The DNA Gyrase-Quinolone Complex

ATP HYDROLYSIS AND THE MECHANISM OF DNA CLEAVAGE

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Quinolone binding to the gyrase-DNA complex induces a conformational change that results in the blocking of supercoiling. Under these conditions, gyrase is still capable of ATP hydrolysis which now proceeds through an alternative pathway involving two different conformations of the enzyme (Kampranis, S. C., and Maxwell, A. (1998) J. Biol. Chem. 269, 22606–22614). The kinetics of ATP hydrolysis via this pathway have been studied and found to differ from those of the reaction of the drug-free enzyme. The quinolone-characteristic ATPase rate is DNA-dependent and can be induced in the presence of DNA fragments as small as 20 base pairs. By observing the conversion of the ATPase rate to the quinolone characteristic rate, the formation and dissociation of the gyrase-DNA-quinolone complex can be monitored. Comparison of the time dependence of the conversion of the gyrase ATPase with that of DNA cleavage reveals that formation of the gyrase-DNA-quinolone complex does not correspond to the formation of cleaved DNA. Quinolone-induced DNA cleavage proceeds via a mechanism consisting of two cleavage events that is modulated in the presence of a nucleotide cofactor. We demonstrate that quinolone binding and drug-induced DNA cleavage are separate processes constituting two sequential steps in the mechanism of action of quinolones on DNA gyrase.

DNA gyrase is the intracellular target of the quinolone group of antibacterial agents. The addition of quinolone drugs to an in vitro reaction containing DNA gyrase, relaxed, closed-circular DNA, and ATP leads to the inhibition of supercoiling (2). If this reaction is terminated by the addition of a protein denaturant, like SDS, the DNA is found to be cleaved in both strands with a gyrase A subunit (GyrA) covalently attached to each 5'-phosphoryl terminus (3). Quinolones also inhibit the relaxation, catenation, and decatenation reactions of gyrase (2, 4).

The molecular details of the interaction of the drugs with the gyrase-DNA complex are unknown. When the binding of the drugs to the enzyme was investigated, insignificant binding of quinolones to the A or B subunit (GyrB) or to the gyrase holoenzyme (A₂B₂) was detected (5–7). Binding of the drugs to the DNA is weak, and more efficient in the case of single-stranded DNA (8, 9). Quinolones were found to bind strongly to the complex formed by gyrase and DNA, and ATP appeared to assist this interaction (5). These results led to the proposal that the drugs bind to the single-stranded DNA region that is revealed when gyrase cleaves DNA during turnover (10). This proposal was addressed by site-directed mutagenesis of the gyrase active site. Mutation of tyrosine 122 to serine or phenylalanine abolished supercoiling and quinolone-induced DNA cleavage but the mutants could still bind the drugs equally well as wild-type (11). Clearly DNA cleavage is not required for drug binding. Similar results have also been obtained with topoisomerase IV (12).

Using limited proteolysis we found that quinolones stabilize a conformational change in the gyrase-DNA complex (1). DNA cleavage is not required for the enzyme to undergo this conformational change since a mutant that binds quinolones but cannot cleave DNA gives the same proteolytic fingerprints in the presence of the drugs as the wild-type enzyme. We suggested that this quinolone-stabilized conformation is responsible for the inhibition of the gyrase functions. At the molecular level this conformation was interpreted as a state of the enzyme where the DNA gate is trapped in the closed form irrespective of the DNA cleavage-religation state. ATP hydrolysis could still occur when gyrase was in the quinolone-trapped state but the complex exhibited a rate of hydrolysis that was lower than that of the drug-free enzyme. In order to shed more light on the interaction of quinolones with gyrase we have studied the characteristics of ATP hydrolysis by the enzyme-drug complex. We show that the rate of ATP hydrolysis can provide a sensitive tool for monitoring of the formation of the gyrase-quinolone complex and we use this tool to probe the relationship between drug binding and drug-induced DNA cleavage.

EXPERIMENTAL PROCEDURES

Enzymes and DNA—GyrA, GyrB, and the 59-kDa domain of GyrA (GyrA59, gift of C. Smith) were purified as described previously (13, 14). Negatively supercoiled and relaxed forms of plasmid pBR322 were provided by A. J. Howells (University of Leicester). Linear pBR322 was prepared by digestion of the supercoiled form with EcoRI. The 198-bp fragment was amplified by the polymerase chain reaction using pBS147 as a template (15). The 147-, 90-, and 80-bp fragments containing the major gyrase cleavage site of plasmid pBR322 (16) were prepared by polymerase chain reaction using pBR322 as a template. The 40- and 20-bp fragments, based on the same pBR322 site, were prepared by the annealing of complementary chemically synthesized oligonucleotides that had been purified by high performance liquid chromatography (PNACL, University of Leicester).

Enzyme Assays—ATPase assays were performed as described previously (17, 18). DNA supercoiling and quinolone-induced cleavage reactions were carried out under the conditions previously described (19). In DNA cleavage assays, 10 nm relaxed pBR322 was incubated with 18 nm GyrA, 54 nm GyrB (giving a final concentration of A₂B₂ of 9 nm), and the indicated concentrations of ciprofloxacin (CFX) at 25 °C for the times shown. Cleavage was revealed by addition of 0.2% SDS and 0.1 mg/ml proteinase K and incubation at 37 °C for 30 min. The results were analyzed by electrophoresis on 1% agarose gels, containing 3 μg/ml

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RESULTS

The Kinetics of ATP Hydrolysis in the Presence of Quinolones—In the preceding paper (1), we described the formation of two different complexes in the presence of quinolones and suggested that the drug-inhibited enzyme can hydrolyze ATP by undergoing a different catalytic cycle entailing these two complexes. To provide further support for the existence of this alternative pathway, we have analyzed the kinetics of ATP hydrolysis in the presence of the drugs (Fig. 1). The interaction of gyrase with ATP leading to nucleotide hydrolysis is far from simple, and it has been shown that the Michaelis-Menten paradigm cannot describe sufficiently the experimental data obtained (20, 21). This is mainly due to the presence of two binding sites for ATP and the requirement for B subunit dimerization in order for nucleotide hydrolysis to take place (17). A more comprehensive model describing the ATP hydrolysis by DNA gyrase is shown below,

\[
E + ATP \rightarrow E \cdot ATP \rightarrow E \cdot ATP_1 \rightarrow E + 2ADP + 2P_i
\]

(Eq. 1)

There is presently no experimental evidence for ATP hydrolysis taking place when the nucleotide is bound to only one of the two ATP-binding sites. Moreover, when the A2B2 tetramer was reconstituted with a mixture of wild-type GyrB and a mutant that cannot bind the nucleotide it was found that both GyrB subunits must be functional in nucleotide binding in order for catalytic supercoiling to occur (22). Therefore, in this mechanism we assume that both ATP-binding sites have to be filled before hydrolysis can take place. The steady-state equation describing this interaction is shown in the “Appendix.” In Equation 1, \( k_3 \) reflects the apparent rate constant of the ATP hydrolysis/product release step of the mechanism. Because of the experimental setup used in these experiments these two steps are indistinguishable. Our assay measures changes in ADP concentration and therefore the rate of hydrolysis measured depends on the rate by which ADP is released from the complex. By fitting the data in Fig. 1 to Equation 17, we obtained values of \( k_3 \) of 0.56 ± 0.03 s\(^{-1}\) in the absence of CFX and 0.27 ± 0.04 s\(^{-1}\) in the presence of the drug, i.e., the final step of the ATPase mechanism is inhibited by the drugs by a factor of ~2. The first steps of the ATPase mechanism, namely the two successive nucleotide binding steps, are represented in this model by the equilibrium dissociation constants \( K_{d1} \) and \( K_{d2} \), respectively. The interaction of ATP with the two binding sites in the drug-free complex appears to exhibit positive cooperativity with \( K_{d2} \) being approximately 1 order of magnitude lower than \( K_{d1} \). The values of these constants were estimated to be \( K_{d1} \approx 590 \mu M \) and \( K_{d2} \approx 70 \mu M \). The gyrase-drug complex exhibits a similar equilibrium dissociation constant for the first step, \( K_{d1} \approx 530 \mu M \), but the second step has a \( K_{d2} \) of ~260 \mu M; it seems that cooperativity between the two ATP sites is significantly reduced in the presence of quinolones.

The Quinolone Characteristic Rate Is DNA-dependent—The dependence of the quinolone-characteristic ATPase rate on DNA concentration was investigated. A59B2 and the version of gyrase that has been deleted for the 33-kDa domains, A599B2, were incubated with increasing concentrations of linear pBR322 and the ATPase rate in the presence of CFX was measured (Fig. 2). Drug-free gyrase showed the well-documented increase in the rate of hydrolysis with increasing DNA concentrations (23). A59B2 was unable to show any increased ATPase in the absence of CFX in the range of DNA concentrations used in these experiments. This characteristic of A59B2 has been seen previously and was attributed to the weak DNA binding of this enzyme (~10-fold weaker than A2B2 (24)). In the presence of the drug, the ATPase activity of A59B2 exhibited a steep dependence on DNA concentration at low DNA:enzyme ratios and reached a plateau after ~200 bp:enzyme. The plateau rate was ~0.63 s\(^{-1}\). As described previously (1), when incubated with quinolones and DNA, A59B2 exhibits a rate of hydrolysis similar to that of the drug-bound full-length enzyme-DNA complex. The dependence of this rate on DNA concentration is not as steep as in the case of A2B2. At the DNA concentrations used in this experiment the A59B2-DA-CFX rate seemed to reach a plateau value which approaches the maximum A2B2-DA-quinolone rate.

We investigated the effect of DNA length on the quinolone rate. Fragments in the range 20–198 bp based on the preferred cleavage site of pBR322 were constructed as described under “Experimental Procedures.” Large DNA fragments, such as the...
198- and 147-bp fragment, when incubated with A₂B₂ and CFX, showed the quinolone characteristic rate at stoichiometric ratios of enzyme:DNA (Fig. 3A). In the case of smaller size fragments, such as a 90-mer or a 80-mer, higher concentrations of DNA were required for reaching the maximum rate. We found that DNA fragments as short as 20 bp are sufficient for inducing the quinolone-characteristic rate (Fig. 3B). The ability of short fragments to form the enzyme-DNA-quinolone complex was observed for both A₂B₂ and A₅₉₂B₂ (Fig. 3B). Again the concentration of 20-mer or 40-mer required for reaching the maximum quinolone rate was higher than stoichiometric.

Conversion to the Quinolone-characteristic Rate Monitors Enzyme-Drug Complex Formation—DNA gyrase and linear pBR322 were incubated with increasing concentrations of CFX (1 nM to 10 μM) and the ATPase rate was measured. The rate of hydrolysis was reduced from 0.98 s⁻¹ in the absence of CFX to 0.6 s⁻¹ at the highest drug concentration (Fig. 4A). We fitted these data to a rectangular hyperbola (see Equation 29 in the “Appendix”) and estimated the inhibitory effect of CFX. An inhibition constant (Kiₚ) was defined as the drug concentration at which half-maximal conversion to the quinolone rate occurs.

This constant was measured to be Kiₚ = 31 ± 5 nM.² As seen before, A₅₉₂B₂ does not have a DNA-dependent ATPase activity, while in the presence of quinolones it exhibits the quinolone-dependent ATPase activity. In these experiments, the concentration of the enzyme-DNA complex was chosen such that the formation and dissociation of the complex was observed in a 10-minute period. The ATPase reaction was initiated by adding ATP to the complex, and the rate of hydrolysis was monitored over a 10-minute period. The turns-over numbers were then estimated relative to the control sample and displayed versus time. The times at the middle of each 10-minute period were used as time points for each of these rates. The apparent dissociation rate constant was determined by fitting the results to a simple exponential equation.

² The gyrase concentration present in this determination (20 nM) is too close to the measured value of Kᵣ and at low CFX concentrations the concentration of added drug would not be equal to the concentration of free drug in solution. For this, the above results are better interpreted using Equation 34 which takes into account this effect (see “Appendix”). Analysis of the above data with Equation 34 yielded a value of kᵣ = 8.4 ± 1.3 nM.
characteristic rate. When the same experiment was performed with the A59B2 complex the rate increased with increasing concentrations of drug (Fig. 4A, inset). The quinolone-induced rate reached a maximum at \( -0.5 \text{s}^{-1} \). The A59B2-DNA-quinolone complex appeared to be significantly less stable than the respective A2B2 complex requiring much higher concentrations of the drug for its formation. The inhibition constant for this complex was determined to be \( K_i = 36 \pm 8 \text{mM} \). As seen previously, the impaired affinity of short DNA fragments for gyrase requires higher DNA concentrations for the formation of the complex. We reported earlier that the affinity of A59B2 for DNA is approximately 10 times lower than the A2B2 complex (24). Therefore, the result obtained with A59B2 is likely to be a manifestation of the weaker stability of the A59B2-DNA complex.

We studied the dissociation of quinolones from the enzyme-drug complex. The gyrase-DNA-quinolone complex was formed and the enzyme-DNA complexes were separated from the free drug by gel filtration through a spin column. The enzyme complex was then introduced into an ATPase reaction and the rate was monitored. Under these conditions the rate of ATP hydrolysis reverted from the quinolone rate to the drug-free one. The rate of hydrolysis was plotted against time and this plot was used to estimate the dissociation rate constant of the drug from the complex (Fig. 4B). The apparent dissociation rate constant was measured to be \( k_{\text{off}} = 3 \times 10^{-4} \text{s}^{-1} \). As we will discuss later, the interaction of quinolones with gyrase appears to involve a number of different steps. Therefore, \( k_{\text{off}} \) is an observed dissociation rate constant representing the overall dissociation process. The value of \( k_{\text{off}} \) determined by this method represents a lower limit in the apparent dissociation rate since the drug was not fully removed from the reaction after it had dissociated from the complex. Rebinding would result in a slower apparent dissociation phase.

The Interaction of Gyrase with Small DNA Fragments Is Slow—We examined the time dependence of the appearance of the quinolone-characteristic ATPase rate. With large fragments (198 and 147 bp) or pBR322 DNA the interaction between the enzyme-DNA complex and the drugs was completed quickly after the addition of the drug so no change in the ATPase rate could be observed (data not shown). When we studied the time dependence of the interaction of gyrase with a 40-mer we found that the appearance of the quinolone characteristic rate was a slow process (Fig. 5). Similar results were found for a 20-bp fragment. This phenomenon was common for both A2B2 and A59B2 and the appearance of the quinolone rate showed the same time dependence for both complexes (data not shown). We measured the rate of ATP hydrolysis over a period of 3 h and plotted the values against time (Fig. 5). From this plot we could estimate the apparent rate of the formation of the enzyme-drug complex by fitting to a simple exponential. The half-life of the conversion to the quinolone complex was \( -20 \text{min} \) at 300 nM 40-mer and 20 nM A59B2. When the concentration of the 40-bp fragment was varied (50–300 nM) the apparent rate of complex formation increased with DNA concentration (Fig. 5). However, the relationship between rate of complex formation and DNA concentration was not linear. A 3-fold increase in DNA concentration, from 100 to 300 nM, resulted only in 50% increase in the rate of complex formation, from \( 4 \times 10^{-4} \text{s}^{-1} \) to \( 6 \times 10^{-4} \text{s}^{-1} \).

Quinolone Binding Does Not Correspond to the Formation of the Cleaved DNA Complex—We investigated the time dependence of the conversion to the quinolone ATPase rate and that of drug-induced DNA cleavage using linear or negatively supercoiled pBR322 as substrate and concentrations of CFX in the range 50 nM to 15 \text{\mu M}. Conversion to the quinolone character-
fied and plotted against time (Fig. 6B). The plot is typical of a reaction proceeding through an intermediate product. We can describe the cleavage reaction using the following simple scheme,

\[
\text{Closed circular DNA} \rightarrow \text{singly nicked DNA} \rightarrow \text{doubly nicked DNA}
\]  
(Eq. 2)

where \( k_1 \) and \( k_2 \) are the apparent rate constants for the formation of the singly and doubly nicked DNA products, respectively. As seen in Fig. 6A only a small population of enzyme-DNA complexes was in the cleaved state at the end of the reaction. The concentration of each of these species at any time is described by the following equations,

\[
[\text{ccDNA}] = [\text{ccDNA}]_0 e^{-k_1 t}
\]  
(Eq. 3)

\[
[\text{snDNA}] = [\text{ccDNA}]_0 \frac{k_1}{k_2-k_1} (e^{-k_1 t} - e^{-k_2 t})
\]  
(Eq. 4)

\[
[\text{dnDNA}] = [\text{ccDNA}]_0 \left( 1 - \frac{k_2 e^{-k_2 t} - k_1 e^{-k_1 t}}{k_2 - k_1} \right)
\]  
(Eq. 5)

\[
[\text{snDNA}] + [\text{dnDNA}] = [\text{ccDNA}]_0 (1 - e^{-k_2 t})
\]  
(Eq. 6)

The abbreviations ccDNA, snDNA, and dnDNA represent the closed circular, singly nicked, and doubly nicked forms of DNA described in Equation 2. \([\text{ccDNA}]_0\) is the concentration of the cleavage-proficient gyrase-quinolone complexes at the beginning of the reaction. By fitting the data obtained to the above kinetic model we were able to determine the values of the two apparent rate constants. The apparent rate constant for the first cleavage event was readily measured from Equation 6 to be \( k_1 = 1.6 \pm 0.1 \times 10^{-2} \text{ s}^{-1} \). However, the apparent rate constant for the second step could not be uniquely defined when \( k_1 \) was fixed at the above value. For this reason, the interaction was simulated using the program KFitSim (D. Thomas and N. Millar) and the value of \( k_2 \) giving the best fit was determined to be \(-1.9 \times 10^{-2} \text{ s}^{-1}\).

We studied the cleavage reaction in the presence of ATP (Fig. 6C). In this experiment the fast binding and inhibition of gyrase by the drugs was clearly evident; the strand-passage reaction was blocked immediately after addition of the drug and no supercoiling was observed (data not shown). DNA cleavage was again slow and proceeded via a singly nicked intermediate as seen previously. The relative amounts of the three DNA species were quantified and the apparent rate constants for the

![Fig. 6](image-url)

**Fig. 6.** DNA cleavage is a slow reaction proceeding via two single-strand scission events. Cleavage experiments were performed as described under “Experimental Procedures.” A, typical experiment showing the cleavage of relaxed pBR322 at 5 \( \mu \text{M} \) CFX in the absence of ATP. The 1% agarose gel contains 3 \( \mu \text{g/ml} \) chloroquine. B, the intensity of the nicked and linear bands was quantified and the extent of single- or double-stranded cleavage caused by the enzyme-quinolone complex was estimated. The results are plotted as percentage of maximum total cleavage. Filled squares (■) indicate the singly nicked product, open circles (○) represent the doubly nicked product, and filled circles (●) represent the sum of the two products. C, the results of a similar experiment carried out in the presence of 2 mM ATP and 5 \( \mu \text{M} \) CFX. D, the enzyme-DNA complex was incubated with 2 mM ADPNP for 1 h and then 500 nM CFX was added and the cleavage reaction was monitored with time. The results were fitted to Equations 4–6 by allowing both rate constants to fluctuate.
two cleavage steps were determined. The cleavage reaction in the presence of ATP was faster than in the absence of the nucleotide. At 5 μM CFX, the values of the rate constants for the two cleavage steps were $k_1 = 2.2 \pm 0.5 \times 10^{-3} \text{ s}^{-1}$ and $k_2 = 4.2 \times 10^{-8} \text{ s}^{-1}$. These rates are faster than those observed in the absence of nucleotide at the same drug concentration (Fig. 6 A and B) suggesting that the presence of the nucleotide increases the rate of the reaction. We examined the cleavage reaction in the presence of ATP over a range of drug concentrations. It was found that the rate of cleavage increased with quinolone concentration. Values for $k_1$ were determined to be 2.5 ± 0.8 and 3.8 ± 0.5 × 10⁻³ s⁻¹ at 10 and 15 μM CFX, respectively, while $k_2$ increased to ~5.3 and ~7.1 × 10⁻³ s⁻¹ at these drug concentrations. A similar investigation was carried out in the presence of the non-hydrolyzable ATP analog, ADP·PNP. The two steps of the cleavage reaction were again apparent in the presence of the nucleotide analogue (Fig. 6D). The rate constants representing the two cleavage steps were also dependent on drug concentration and at 0.5 μM CFX were determined to be $k_1 = 7.5 \pm 0.5 \times 10^{-4} \text{ s}^{-1}$ and $k_2 = 3.2 \times 10^{-8} \text{ s}^{-1}$.

**DISCUSSION**

*The Kinetics of ATP Hydrolysis—* Using limited proteolysis we identified two conformational states of DNA gyrase in the presence of quinolone drugs (complex III and complex IV) and suggested that ATP hydrolysis in this case proceeds through an alternative pathway involving complexes III and IV (1). We have now investigated the kinetic characteristics of hydrolysis by the drug-bound complex, which can be described by Equation 1. Essentially the two nucleotide-binding steps are represented by the respective equilibrium dissociation constants, $K_{D1}$ and $K_{D2}$, and the hydrolysis/product release step with the apparent first-order rate constant $k_s$. Drug binding reduces the rate of the overall hydrolysis step ($k_s$) by a factor of ~2. The equilibrium dissociation constants determined by this method should be treated as estimates as the data are inadequate to uniquely define these values. Nevertheless, the results suggest that the two successive nucleotide binding steps exhibit positive cooperativity. By contrast, in the case of the drug-bound enzyme, cooperativity seems to be less pronounced. This effect can be rationalized on the basis of the conformational change induced by the drug. For cooperativity to take place a mechanism of allosteric interaction must operate between the two GyrB proteins. As the conformational change observed by proteolysis has its major effect on the C-terminal domain of GyrB (1), it is not difficult to imagine how such a change in protein conformation could affect allosteric communication between the two subunits.

*The DNA Dependence of the Quinolone Characteristic ATPase Rate—* We studied the DNA dependence of the formation of the gyrase-DNA-quinolone complex by looking at the conversion of the ATPase rate. The formation of the complex was very efficient at low DNA concentrations and reached a plateau when the DNA to enzyme ratio exceeded 200 bp per gyrase molecule. The footprint of gyrase is ~130 bp (15). Nevertheless, two enzyme molecules that are bound at sites that are separated by a stretch of DNA smaller than the footprint of the protein exclude this part of the DNA from being bound, so that the effective length of DNA occupied by a DNA-binding protein is much larger than its footprint (26). Therefore, the “critical” concentration of ~200 bp per enzyme, for the drug-bound complex, represents here the minimum amount of DNA required for all the gyrase molecules to be bound. These results indicate that at low DNA concentration the formation of the enzyme-DNA-drug complex is limited by the amount of DNA present in the reaction and as soon as there is enough DNA for all the enzyme molecules to be bound, the maximum rate is reached. The fact that the quinolone rate plateaus at this critical DNA concentration suggests that the gyrase-DNA-quinolone complex does not form only at certain preferred sites on DNA. If this was the case, a lot more DNA would have been required for the ATPase rate to level out. In contrast to quinolone-induced DNA cleavage that takes place at preferred DNA sequences (27–29), any sequence of DNA long enough to accommodate a gyrase molecule can form the enzyme-DNA-quinolone complex. The dependence of the formation of the enzyme-DNA complex on DNA concentration is steeper in the presence of the drug. This can be explained in terms of the higher stability of the quinolone-gyrase-DNA complex as opposed to the drug-free one. Being that the footprint of A59,B2 is much smaller than that of full-length gyrase (24), it would be expected that the rate of this complex in the presence of the drugs would have reached a plateau at DNA concentrations lower than those required in the case of the A59,B2-quinolone complex. As we will discuss below the stability of the A59,B2-DNA-quinolone complex is impaired due to the weaker affinity of this enzyme for DNA. The requirement for higher DNA concentrations for reaching the maximum ATPase rate in this case is probably a manifestation of this poor stability.

*The Effect of DNA Length—* We found that a DNA fragment as short as 20 bp is sufficient for inducing the quinolone-characteristic ATPase rate. This is in agreement with the results of Cove et al. (30) and Gminder et al. (31) who found that gyrase can cleave DNA fragments as small as 20 bp in the presence of quinolones. DNA fragments smaller than 100 bp were shown to be unable to stimulate efficiently the ATPase of drug-free gyrase and when they did so, the need for DNA binding at two sites on the enzyme was evident (23). By contrast, the results obtained here with the 20-bp fragment suggest that DNA wrapping or occupation of two sites is not required for the induction of the quinolone complex characteristic rate.

The affinity of the enzyme for DNA seems to be an important factor affecting the stability of the gyrase-quinolone complex. First, induction of the quinolone characteristic rate in the presence of small DNA fragments (<100 bp), that are known to have reduced affinity for the enzyme (23), requires much higher DNA concentrations than when larger (>147 bp) fragments were used (Fig. 3, A and B). Second, deletion of the 33-kDa domains in the case of A59,B2 results in significant reduction in the ability of this enzyme to bind DNA (24). When we looked at the appearance of the quinolone characteristic rate in the case of A59,B2 we found that the A59,B2-DNA-quinolone complex requires much higher concentrations of DNA and drug for its formation than full-length gyrase (Figs. 2 and 4B). We reported above that gyrase requires approximately 200 bp of linear DNA for quinolone complex formation, which appears to contradict the requirement for only 20 bp found with short fragments. However, being that complexes formed with small DNA fragments are less stable than the complexes formed with larger fragments, it seems reasonable that although only 20 bp are sufficient for complex formation; this complex is not stable enough to be the major species when linear pBR322 DNA is used.

*The Gyrase-Quinolone Complex—* Taking into account the association of the enzyme-DNA complex and the slow cleavage step seen under “Results,” the formation of the gyrase-quinolone complex can be described by the following simple equation,

\[ K_D E + DNA \rightarrow E \cdot DNA + Q \rightarrow E \cdot DNA \cdot Q \rightarrow E \cdot DNA \cdot Q^* \]  

(Eq. 7)

In this equation, $K_D$, $K_Q$, and $K_C$ describe the apparent equilibrium dissociation constants for the DNA binding, quinolone
binding, and DNA cleavage steps, respectively. E-DNAQ and E-DNAQ* represent the drug-bound complex in the DNA ligated and cleaved form, respectively. A similar scheme has been proposed by Scheirer and Higgins (25). In this case only a single form of the enzyme-DNA-quinolone complex was proposed. We believe we have evidence for the existence of at least 2 distinct quinolone-bound complexes.

For simplicity, the binding of quinolones to the enzyme-DNA complex is represented in Equation 7 by a single step although the interaction is clearly more complicated. Although no definitive experimental evidence exists for the exact stoichiometry of the complex, recent data suggest that this is probably two quinolone molecules per enzyme-DNA complex (1, 11). In the recent crystal structure of GyrA59 the known quinolone-resistance mutations in GyrA are located in a discrete region in the proximity of the active site tyrosine (32). This region probably represents part of the quinolone-binding site in the enzyme-DNA complex; one such region is present in each GyrA59 monomer. Therefore, we expect that binding of the drugs would include at least two steps and that cooperativity might exist between these two binding events. In the above scheme, \( K_Q \) represents the apparent equilibrium dissociation constant describing the overall quinolone binding process. We showed in this paper that DNA cleavage is a slow reaction that consists of two independent cleavage events. Therefore, \( K_C \) here is the apparent equilibrium constant for the overall cleavage process. We should stress here that Equation 7 represents an equilibrium and E-DNAQ* (the cleaved complex) is not an end-product. The above reaction is fully reversible as indicated by the ability of the gyrase-DNA complex to revert back to the quinolone-free ATPase rate once the drug is removed (Fig. 4C). A similar experiment to the one described in Fig. 4C, where drug-induced DNA cleavage was monitored instead of ATPase, also revealed that cleavage is fully reversible upon drug dissociation.\(^3\) Based on the above scheme, a steady-state equation can be derived that relates the rate of ATP hydrolysis to the formation of the gyrase-quinolone complex (see “Appendix”). In deriving this equation we consider that all quinolone-bound complexes, irrespective of the cleavage-religation state of the DNA, exhibit the quinolone characteristic rate. In support of this we found that, first, the quinolone-induced rate in the case of short fragments containing a preferred cleavage site (147, 198, 90, and 80 bp fragments) is similar to the rate exhibited by the gyrase-quinolone complex formed on linear pBR322 (147). Second, studying the time dependence of cleavage we found that the quinolone characteristic rate appeared immediately after the addition of the drug and remained constant throughout the slow process of DNA cleavage. Third, when the dependence of the quinolone-induced rate on DNA concentration was investigated we determined an inhibitory constant \( K_{iq} \) as the concentration of quinolone required for half-maximal conversion of the ATPase rate. The relationship between \( K_Q \) and the apparent equilibrium association constant for the enzyme-quinolone complex \( K_Q \) can be determined from the above steady-state equation (see “Appendix”),

\[
K_{iq} = \frac{1 + [DNA]}{1 + \frac{1}{K_C}} K_Q
\]

(Eq. 8)

In the experiment described in Fig. 4A we studied the quinolone concentration dependence of the interaction of \( \lambda_2B_2 \) with linear pBR322. Given the experimentally determined value of the equilibrium constant for the binding of gyrase to DNA (\( K_D \approx 0.5 \times 10^{-9} \text{ M} \)) and the concentrations of enzyme and DNA present, most of the enzyme would be in the DNA-bound form. Therefore, \( [E]/[E-DNA] = K_D/[DNA] \ll 1 \). Examination of the cleavage reaction of gyrase with pBR322 at conditions of full inhibition of the enzyme supercoiling activity reveals that the amount of enzyme-quinolone complexes that have induced DNA cleavage is much lower than the amount of enzyme molecules present in the reaction (Fig. 6A).\(^4\) Therefore, in this case, \( [E-DNA]*]/[E-DNA-Q] = 1/K_Q < 1 \). Under these conditions Equation 8 becomes,

\[
K_{iq} = K_Q
\]

(Eq. 9)

Thus, in the particular case of the interaction of \( \lambda_2B_2 \) with linear pBR322, \( K_Q \), reflects the apparent equilibrium constant for the dissociation of the enzyme-quinolone complex. Clearly this is not true in the case of A59B2, where the affinity of the complex for DNA (\( K_Q \)) is weaker (24). Using the same system (\( \lambda_2B_2 \) and linear pBR322) we found that the apparent dissociation rate constant for this complex is \( k_{off} = 3 \times 10^{-4} \text{ s}^{-1} \) (Fig. 4B). From the values of the equilibrium inhibitory constant, \( K_{iq} \), and the observed dissociation rate constant, \( k_{off} \), we can estimate the overall association rate constant of the formation of the gyrase-linear DNA-quinolone complex \( k_{on} \) to be at least \( 10^4 \text{ M}^{-1} \text{ s}^{-1} \).

The Slow Interaction of Gyrase with Short DNA Fragments—When we studied the interaction of gyrase with small DNA fragments we found that the conversion to the quinolone-characteristic ATPase rate was slow. We consider this to be direct evidence for the slow formation of the enzyme-cleaved DNA complex. In the presence of long DNA fragments, conversion to the quinolone-characteristic rate is immediate since both the covalent and noncovalent enzyme-DNA-quinolone complexes are very stable and the conversion of one complex to the other cannot be observed as they both exhibit the same rate of hydrolysis. However, with short fragments the noncovalent complex is very unstable and the appearance of the quinolone characteristic rate depends mainly on the formation of the stable covalent complex. To describe these results, a pre-steady state equation was derived based on Equation 7. In this model, DNA cleavage is considered to be rate-limiting step of the reaction and the first two steps of this equation are considered to be significantly faster so that equilibrium between them is always established (see “Appendix”). This equation takes into account the effect of DNA concentration on the rate of complex formation and explains the observation that their relationship is not linear (see “Results” and factor \( F \) in Equation 43 in the “Appendix”). Application of this equation to the data obtained in these experiments allows us to determine values for the apparent equilibrium constant for DNA cleavage \( K_{C} \) and the apparent rate constants \( k_{c,1}, k_{c,1} \) for the interconversion.

\(^3\) C. Willmott and A. Maxwell, unpublished results.

\(^4\) S. C. Kampranis, unpublished observations.
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FIG. 7. A scheme describing the cleavage reaction of DNA gyrase. DNA-free gyrase is represented as AB-BA and the enzyme-DNA complex is represented as E-E to indicate the dyadic symmetry of the complex. The star sign (*) indicates single-strand DNA cleavage, while Q indicates a quinolone molecule. The vertical pathway on the left represents the cleavage-religation equilibrium in the absence of the drug. Thus, $E-E^*$ and $E-E^*$ indicate spontaneous cleavage at one or both strands, respectively. Similarly, $E-E^*$ represents the complex where one strand has undergone spontaneous cleavage and the other quinolone-induced cleavage, while $QE-EQ^*$ is the complex where drug is bound at both halves of the molecule but only one of the strands is cleaved. The scheme on the left indicates the DNA product that each of these complexes would give upon treatment with SDS and protease K.

between the cleaved and ligated forms of the enzyme-DNA-quinolone complex. Under these conditions, $K_c$ had an estimated value of $\sim 5.9 \times 10^{-2}$ while the apparent rate constant for DNA cleavage $k_{C-1}$ was found to be $\sim 2.2 \times 10^{-3}$ s$^{-1}$ ($k_{C-1} \sim 1.3 \times 10^{-4}$ s$^{-1}$). This ($k_{C-1}$) is in good agreement with the values of the apparent rate constants ($k_1$ and $k_2$) obtained for the two successive cleavage events, although the difference in the substrate used does not allow for further comparison. The value of the equilibrium constant $K_c$ suggests that, in this particular situation, most of the complexes in equilibrium would be in the cleaved state ($E$-DNA-$Q^*$). This is in agreement with experimental data on the cleavage of this site. Cleavage of the 147-bp fragment used in this study (which is centered around the same preferred cleavage site as the 20- and 40-bp fragments) is full at stoichiometric concentrations of gyrase and 25 μM CFX (data not shown). However, the fact that the gyrase-quinolone complex can form on different DNA sequences but only a number of these complexes would reveal DNA cleavage (this work and Refs. 27–29), suggests that the state of the cleavage-religation equilibrium ($K_c$) depends on the sequence of the DNA segment on which the complex is formed.

Quinolone-induced DNA Cleavage Consists of Two Separate Scission Events—From the results presented in this paper it is clear that quinolone binding and DNA cleavage are two different processes. Drug binding is quite fast ($k^{obs}_{dna} > 10^4$ M$^{-1}$ s$^{-1}$) and largely independent of DNA sequence, while drug-induced cleavage is relatively slow and occurs with different efficiency at different DNA sites. The DNA cleavage reaction appears to consist of two independent cleavage steps. Examination of time courses of quinolone-induced cleavage under various conditions revealed that double-stranded cleavage proceeds by two successive scission events, one on each strand of the DNA substrate. This resulted in the appearance of both singly and doubly nicked DNA species in the process of the cleavage reaction (Fig. 6). (Investigation of the cleavage reaction of eukaryotic topoisomerase II in the absence of drugs produced similar results, suggesting that double-stranded DNA cleavage in the absence of the drugs is also a two-step process (33).) The overall interaction leading to DNA cleavage can be summarized in the scheme shown in Fig. 7. By measuring the concentration of the different cleaved DNA species we were able to determine rate constants representing these two cleavage events. Due to the complicated nature of this interaction the constants determined by this method, $k_1$ and $k_2$, represent apparent rate constants for the conversion between the three DNA species in Fig. 7, rather than any specific pathway in this scheme. We found that the value of these rate constants was dependent on quinolone concentration. From the scheme in Fig. 7 the involvement of quinolone-dependent steps in the overall interaction is evident. The nature of this interaction suggests that cooperativity could play an important role in the process of drug binding. Moreover, the possibility of a stoichiometry of more than two quinolone molecules per complex or the existence of different modes of drug binding cannot be ruled out. Although these experiments were performed under conditions where only one cleavage event occurs per plasmid, this does not necessarily involve the same site for all the enzyme-DNA-quinolone complexes in the reaction. Indeed when a similar experiment was performed using linear pBR322 a number of sites appeared to be cleaved more efficiently than others (data not shown). Therefore the rates observed here represent the overall result of DNA cleavage events occurring at a number of different complexes which are likely to exhibit different kinetic parameters.

Despite these complexities, a number of conclusions can be drawn from these results. First, cleavage on one DNA strand seems to have a positive effect on the cleavage of the other strand. Being that the first cleavage event has the possibility to occur in either DNA strand while the second step could only occur in the strand that has not been already cleaved, the rate constant describing the first step of the reaction should be divided by a factor of 2 in order to accurately represent this interaction. This makes the second cleavage step from 2 to 8 times faster in all the cases studied in these experiments. Second, ATP appears to increase the rate of the cleavage reaction. Comparison of the rate of ATP hydrolysis in the presence
of the drugs (0.6 s⁻¹) with the rate of cleavage in the presence of the nucleotide (2.2 × 10⁻³ s⁻¹) suggests that ~300 ATP molecules have been hydrolyzed by the time one cleavage event has been completed (the numbers quoted here reflect the situation at 5 μM CFX where inhibition of the enzyme is complete and fast). This suggests that during that time the gyrase-quinolone complex interconverts between the conformations of complexes III and IV undergoing the uncoupled cycle of ATP hydrolysis suggested in the previous paper (1). It is possible that the deformation imposed on the enzyme-bound DNA during this interchange between conformational states plays an important role in quinolone-induced DNA cleavage.

A Model for the Action of Quinolones—Based on the results of this work, a model can be devised to describe the interaction of quinolones with the gyrase-DNA complex. Examination of the recent crystal structure of GyrA59 reveals that a certain amount of unwinding may occur upon binding of the DNA to gyrase (32). It can therefore be envisaged that formation of the gyrase-DNA complex results in the deformation of the part of the DNA that is bound across the active site so that a “DNA bubble” is formed (Fig. 8). Quinolones bind more favorably to negatively supercoiled or single-stranded DNA (8, 9). Moreover, binding of the quinolones to the gyrase-DNA complex formed with negatively supercoiled DNA is stronger than when it takes place in the drug-free enzyme (complex I). In certain cases, when this is supported by the DNA sequence bound at the active site, the deformation of the DNA molecule would be such that the cleavage-religation equilibrium would be shifted to the cleaved form, and DNA cleavage would be revealed in the presence of a denaturant.

We found that the quinolone-induced cleavage reaction is slow. By contrast the Ca²⁺-induced cleavage reaction is much faster. We believe that Ca²⁺ ions act by shifting the normal (complex I) cleavage-religation equilibrium to the cleaved form without this being a result of a profound effect on the conformation of the complex (1). This suggests that the cleavage-religation equilibrium in the absence of the drugs is relatively fast, with the complex predominantly in the ligated form. In contrast, the binding of quinolones alters the enzyme conformation around the active site and disfavors the cleavage reac-
tion. Drug-induced cleavage is therefore a slow reaction that occurs only when the base sequence of the DNA in the active site is appropriate and is associated with an even slower relaxation step. We found that DNA cleavage proceeds via two scission events. Cleavage on one DNA strand may allow the other strand to deform more readily thus making the second cleavage event faster. It has been shown that ATP or ADPNP alter the efficiency and site specificity of quinolone-induced cleavage (28, 29). This could be explained by a mechanism where different DNA sequences would be subjected to different structural deformations when the enzyme is in the complex IV state. Therefore, some sites would be deformed in a way that prevents DNA cleavage and thus being protected while others would have resumed a conformation that supports the cleavage reaction.

Conclusions—Quinolone binding is followed by a conformational change in the gyrase-DNA complex that is responsible for the inhibition of the enzyme. When the complex is in the quinolone-trapped state, it can hydrolyze ATP with a characteristic rate. We have studied this quinolone-characteristic ATPase rate and used it as a sensitive tool for monitoring the formation of the gyrase-quinolone complex. We found that binding of the drugs to the gyrase-DNA complex is a relatively fast reaction while DNA cleavage is a subsequent slow step. The cleavage reaction consists of two scission events, one in each DNA strand. Based on these results we suggest that DNA cleavage is the result rather than a prerequisite of quinolone binding.

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APPENDIX

The steady-state equation describing ATP hydrolysis is derived based on the following model,

\[ \text{E} + \text{ATP} \rightleftharpoons \text{E} \cdot \text{ATP} \rightleftharpoons \text{E} \cdot 2\text{ADP} + 2\text{P}, \]

(Eq. 10)

In steady state,

\[ K_{a1} = \frac{[\text{E}] [\text{ATP}]}{[\text{E} \cdot \text{ATP}]}, \]

(Eq. 11)

\[ K_{a2} = \frac{[\text{E} \cdot \text{ATP}] [\text{ATP}]}{[\text{E} \cdot 2\text{ADP}][\text{ATP}]} \]

(Eq. 12)

and,

\[ [\text{E}]_0 = [\text{E}] + [\text{E} \cdot \text{ATP}] + [\text{E} \cdot 2\text{ATP}], \]

(Eq. 13)

where \([\text{E}]_0\) is the total concentration of enzyme in the reaction. From Equations 11–13, Equations 11–13 \(\Rightarrow [\text{E} \cdot 2\text{ATP}] = \frac{[\text{E}] [\text{ATP}]}{[\text{ATP}] + K_{a2}[\text{ATP}] + K_{a1} K_{a2}} \)

(Eq. 14)

The rate of ATP hydrolysis \((v)\) is,

\[ v = \frac{d[\text{ADP}]}{dt} = \frac{d[\text{P}]}{dt} = 2k_3 [\text{E} \cdot 2\text{ATP}], \]

(Eq. 15)

From,

\[ v = \frac{d[\text{ADP}]}{[\text{E}]_0} = \frac{d[\text{ADP}]}{[\text{E}]_0} = \frac{[\text{E}]_0 [\text{ATP}]^2}{(2k_3)[\text{ATP}] + K_{a2} [\text{ATP}] + K_{a1} K_{a2}} \]

(Eq. 17)

The steady-state equation describing the binding of quinolones is derived from the following scheme,

\[ \text{E} + \text{DNA} \rightleftharpoons \text{E} \cdot \text{DNA} + Q \rightleftharpoons \text{E} \cdot \text{DNA} \cdot Q \rightleftharpoons \text{E} \cdot \text{DNA} \cdot Q^* \]

(Eq. 18)

At equilibrium,

\[ K_D = \frac{[\text{E}] [\text{DNA}]}{[\text{E} \cdot \text{DNA}]}, \]

(Eq. 19)

\[ K_Q = \frac{[\text{E} \cdot \text{DNA}] [\text{Q}]}{[\text{E} \cdot \text{DNA} \cdot \text{Q}]}, \]

(Eq. 20)

and

\[ K_C = \frac{[\text{E} \cdot \text{DNA} \cdot \text{Q}]}{[\text{E} \cdot \text{DNA} \cdot \text{Q}^*]}, \]

(Eq. 21)

\[ [\text{E}]_0 = [\text{E}] + [\text{E} \cdot \text{DNA}] + [\text{E} \cdot \text{DNA} \cdot \text{Q}] + [\text{E} \cdot \text{DNA} \cdot \text{Q}^*] \]

(Eq. 22)

From Equations 19–22,

\[ [\text{E} \cdot \text{DNA} \cdot \text{Q}] + [\text{E} \cdot \text{DNA} \cdot \text{Q}^*] = (1 + 1/K_C) [\text{E} \cdot \text{DNA} \cdot \text{Q}] \]

(Eq. 23)

In the experiments shown in Fig. 4 the concentration of linear pBR322 DNA is constant while the concentration of drug is varied. In this case the observed rate of ATP hydrolysis \((u)\) will be the sum of the rates of the DNA-free enzyme, the DNA-bound enzyme, and the gyrase-quinolone complex.

\[ v = u_E + u_{\text{EDNA}} + u_{\text{EDNAQ}} \]

(Eq. 24)

If \(k_E, k_{E\text{DNA}}, k_Q\) are the turnover numbers of the DNA-free enzyme, the enzyme-DNA complex, and the drug-bound enzyme-DNA complexes, respectively, then

\[ v = k_E [\text{E}] + k_{E\text{DNA}} [\text{E} \cdot \text{DNA}] + k_Q [\text{E} \cdot \text{DNA} \cdot \text{Q}] + [\text{E} \cdot \text{DNA} \cdot \text{Q}^*] \]

(Eq. 25)

Fig. 4A describes the interaction of \(\Lambda_{59} B_2\) with CFX. Due to the high affinity of \(\Lambda_{59} B_2\) for DNA, the concentration of DNA-free enzyme would be very small compared with the concentration of the DNA-bound form (i.e. \(v_E + v_{\text{EDNA}} = v_{\text{EDNA}} = k_{E\text{DNA}}[\text{DNA}])\). In the case of the A59B2 complex (Fig. 4A, inset), linear DNA cannot stimulate the ATPase of this enzyme (24), i.e. \(v_E + v_{\text{EDNA}} = v_E = \kappa_E[E]\). If the turnover number of the drug-free complexes is \(k_E\) (either \(\Lambda_{59} B_2\) or \(A59\;\Lambda B_2\)) then the rate of hydrolysis at any [CFX] would be,

\[ v = \frac{k_E [\text{E}] + [\text{E} \cdot \text{DNA}] + [\text{E} \cdot \text{DNA} \cdot \text{Q}] + [\text{E} \cdot \text{DNA} \cdot \text{Q}^*]}{[\text{E}]_0} \]

(Eq. 26)
From Equations 22 and 24 and Equation 27 becomes,

\[
\frac{v}{[E_0]} = k_b + (k_q - k_b)(1 + 1/K_c)[Q][DNA] + K_q[DNA] + K_cK_D
\]  
(Eq. 28)

Equation 28 can be written in the form of the following rectangular hyperbola,

\[
\frac{v}{[E_0]} = k_b + (k_q - k_b)\frac{[Q]}{1 + K_c[Q]} + \frac{K_q}{1 + K_c[Q]}[DNA]
\]  
(Eq. 29)

The quinolone concentration resulting in 50% inhibition \((K_{q0})\) is therefore,

\[
K_{q0} = \frac{1}{1 + K_c[Q]}K_q
\]  
(Eq. 30)

Because at low quinolone concentrations (close to the concentration of enzyme) the concentration of free drug \([Q]\) cannot be approximated to the total drug concentration \([Q]_t\), the interaction is better described if in Equation 24 \([Q]\) is substituted by \([Q]_t - ([E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+])\). Thus,

\[
[E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+] = \frac{(1 + 1/K_c)[Q] - ([E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+])([DNA][E])}{(1 + 1/K_c)[Q] - ([E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+])} + K_q[DNA] + K_cK_D
\]  
(Eq. 31)

This can be written in the form,

\[
([E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+])^2 - (K_{q0} + [Q]_t + [Q]_t)([E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+] + [Q]_t[E]) = 0
\]  
(Eq. 32)

From the solutions of this equation only one has a finite limit (note that \([E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+]\) cannot exceed \([E]_0\)). This is,

\[
[E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+] = \frac{K_{q0} + [Q]_t + [E]_0 - \sqrt{K_{q0} + [Q]_t + [E]_0^2 - 4[E]_0[Q]}}{2}
\]  
(Eq. 33)

Substituting back to Equation 24,

\[
\frac{v}{[E_0]} = k_b + (k_q - k_b)\frac{[Q]_t}{2K_{q0} - \sqrt{[Q]_t^2 - 4[E]_0[Q]}}
\]  
(Eq. 34)

On the pre-steady state level, \(K_{c} = k_{c,1}/k_{c,1-1}\), where \(k_{c,1}\) and \(k_{c,1-1}\) represent the apparent first-order rate constants for the quinolone-induced DNA cleavage and religation reactions, respectively. Supposing that the DNA and quinolone binding steps are much faster than the DNA cleavage event, as illustrated by experimental data, then the equilibrium involving the first two steps of this reaction would be established very fast compared with any conversion to the cleaved form. Then at any time Equations 19–22 would be obtained. By combining those four equations,

\[
[E\cdot DNA\cdot Q] = \frac{[Q][DNA][E]_0 - [E\cdot DNA\cdot Q^+]}{[Q] + K_q[DNA] + K_cK_D}
\]  
(Eq. 35)

When \([DNA]\) and \([Q]\) are in excess over \([E]_0\), then they can be considered to remain constant throughout the reaction. In this case,

\[
\frac{[Q][DNA]}{[Q] + K_q[DNA]} + K_cK_D = F
\]  
(constant)  
(Eq. 36)

In this case Equation 32 becomes,

\[
\frac{v}{[E_0]} = k_b + (k_q - k_b)\frac{F}{1 + K_c[Q]}
\]  
(Eq. 37)

The rate of formation of the cleaved complex is,

\[
\frac{d[E\cdot DNA\cdot Q]}{dt} = k_{c,1}[E\cdot DNA\cdot Q] - k_{c,1-1}[E\cdot DNA\cdot Q^+]
\]  
(Eq. 38)

This is a linear first-order differential equation whose solution is,

\[
[E\cdot DNA\cdot Q] = e^{-k_{c,1}F[Q] + k_{c,1-1}}[E]_0
\]  
(Eq. 39)

At \(t = 0\), \([E\cdot DNA\cdot Q] = 0\) and from Equation 39,

\[
c = -k_{c,1}F[Q]_0 + k_{c,1-1}
\]

By replacing \(c\) back to Equation 39,

\[
[E\cdot DNA\cdot Q] = \frac{k_{c,1}}{k_{c,1-1}}[E]_0 - e^{-k_{c,1}F[Q] + k_{c,1-1}}
\]  
(Eq. 41)

The total concentration of quinolone bound complexes is,

\[
[E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+] = [E\cdot DNA\cdot Q] + [E\cdot DNA\cdot Q^+]
\]  
(Eq. 42)

Since a short DNA fragment, at these concentrations, is unable to stimulate the ATPase of gyrase (i.e. \(k_{c} = k_{c,1}\)), the rate of ATP hydrolysis at any time would be,

\[
\frac{v}{[E_0]} = k_b + (k_q - k_b)\left(F + (1 + F)\frac{k_{c,1}}{k_{c,1-1} + F + k_{c,1-1}}(1 - e^{-k_{c,1}F[Q] + k_{c,1-1}})\right)
\]

Where \(k_0\) is the turnover number of the drug-free complexes \((k_0 = k_{c,1-1}\)), while \(Q_{0}\) is again the turnover number of the drug-bound complexes.

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