavage. Reactions contained 5 nM pBR322 plasmid DNA and 150 n M human topoisomerase IIa (Hum α), 75 nM Drosophila topoisomerase II (D. mel), or 7.5 nM topoisomerase IV (Topo IV). Double-stranded DNA cleavage converts negatively supercoiled plasmid (form I, F1I) to linear molecules (form III, F1II). The position of nicked circular DNA (form II, F1II) is shown for reference. The bar graph quantitates the relative cleavage per mol of enzyme for an average of three independent experiments. Standard deviations averaged less than 2%. The relative cleavage of topoisomerase IV was set to 100%.

FIG. 2. ATP does not stimulate topoisomerase IV-mediated DNA cleavage. Assays containing 0–30 nM topoisomerase IV were incubated in the absence of a nucleoside triphosphate cofactor (closed bar) or in the presence of 1 mM ATP (opened bar) or APP(NH)P (stippled bar). The percent DNA cleaved was quantitated by dividing the amount of topoisomerase IV-generated linear DNA by the total amount of DNA present in the absence of enzyme. Data represent the average of two independent experiments. Standard deviations are shown as error bars.

density slower than that previously reported for Drosophila topoisomerase II (0.028 s⁻¹ versus 0.248 s⁻¹) (37). Furthermore, unlike the Drosophila enzyme, religation rates did not drop (and actually increased slightly) following strand passage. (Apparent first order rates of post-strand passage DNA religation were 0.035 s⁻¹ and 0.085 s⁻¹ for the E. coli and Drosophila enzymes, respectively.) Taken together, these results strongly suggest that differences in DNA cleavage/religation equilibria observed for topoisomerase IV and the eukaryotic type II topoisomerases are due (at least in part) to differences in DNA religation rates. Preference of Topoisomerase IV for Intermolecular DNA Strand Passage—Topoisomerase IV displays a pronounced preference for catalyzing in vitro strand passage reactions in which the two DNA helices are on separate molecules (i.e.

decatenation as opposed to relaxation reactions) (13–16). However, the mechanistic basis that underlies this preference for intermolecular strand passage is unknown.

The ability of topoisomerase IV to distinguish between inter- and intramolecular DNA substrates may be explained by two distinct (but not mutually exclusive) mechanisms. Either the enzyme preferentially recognizes and binds DNA molecules that contain intermolecular crossovers or it catalyzes higher rates of DNA strand passage with intermolecular substrates. Two experiments were carried out to distinguish between these possibilities. In the first, the ability of topoisomerase IV to recognize intermolecular substrates was assessed by a DNA cleavage assay. Topoisomerase IV displayed virtually no difference in its propensity to cleave negatively supercoiled monomeric (i.e. intramolecular substrate) or catenated (i.e. intermolecular substrate) pBR322 molecules (Fig. 4). If anything, levels of DNA scission were slightly decreased with catenated substrates. Since the efficiency of cleavage within a given DNA substrate reflects (to a large extent) levels of enzyme-DNA substrate binding (45), this result indicates that the strong decatenation activity of topoisomerase IV is not due to a heightened recognition of substrates with intermolecular DNA helices.

In the second experiment, the effects of inter- versus intramolecular DNA substrates on the ATPase activity of topoisomerase IV were assessed. ATP hydrolysis represents the penultimate step in the topoisomerase IV catalytic cycle and triggers enzyme recycling (3, 38). As seen in Fig. 5, the rate of ATP hydrolysis was >4-fold higher in the presence of kDNA networks than it was with monomeric kDNA molecules. A more dramatic increase was observed when catenated pBR322 molecules were compared with monomeric pBR322 substrates. These data strongly suggest that the preferential decatenase activity of topoisomerase IV results from higher rates of strand passage and enzyme recycling with intermolecular substrates. Quinolone Mechanism

Prokaryotic type II topoisomerases are the cytotoxic targets for a clinically important class of antibacterials known as quinolones (18–21, 23–25). One member of this drug class, ciprofloxacin (shown in Fig. 6), is widely prescribed and displays potent activity against a spectrum of pathogenic bacteria (23, 26, 27). In Gram-negative species, DNA gyrase appears to
experiments. Standard deviations are shown as average of three independent experiments. Standard deviations are shown as error bars.

be the primary target of quinolone-based agents, whereas topoisomerase IV serves as a secondary target and contributes to drug efficacy (10, 18, 19, 23–25, 29). Beyond the effects of quinolones on enzyme-mediated DNA cleavage, it has been suggested that the ability of these drugs to block the catalytic activity of topoisomerase IV also contributes to their cytotoxicity (20, 21, 28, 29). Finally, in Gram-positive bacterial species, topoisomerase IV rather than DNA gyrase appears to be the primary cellular target of most quinolones (23, 46–50).

Stimulation of Topoisomerase IV-mediated DNA Cleavage by Ciprofloxacin—Consistent with previous reports (25, 29, 51, 52), ciprofloxacin was a potent enhancer of DNA scission mediated by topoisomerase IV (Fig. 7). Levels of DNA cleavage doubled at a drug concentration of ∼500 nM (Table I) and plateaued in the 10–20 μM range. In addition, the concentration dependence of ciprofloxacin-induced DNA cleavage was identical in the absence or presence of APP(NH)p (Fig. 7), indicating that quinolone efficacy is not dependent on the DNA strand passage state of topoisomerase IV.

No previous reports have determined the mechanism by which antibacterial agents stimulate DNA cleavage mediated by prokaryotic type II topoisomerases. However, a body of literature indicates that anticancer drugs targeted to eukaryotic type II topoisomerases stimulate DNA cleavage in one of two primary fashions. They act either by inhibiting the ability of the enzyme to religate cleaved DNA molecules, or by increasing rates of enzyme-mediated DNA cleavage (37, 53–58).

To determine the mechanistic basis for the enhancement of topoisomerase IV-mediated DNA cleavage by quinolones, the effects of ciprofloxacin on the rate of DNA religation generated by the enzyme were characterized (Fig. 8). Under conditions in which ciprofloxacin stimulated DNA scission 4–5-fold, the drug decreased the apparent first order rate of pre-strand passage religation from 0.028 s⁻¹ to 0.018 s⁻¹ and post-strand passage religation from 0.035 s⁻¹ to 0.016 s⁻¹. Since this inhibition cannot fully account for the levels of cleavage stimulation observed, it is proposed that ciprofloxacin acts both by increasing rates of DNA scission and by inhibiting the ability of topoisomerase IV to religate cleaved DNA.

Sensitivity of Topoisomerase IV to the C-7 Quinolone Ring Substituent—Of the DNA cleavage-enhancing agents described to date, quinolones are the only drugs that display potent activity toward both bacterial and eukaryotic type II topoisomerases (23–25, 59–62). A distinguishing feature, however, between bacterial DNA gyrase and eukaryotic topoisomerase II is their response to the nature of the substituent at the C-7 position of the quinolone ring (56, 60–63). Whereas the sensitivity of DNA gyrase is only mildly increased (~2.5-fold) by the substitution of an aromatic hydroxyphenyl ring for an aliphatic piperazine ring (converting ciprofloxacin to CP-115,955, Fig. 6), the sensitivity of human topoisomerase IIα is dramatically enhanced (~180-fold) by this ring modification (Table I). Similar trends are observed for other quinolones containing a hydroxyphenyl group at C-7.

To determine the response of topoisomerase IV to aromatic substituents in the C-7 quinolone ring position, the sensitivity of the enzyme toward a panel of quinolones containing a C-7 hydroxyphenyl ring was characterized (see Fig. 6). As seen in Table I, topoisomerase IV was relatively insensitive to this
Assays containing 7.5 nM topoisomerase IV and 5 nM cleaved DNA.

FIG. 7. Ciprofloxacin enhances topoisomerase IV-mediated DNA cleavage. Topoisomerase IV was incubated with 5 nM pBR322 plasmid DNA and 0–50 μM ciprofloxacin. Pre-strand passage reactions (●) lacked a nucleoside triphosphate, and reactions monitoring post-strand passage DNA cleavage (○) contained 1 mM ATP/NH₃. The relative level of DNA cleavage in the absence of drug was set to 1.0. Data represent the average of three independent experiments. Standard deviations are shown as error bars.

FIG. 8. Ciprofloxacin inhibits enzyme-mediated religation of cleaved DNA. Assays containing 7.5 nM topoisomerase IV and 5 nM pBR322 plasmid DNA were incubated in the absence (●) or presence (○) of 20 μM ciprofloxacin (Cipro). The left panel shows religation occurring before the DNA strand passage event (Pre), and the right panel depicts religation following strand passage (Post) and contained 1 mM ATP/NH₃. Religation was initiated by shifting the temperature from 37 to 75°C. At time 0, levels of double-stranded cleavage were set to 100%. Data represent the average of three independent experiments. Standard deviations are shown as error bars.

Table I: Relative activities of quinolones toward type II topoisomerases

| Compound       | Human Topo IIα | E. coli Topo IV | E. coli DNA gyrase |
|----------------|----------------|-----------------|-------------------|
| Ciprofloxacin  | 1.00 (500)     | 1.0 (0.54)      | 1.0               |
| CP-115,953     | >330 (1.5)     | 0.8 (0.67)      | 4.3               |
| CP-115,955     | >180 (2.8)     | 0.7 (0.70)      | 2.5               |
| CP-67,804      | >40 (12)       | 0.5 (0.99)      | 1.0               |

a Relative potency values are from Robinson et al. (56, 61).
b The relative potency of ciprofloxacin was set to 1.0.

Values in parentheses represent the micromolar drug concentration necessary to stimulate DNA cleavage 2-fold.

distinguishing modification. Thus, while its decatenation (and lack of supercoiling) activity aligns topoisomerase IV "enzymatically" with the eukaryotic type II topoisomerases, its insensitivity to aromatic C-7 quinolone ring substituents aligns the enzyme "pharmacologically" with bacterial DNA gyrase.

Inhibition of Topoisomerase IV Catalysis by Ciprofloxa-

cin—in addition to the DNA cleavage-enhancing properties of quinolones, these drugs also inhibit the catalytic DNA strand passage activity of topoisomerase IV (Fig. 9). It has been suggested that this inhibition contributes to the cytotoxicity of quinolone-based drugs to E. coli by blocking chromosome segregation (20, 21, 28, 29). However, the mechanistic basis for this potentially lethal drug effect has yet to be described.

The inhibition of topoisomerase IV-catalyzed DNA decatena-

tion by ciprofloxacin can be explained, at least in part, by the ability of the drug to interfere with enzyme-mediated DNA religation (see Fig. 8). However, quinolones may also affect topoisomerase IV at several additional steps of the catalytic cycle of the enzyme. Previous studies on the mechanism of action of antineoplastic agents targeted to eukaryotic type II topoisomerases indicate that many of these DNA cleavage-enhancing drugs (including quinolones) disrupt interactions between the enzyme and its ATP cofactor (64–66). Therefore, to further our understanding of quinolone inhibition, the effects of ciprofloxacin on the ATPase activity of topoisomerase IV were assessed (Fig. 10).

Ciprofloxacin was a potent inhibitor of ATP hydrolysis (IC₅₀ ≈7 μM; where IC₅₀ indicates drug concentration required to inhibit catalysis by 50%). Since this IC₅₀ value is similar to that observed for inhibition of decatena-

tion (IC₅₀ ≈12 μM, Fig. 9), it is likely that obstruction of topoisomerase IV-ATP interactions by quinolones contributes significantly to the inhibition of overall catalytic activity by these drugs.

DISCUSSION

Topoisomerase IV is ubiquitous among bacteria and is essen-

tial for chromosomal segregation in prokaryotic species (10–12). Despite its importance to the bacterial cell, considerably less is known regarding its catalytic mechanism than that of its sister prokaryotic enzyme, DNA gyrase, or its eukaryotic counterparts.

Of the type II topoisomerases examined to date, E. coli topo-

isomerase IV generates (by far) the highest levels of DNA scission at equilibrium. This prolific cleavage activity is due in large part to decreased rates of DNA religation. It is not clear how E. coli cells tolerate a topoisomerase that has the potential to create such a large number of breaks in the genetic material. However, it may be related to the fact that topoisomerase IV specifically acts behind replication forks during DNA synthesis, decreasing the probability that transient DNA cleavage com-
Topoisomerase IV Catalysis and Mechanism of Quinoline Action

Topoisomerase IV displays a considerable preference for DNA C-7 ring position. As observed previously for DNA gyrase (56, sensitivity to quinolones that contain aromatic groups in the domain for quinolones appears to be conserved throughout to mammalian species. Furthermore, the enzyme interaction somerases (23–25, 59–62). Members of this drug class display DNA cleavage-enhancing drugs known to target type II topoisomerases (23, 60, 61, 63), topoisomerase IV was relatively insensitive to the C-7 substituent. This response to quinolones clearly marks topoisomerase IV as a member of the prokaryotic family, despite the fact that the catalytic activity of the enzyme appears to be more closely aligned with the eukaryotic type II topoisomerases than it is with bacterial DNA gyrase.

Topoisomerase IV is critical to the survival of all bacterial species (10, 12, 13). It plays fundamental roles in many aspects of DNA metabolism and appears to be an emerging target for antibacterial therapy (10, 23, 29, 46–49). Despite its importance, it remains one of the least well characterized of all the type II topoisomerases. Results of the present study increase our understanding of the mechanism of action of this essential enzyme and further define interactions between topoisomerase IV and quinolone-based therapeutics.

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