Distinct Effects of the UvrD Helicase on Topoisomerase-Quinolone-DNA Ternary Complexes*

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Quinolone antibacterial drugs target both DNA gyrase (Gyr) and topoisomerase IV (Topo IV) and form topoisomerase-quinolone-DNA ternary complexes. The formation of ternary complexes results in the inhibition of DNA replication and leads to the generation of double-strand breaks and subsequent cell death. Here, we have studied the consequences of collisions between the UvrD helicase and the ternary complexes formed with either Gyr, Topo IV, or a mutant Gyr, Gyr (A59), which does not wrap the DNA strand around itself. We show (i) that Gyr-norfloxacin (Norf)-DNA and Topo IV-Norf-DNA, but not Gyr (A59)-Norf-DNA, ternary complexes inhibit the UvrD-catalyzed strand-displacement activity, (ii) that a single-strand break is generated at small portions of the ternary complexes upon their collisions with UvrD, and (iii) that the majority of Topo IV-Norf-DNA ternary complexes become nonreversible when UvrD collides with the Topo IV-Norf-DNA ternary complexes, whereas the majority of Gyr-Norf-DNA ternary complexes remain reversible after their collision with the UvrD helicase. These results indicated that different DNA repair mechanisms might be involved in the repair of Gyr-Norf-DNA and Topo IV-Norf-DNA ternary complexes.

Topoisomerases are responsible for altering the linking number of DNA. As such, they play essential roles in DNA replication, transcription, and DNA recombination (1). There are two classes of topoisomerases, type I and type II enzymes. Type I topoisomerases alter the linking number in steps of one by breaking one strand of the duplex DNA, passing the other strand through the break, and then resealing the broken strand. On the other hand, type II topoisomerases alter the linking number in steps of two by breaking both strands, passing another segment of the helix through the break, and then resealing the broken strands (1). Both DNA gyrase (Gyr) and topoisomerase IV (Topo IV) are type II topoisomerases.

Quinolones are synthetic antibacterial drugs based on the 4-oxo-1,4-dihydroquinolone skeleton (2). After the discovery of nalidixic acid as an antibacterial agent, successive generations of drugs have brought orders of magnitude increases in antibacterial activity. Quinolone drugs, such as ciprofloxacin, are now one of the most widely prescribed antibacterial drugs. It has been demonstrated that, shortly after the identification of *Escherichia coli* Gyr (3–5), quinolones target Gyr (3, 6) and convert it to a poison that inhibits DNA replication (7). These drugs form a topoisomerase-quinolone-DNA ternary complex. As a result, the covalent topoisomerase-DNA complex (often referred to as the “cleavable complex”) that contains broken DNA strands can persist on the DNA, as if the topoisomerase were trapped in the cleavable complex. Ultimate denaturation or disruption of the topoisomerase in the ternary complex therefore results in the generation of a double-strand break (DSB) and subsequent cell death (8–10). Based on this unique mode of action, this class of topoisomerase inhibitors is often called the “topoisomerase poison.” Some anticancer drugs targeting human type II topoisomerases also convert these enzymes to poisons in the same fashion (11).

The formation of topoisomerase-quinolone-DNA ternary complexes is necessary but not sufficient for the cytotoxicity of topoisomerase poisons. These ternary complexes are normally reversible. In addition to the formation of the topoisomerase-quinolone-DNA ternary complexes, an active DNA transaction is required for the disruption of ternary complexes and the generation of a DSB (8–10). The reconstitution of the collision between a replication fork and a Topo IV-norfloxacin (Norf)-DNA ternary complex has revealed that the collision of a replication fork with a Topo IV-Norf-DNA ternary complex converts the ternary complex to a nonreversible form but does not generate a DSB (12). An additional step, perhaps an aborted repair attempt, is required for the generation of a DSB.

Khodursky and Cozzarelli (13) have recently demonstrated that quinolone drugs poison both Gyr and Topo IV in the same manner and that the cytotoxicity of these drugs correlates with the inhibition of DNA replication. These studies (13) have also shown that the recombinational repair system is the main pathway for the repair of quinolone-induced covalent topoisomerase-DNA complex. Interestingly, the loss of the UvrD function affects Topo IV-targeted cell killing but not Gyr-targeted cell killing. These results suggest that the postreplicative repair system is effective for the repair of ternary complexes formed with Topo IV but is not effective for the repair of ternary complexes formed with Gyr. It is concluded that the UvrD-directed postreplicative repair system can repair Topo IV-quinolone-DNA ternary complexes, but not Gyr-quinolone-DNA ternary complexes, because of the positions of Gyr and Topo IV during DNA replication (13). Gyr acts ahead of the advancing replication forks, and Topo IV acts behind the forks (14, 15). Thus, ternary complexes formed with Topo IV have a greater chance to be repaired by the postreplicative repair system than those formed with Gyr. In addition, the ternary complexes formed with Gyr more frequently collide with the replication forks than those formed with Topo IV to trigger the quinolone-induced cytotoxic events.

We have previously studied the interactions between Topo...
IV-Norf-DNA ternary complexes and various DNA helicases (16). We have found that the collision of the UvrD helicase with a Topo IV-Norf-DNA ternary complex results in the conversion of the ternary complex to a nonreversible form, which seems to be critical for the generation of a DSB. It is suggested that this conversion of Topo IV-Norf-DNA ternary complex to a nonreversible form may be a critical step to initiate the removal of the ternary complex and the repair of the DNA damage by the postreplicative repair system (16).

Here, we further investigated the effects of the UvrD helicase on the topoisomerase-quinolone-DNA ternary complexes formed with either Gyr, Topo IV, or Gyr (A59), a mutant Gyr that does not wrap the DNA strand around itself (17). The ternary complexes formed with either Gyr or Topo IV, but not those formed with Gyr (A59), inhibited the UvrD helicase activity. Collisions of UvrD with these ternary complexes resulted in the formation of a single-strand break (SSB) at small portions of ternary complexes. Interestingly, the majority of Gyr-Norf-DNA ternary complexes remained reversible after their collisions with UvrD, whereas the majority of Topo IV-Norf-DNA ternary complexes was converted to a nonreversible form upon their collisions with UvrD. These results demonstrated that the UvrD helicase could affect the ternary complexes formed with Gyr and those formed with Topo IV in a distinct manner. Furthermore, these results indicated that different DNA repair mechanisms might be involved in the repair of Gyr-Norf-DNA and Topo IV-Norf-DNA ternary complexes.

MATERIALS AND METHODS

DNAs and Proteins—The construction of a recombinant M13 containing a defined Topo IV-binding site (M13-T440) (16) and the preparation of the single-stranded circular DNA of M13-T440 (18, 19) were as described previously. Purified UvrD and the subunits of E. coli Gyr and Topo IV were generous gifts of Kenneth Marians (Memorial Sloan-Kettering Cancer Center). GyrA and GyrB and ParC and ParE proteins were mixed on ice to form active tetramers of Gyr and Topo IV, respectively. Purified UvrD, a gift of Steve Matson (University of North Carolina), was also used. A deletion mutant of gyrA encoding the amino acids from 1 to 523 (17) was generated by PCR and cloned into pET-11c. The DNA sequence analysis confirmed that this deletion mutant of gyrA gene encoded a 59-kDa A. Page 1 of 14650

Partial Duplex DNAs—The preparation of the partial duplex DNA was prepared as described previously (16). Briefly, a 62-nucleotide (nt) oligonucleotide (oligo), T440C, 5'-GCTCGTATCTAGACTCCTAAAAATCA-3' (10) was generated by PCR and cloned into pET-11c. The DNA sequence containing a defined Topo IV-binding site (M13-T440) (16) and the prepa-

RESULTS

Partial Duplex DNAs—One of the basic differences between Gyr and Topo IV is their mode of DNA binding. Gyr is unique among the type II topoisomerases. This enzyme wraps the DNA strand around itself (20–23) and protects about 140 base pairs (bp) of the duplex DNA (21–23). This unique mode of DNA binding enables Gyr to catalyze the supercoiling reaction. In contrast, Topo IV and other type II topoisomerases do not wrap the DNA strand and require shorter DNA fragments for their binding. The footprinting analysis of Topo IV has revealed that
it protects only about 35–40 bp (24). Kampranis and Maxwell (17) have demonstrated that a deletion of the C-terminal DNA-binding domain of the GyrA subunit, GyrA (59), gives rise to an enzyme that does not wrap the DNA strand around itself and that cannot catalyze the supercoiling reaction. Thus, the DNA binding mode of Gyr (A59) is likely to be similar, if not identical, to that of Topo IV (17).

To perform the strand-displacement assay, we prepared two partial duplex DNAs, which differed only by the length of the duplex region (Fig. 1A). T440 DNA was prepared by annealing a 62-nt oligo and labeling the annealed oligo at the 3'-end. As a result, this partial duplex DNA contained a 64-bp duplex region, and a defined Topo IV-binding site (25) was located in the middle of the duplex region (Fig. 1A). We have previously shown that the T440 DNA serves as a good substrate for Topo IV binding (16). It has been shown that many of the binding sites, as Norf-stimulated cleavage sites, of Gyr and Topo IV overlap, although site preferences of these enzymes are distinct (26). Thus, it seemed reasonable to assume that the defined Topo IV-binding site (25) serves as a good binding site for Gyr. However, we expected the 64-bp duplex region in the T440 DNA to be too short for Gyr to bind. Thus, the annealed and labeled oligo in the T440 DNA was further elongated using the Klenow enzyme to an average length of 175 nt with a range of 150–200 nt (Fig. 1B). This partial duplex with an elongated duplex region was referred to as T440E.

The occupancy of the topoisomerase on the DNA substrate is one of the determining factors of the probability of the collision between the UvrD helicase and the topoisomerase-DNA complex. In order to determine the amounts of topoisomerase-DNA complexes formed on the partial duplex DNA, we measured the topoisomerase-catalyzed cleavages in the presence of various concentrations of Norf. The relative amounts of topoisomerase-catalyzed cleavages represent the occupancy of the DNA substrate by the topoisomerase-DNA complex.

The Wrapping of the DNA Strand Prevents Gyr from Binding to a Short Duplex DNA—First, we measured Gyr-, Topo IV-, and Gyr (A59)-catalyzed cleavages using T440 as a substrate.

The T440 DNA contains a 64-bp duplex region including a 40-bp defined Topo IV-binding site (Fig. 1A). Topo IV cleaved the T440 DNA at a unique site, and Norf stimulated Topo IV-catalyzed cleavages (Fig. 2A). It seemed to be an interesting question to ask if Gyr (A59), which does not wrap a DNA strand around itself (17), could bind to a short duplex. To address this question directly, Gyr- and Gyr (A59)-catalyzed cleavages in the presence of various concentrations of Norf were performed in the presence of indicated concentrations of Norf, and the DNA products were analyzed as described under "Materials and Methods." B, the extent of DNA cleavages as a function of the Norf concentration was quantitated using a phosphorimager. Open circles, closed circles, and open squares represent Gyr-, Gyr (A59)-, and Topo IV-catalyzed cleavages, respectively.
Next, we measured the Gyr- and Topo IV-catalyzed cleavages using T440E as a substrate (Fig. 3). All three topoisomerases cleaved the elongated duplex region and generated DNA fragments with various sizes. These cleavages were greatly stimulated by Norf. The pattern of Gyr (A59)-catalyzed cleavages was somewhat similar to that of Topo IV-catalyzed cleavages but not that of Gyr-catalyzed cleavages (Fig. 3). To maximize the formation of covalent topoisomerase-DNA ternary complexes formed, the highest concentrations of Norf and the topoisomerases were used in the following experiments. Under these conditions, more than 80, 80, or 90% of the DNA substrate was bound by at least one molecule of Gyr (Fig. 3, lane 6), Topo IV (Fig. 3, lane 11), or Gyr (A59) (Fig. 3, lane 16), respectively. The elongated duplex region of T440E could provide more than one binding site for each topoisomerase. Thus, the generation of cleaved fragments with various sizes was likely due to the binding of multiple topoisomerases to one DNA substrate and/or the binding of one topoisomerase per DNA molecule at different sites.

**Ternary Complexes Formed with Either Gyr or Topo IV Inhibit the UvrD Helicase Activity**—We have demonstrated that the Topo IV-Norf-DNA ternary complex on T440 inhibits the strand-displacement activities of various DNA helicases including UvrD (16). We have concluded that the Topo IV-Norf-DNA ternary complex blocks the passage of DNA helicases. Because Gyr could form the ternary complex with the T440 DNA (Fig. 2), we examined if the Gyr complex formed at the short duplex region of T440 could inhibit the UvrD-catalyzed strand-displacement activity.

The UvrD helicase activity was assessed in the absence and presence of Gyr, Topo IV, and Norf, using T440 as a substrate (Fig. 4). Under the conditions used, UvrD displaced 78% of the annealed oligo (Fig. 4A, lane 3). Gyr (Fig. 4A, lane 6), Topo IV (Fig. 4A, lane 9), or Norf alone (Fig. 4A, lane 4) did not affect the UvrD-catalyzed strand displacement. The Gyr-Norf-DNA ternary complex reduced the UvrD-catalyzed strand-displacement activity by 25% (Fig. 4A, lane 7), whereas the Topo IV-Norf-DNA ternary complex inhibited the UvrD helicase activity by 67% (Fig. 4A, lane 10). The extent of inhibition by Gyr and Topo IV complexes correlated well with the relative amounts of Gyr (28%) and Topo IV-catalyzed (61%) DNA cleavages (Fig. 2). Thus, the passage of UvrD was blocked when UvrD collided with a topoisomerase-quinolone-DNA ternary complex formed with either Gyr or Topo IV.

Howard et al. (27) have demonstrated that the collision between UvrD and a T4 topoisomerase-m-AMSA-DNA ternary complex results in the generation of an SSB and the release of a broken DNA strand from the ternary complex. To examine if there were any strand breaks at the ternary complexes when UvrD collided with the ternary complexes formed with either Gyr or Topo IV, DNA products were analyzed on polyacrylamide gels (Fig. 4B). Only the DNA strand detected was the intact annealed oligo displaced by UvrD, suggesting that UvrD did not disrupt either Gyr-Norf-DNA or Topo IV-Norf-DNA ternary complex to generate an SSB. One possible explanation of these apparent differences was that, as proposed by Howard et al. (27), the disruption of the ternary complex and the generation of an SSB by UvrD might require the binding of UvrD...
to the partially unwound DNA strand. The annealed oligo may be too short for UvrD to bind when it is partially unwound. Therefore, we further investigated the molecular events during the collision between the UvrD helicase and the ternary complexes formed with either Gyr or Topo IV, and Norf, as indicated, were performed, and the DNA products were analyzed by electrophoresis through 1% agarose gels (A) and 8% polyacrylamide gels (B) as described under “Materials and Methods.” T440E was used as a substrate, and each experiment was duplicated. In the absence of topoisomerase, UvrD-catalyzed strand displacement, either in the presence or absence of Norf, was 64% (±5%). Representative results are shown, but the S.D. was derived from the results of two experiments. Lane 1 in both panels was heat-denatured DNA substrate. Gyr (as tetramer), Topo IV (as tetramer), and UvrD (as monomer) were present at 30-, 30-, and 25-fold molar excess over DNA substrate, respectively. Norf was at 400 M when present. Arrows indicated broken DNA strands released from the ternary complexes.

**FIG. 5. Collisions of UvrD with Gyr-Norf-DNA and Topo IV-Norf-DNA ternary complexes formed with the T440E DNA result in the inhibition of UvrD-catalyzed strand-displacement activity and the generation of strand breaks.** The strand-displacement assay for UvrD in the presence and absence of Gyr, Topo IV, and Norf, as indicated, were performed, and the DNA products were analyzed by electrophoresis through 1% agarose gels (A) and 8% polyacrylamide gels (B) as described under “Materials and Methods.” T440E was used as a substrate, and each experiment was duplicated. In the absence of topoisomerase, UvrD-catalyzed strand displacement, either in the presence or absence of Norf, was 64% (±5%). Representative results are shown, but the S.D. was derived from the results of two experiments. Lane 1 in both panels was heat-denatured DNA substrate. Gyr (as tetramer), Topo IV (as tetramer), and UvrD (as monomer) were present at 30-, 30-, and 25-fold molar excess over DNA substrate, respectively. Norf was at 400 M when present. Arrows indicated broken DNA strands released from the ternary complexes.

UvrD-catalyzed strand-displacement was 67% (Fig. 5A, lane 3). Gyr (Fig. 5A, lane 6), Topo IV (Fig. 5A, lane 9), or Norf alone (Fig. 5A, lane 4) did not affect the activity of UvrD.

We expected both Gyr-Norf-DNA and Topo IV-Norf-DNA ternary complexes to block the UvrD-catalyzed strand-displacement activity. However, when we analyzed the DNA products on 1% agarose gels, we detected the partial displacement of the DNA strand in the presence of either the Gyr-Norf-DNA (Fig. 5A, lane 7) or the Topo IV-Norf-DNA ternary complex (Fig. 5A, lane 10). These apparent displaced fragments (Fig. 5A, lanes 7 and 10) migrated faster than the intact elongated DNA strands (Fig. 5A, lane 1). We suspected that the apparent displaced fragments might be broken DNA strands released from ternary complexes as a result of strand breaks. If there were a DSB at the ternary complex, M13 DNA would become linear, which would change the migration of the substrate DNA. On the other hand, if a strand break were an SSB, the M13 DNA would remain circular and the migration of the substrate DNA would not change. Because the migration of the DNA substrate on the agarose gel did not change (Fig. 5A, lanes 7 and 10), the strand break was likely to be an SSB.

To confirm the formation of an SSB and the release of a DNA strand from the ternary complex, the DNA products were analyzed on 8% polyacrylamide gels (Fig. 5B). Short DNA fragments were generated when UvrD collided with the ternary complexes formed with either Gyr (Fig. 5B, lane 7) or Topo IV (Fig. 5B, lane 10). These results demonstrated that the apparent UvrD-catalyzed strand displacement in the presence of either Gyr-Norf-DNA or Topo IV-Norf-DNA ternary complexes was indeed due to the release of broken DNA strands from the ternary complex as a result of an SSB. Based on the amounts of broken DNA strands released from the T440E DNA (Fig. 5A, lanes 7 and 10), the frequency of the formation of an SSB was estimated as 28 and 15% at the ternary complex formed with Gyr and Topo IV, respectively. It was not clear why strand breaks occurred only at small portions of the ternary complexes. The majority of the ternary complexes formed with either Gyr or Topo IV seemed to retain all DNA strands after their collisions with UvrD. Interestingly, portions of the displaced, intact DNA fragments were shifted (Fig. 5B, lanes 3, 4, 6, and 9). This shift was due to the binding of UvrD, which was abolished by either the heat treatment or the deproteinization by the extraction with phenol/chloroform (data not shown).

**UvrD Converts the Topo IV-Norf-DNA Ternary Complex, but Not the Gyr Norf-DNA Ternary Complex, to a Nonreversible Form—**We have previously shown that the Topo IV-Norf-DNA ternary complex formed with the T440 DNA is converted to a nonreversible form when it collides with UvrD (16). This conversion seems to be critical for the DSB formation, either to trigger the cytotoxic process or to remove the covalent topoisomerase-DNA complex and repair DNA damage. It has been demonstrated that the UvrD-directed postreplicative repair system can effectively repair Topo IV-Norf-DNA ternary complexes but not Gyr-Norf-DNA ternary complexes (13). To examine the consequences of the collisions between UvrD and ternary complexes formed with either Gyr or Topo IV, we assessed the effect of the UvrD helicase on the reversibility of the ternary complexes.

The reversal assay was first performed using T440 as a substrate (Fig. 6A). The Topo IV-Norf-DNA ternary complex formed with T440 was converted to a nonreversible form when it collided with UvrD (Fig. 6A, lane 4). In contrast, the Gyr-Norf-DNA ternary complex remained reversible after its collision with UvrD (Fig. 6A, lane 8). Two independent preparations of Gyr, Topo IV, and UvrD were used, and identical results were obtained (data not shown). These results demon-
stratified that the UvrD helicase affected the ternary complexes formed with Gyr and Topo IV in a distinct manner.

We obtained similar results when T440E was used as a substrate in the reversal assay (Fig. 6B). The majority of Gyr-Norf-DNA ternary complexes remained reversible after their collisions with the UvrD helicase. The amount of the reversed DNA strands in the presence of UvrD (Fig. 6B, lane 4) was less than that in the absence of UvrD (Fig. 6B, lane 2) by 22%. This correlated well with the observation that about 28% of the Gyr-Norf-DNA ternary complexes lost a DNA strand as a result of an SSB (Fig. 5A). In contrast, the Topo IV-Norf-DNA complexes were completely nonreversible after their collision with UvrD (Fig. 6B, lane 8). Only a small portion (15%) of these nonreversible ternary complexes became nonreversible because of the formation of an SSB (Fig. 5A). The majority of nonreversible complexes seemed to retain all DNA strands. Thus, UvrD could convert Topo IV-Norf-DNA ternary complexes, but not Gyr-Norf-DNA ternary complexes, to a nonreversible form. These results suggested that UvrD, and most likely the UvrD-directed repair system, could distinguish ternary complexes formed with Topo IV from those formed with Gyr.

The Gyr (A59)-Norf-DNA Ternary Complex Remains Reversible After Its Collision with UvrD—It is not clear what makes the difference between ternary complexes formed with Gyr and those formed with Topo IV. One obvious possibility is that the Gyr-mediated wrapping of the DNA strand makes Gyr different from Topo IV and other type II topoisomerases. We used Gyr (A59) protein to ask this question directly. If the Gyr-Norf-DNA ternary complex remained reversible after its collision with UvrD because of the Gyr-mediated wrapping of the DNA strand, the Gyr (A59)-Norf-DNA ternary complex should be converted to a nonreversible form. In contrast, if the difference between Gyr and Topo IV is not due to the Gyr-mediated wrapping of the DNA strand, the ternary complex formed with Gyr (A59) should remain reversible.

We performed the reversal assay using Gyr (A59) (Fig. 7). The Gyr (A59)-Norf-DNA ternary complexes remained completely reversible when T440 was used as a substrate (Fig. 7A, lane 4). These results showed that, whether Gyr could wrap the DNA strand around itself or not, the Gyr-Norf-DNA ternary complexes remained reversible after their collisions with UvrD. The Gyr (A59)-catalyzed cleavages were inhibited when UvrD was present in the reaction mixtures (Fig. 7, lane 5).

We further examined the consequences of the collisions between UvrD and the Gyr (A59)-Norf-DNA ternary complex using T440E as a substrate (Fig. 7B). Two distinct DNA products, a short DNA strand (38%) and the intact fragments (62%), were generated after the encounter of UvrD with the Gyr (A59)-Norf-DNA ternary complex (Fig. 7B, lane 4). These results were very similar to those using the wild type Gyr (Fig. 6B). These results demonstrated that the collision between UvrD and the Gyr-Norf-DNA ternary complex did not affect the reversibility of the ternary complex formed with Gyr, even when Gyr could no longer wrap the DNA strand around itself.

The Gyr (A59)-Norf-DNA Ternary Complex Does Not Inhibit UvrD-catalyzed Strand Displacement—To confirm the formation of an SSB at the Gyr (A59)-Norf-DNA ternary complex, we performed the strand-displacement assay using Gyr (A59). First, T440 was used as a DNA substrate (Fig. 8, A and B). Under the condition used, about half of the T440 DNA was occupied by the Gyr (A59)-Norf-DNA ternary complex (Fig. 8A, lane 4). These results were very similar to those using the wild type Gyr (Fig. 7B). These results demonstrated that the collision between UvrD and the Gyr-Norf-DNA ternary complex did not affect the reversibility of the ternary complex formed with Gyr, even when Gyr could no longer wrap the DNA strand around itself.

The Gyr (A59)-Norf-DNA Ternary Complex Remains Reversible After Its Collision with UvrD.
was present, however, in addition to the intact DNA strands displaced by UvrD, a short DNA strand was generated (Fig. 8D, lane 5). We used two preparations of Gyr (A59) and obtained essentially identical results (data not shown). These results showed that an SSB was generated at a portion (58%) of the Gyr (A59)-Norf-DNA ternary complexes formed with T440E and that UvrD could displace some (42%) of the intact DNA fragment even in the presence of the Gyr (A59)-Norf-DNA ternary complex. Thus, the UvrD helicase, upon its collision with the Gyr (A59)-Norf-DNA ternary complex, seemed to be able to force Gyr (A59) to reverse the ternary complex formation and religate the DNA strands.

**DISCUSSION**

Topoisomerases are responsible for unlinking the DNA molecules during DNA replication (1). It has been thought that the topological constraint accumulates as positive supercoils in the unreplicated region. However, recent studies have demonstrated that, as originally proposed by Champoux and Been (28), the topological constraint can take two forms, positive supercoils in the unreplicated region and precatenanes in the replicated region (15, 29, 30). Gyr removes positive supercoils in front of the replication forks to support the nascent chain elongation, whereas Topo IV decatenates precatenanes behind the forks to support the nascent chain elongation and the decatenation of daughter DNA molecules (15).

Gyr binds to the DNA by wrapping a DNA strand around itself, which requires about 140-bp duplex DNA (20–23). Thus, we did not expect Gyr to bind to the T440 DNA, because the duplex region of T440 is only 64 bp long (Fig. 2). A mutant Gyr, Gyr (A59), which does not wrap a DNA strand around itself (17), was used to examine the effect of Gyr-mediated wrapping on the ability of Gyr to bind to a short duplex DNA. Gyr (A59) could bind to T440 as well as Topo IV (Fig. 2). Thus, Gyr-mediated wrapping of the DNA strand prevents Gyr from binding to a short duplex DNA. Furthermore, these results may support the finding that the binding sites of Gyr and Topo IV overlap, although site preference for cleavages is distinct for these enzymes (26). In contrast, yeast topoisomerase II could not bind to T440 in the presence of n-AMSA or etoposide.

Topoisomerases are the cellular targets of some antibacterial agents and potent anticancer drugs (8–10). Both Gyr and Topo IV are the targets of quinolone antibacterial drugs. These drugs form a topoisomerase-quinolone-DNA ternary complex, in which the topoisomerase is trapped as a cleavable complex. These ternary complexes are normally reversible, and the formation of ternary complexes is not sufficient, although necessary, for the cytotoxicity of these drugs. An active DNA trans-action, such as the passage of replication forks, is required to convert a ternary complex to a permanent cytotoxic lesion (8–10). It has been proposed that the collision between a replication fork and a topoisomerase-drug-DNA ternary complex disrupts the ternary complex and generates a DSB. We have previously demonstrated that, in fact, the Topo IV-Norf-DNA ternary complex blocks the replication fork progression in *vivo* and this collision converts the ternary complex to a nonreversible form (12). However, an additional step is required to remove the topoisomerase in the dead-end complex and to generate a DSB (12).

In *E. coli*, although both Gyr and Topo IV are the targets of the quinolone drugs, Gyr becomes the primary target and Topo IV is the secondary target (14). The order of the targets seems to be reversed in *Staphylococcus aureus*, where Topo IV becomes the primary target (31). It is not clear what determines which topoisomerase is the primary target in the cell. One

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possibility is that *E. coli* Gyr is more sensitive to the quinolone drugs than *E. coli* Topo IV. However, this is not likely to be the case. Only a slight difference is found between Gyr and Topo IV when the drug sensitivities of these enzymes are measured *in vitro* (14, 26).

Alternatively, although both topoisomerases are poisoned in the same manner, the ternary complex formed with Gyr is more cytotoxic than that formed with Topo IV. It has been proposed that Gyr becomes the primary target in *E. coli* because of the position of Gyr-Norf-DNA ternary complexes relative to the advancing replication forks (13). Gyr functions in front of the replication forks, whereas Topo IV binds behind the forks during the chromosomal DNA replication (14, 15). Thus, the ternary complexes formed with Gyr collide with the replication forks more frequently than those formed with Topo IV. Another possibility is that the differences in repairing the ternary complexes formed with Gyr and those formed with Topo IV make the Gyr-quinolone-DNA ternary complex more cytotoxic than the Topo IV-quinolone-DNA ternary complex. Let us assume that the same number of ternary complexes is formed with Gyr and Topo IV on the chromosome. If the ternary complexes formed with Topo IV, but not those formed with Gyr, can be repaired efficiently, Gyr-quinolone-DNA ternary complexes become more cytotoxic than Topo IV-quinolone-DNA ternary complexes do. Note that, once the collision takes place between a replication fork and a ternary complex formed with either Gyr or Topo IV, both Gyr-Norf-DNA and Topo IV-Norf-DNA ternary complexes block the replication fork progression (12).

We detected an SSB as a result of the collision between the UvrD helicase and the ternary complex formed with either Gyr...

**FIG. 8.** The ternary complex formed with Gyr (A59) does not inhibit the UvrD helicase activity. The strand-displacement assay for UvrD in the presence and absence of Gyr (A59) and Norf, as indicated, were performed, and the DNA products were analyzed by electrophoresis through 1% agarose gels (A and C) and 8% (D) or 10% (B) polyacrylamide gels as described under “Materials and Methods.” Either T440 (A and B) or T440E (C and D) was used as a substrate. Each experiment was either duplicated or triplicated. In the absence of topoisomerase, UvrD-catalyzed strand displacement, either in the presence or absence of Norf, of T440, and T440E were 75 (±4%) and 64% (±5%), respectively. Representative results are shown, but the standard deviations were derived from the results of three experiments. Lane 1 in all panels was heat-denatured DNA substrate. Gyr (A59) (as tetramer) and UvrD (as monomer) were present at 30- and 25-fold molar excess over the DNA substrate, respectively. Norf was at 400 μM when present. An arrow (D) indicates a broken DNA strand released from the ternary complex.
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or Topo IV (Figs. 6 and 7). How is an SSB formed at the ternary complex? One possible mechanism is that the ternary complex formed with either Gyr or Topo IV could block the UvrD-catalyzed unwinding of the duplex DNA. Binding of the UvrD to the displaced DNA strand seems to be essential for the destabilization of the interaction between the displaced DNA strand and the topoisomerase in the ternary complex. The displaced DNA strand is not covalently attached to the topoisomerase. Thus, release of this DNA strand from the ternary complex results in the formation of an SSB. A similar model has been proposed by Howard et al. (27).

What is the role of the UvrD-mediated SSB formation in the repair of the topoisomerase-quinolone-DNA ternary complexes? Ternary complexes formed with either Gyr or Topo IV are mainly repaired by the recombinational repair system (13). The formation of an SSB at the ternary complex may provide a single-stranded DNA with a free 3'-OH. It is interesting to speculate that the SSB formation provides an invading strand to initiate the recombination process, which leads to the repair of the quinolone-induced, topoisomerase-mediated DNA damage.

We found that SSBs were generated only at small portions of the ternary complexes (Figs. 5 and 8). It is not clear what determines if an SSB generates at the ternary complex upon its encounter with the UvrD helicase. One possible explanation is that the DNA sequences of topoisomerase-binding sites, in which the topoisomerase-quinolone-DNA ternary complexes are formed, affect the stability of each ternary complex. Some ternary complexes are more stable than others. The formation of an SSB could occur only at unstable ternary complexes. Stromberg et al. (32) have recently demonstrated that, using yeast topoisomerase II, the DNA sequences of the topoisomerase-binding sites affect the stability of the ternary complex.

Recently, an interesting phenotype of the uvrD deletion has been reported (13). Khodursky and Cozzarelli (13) have developed an assay system to assess the efficiencies of the Gyr- and Topo IV-targeted cell killing by the quinolone drug. The loss of the UvrD function has no effect on the Gyr-targeted cell killing, whereas the efficiency of the Topo IV-targeted cell killing is drastically increased when the uvrD gene is deleted. It is concluded that the postreplicative repair system can repair the Topo IV-Norf-DNA ternary complexes but not Gyr-Norf-DNA ternary complexes in E. coli (13). The observed Topo IV-specific repair by the postreplicative repair system is explained by the positions of Gyr and Topo IV relative to the advancing replication forks. Topo IV-Norf-DNA ternary complexes are formed behind the replication forks, and thus these complexes are repaired by the postreplicative repair system.

We showed here that the UvrD helicase affected the Gyr-Norf-DNA and Topo IV-Norf-DNA ternary complexes in a distinct manner. The UvrD-mediated conversion of Topo IV-Norf-DNA ternary complexes to a nonreversible form may be a critical step for the removal of the covalent Topo IV-DNA complex and the repair of the DNA damage. It is likely that the UvrD helicase can distinguish between Gyr and Topo IV in the ternary complexes. At the Topo IV-Norf-DNA ternary complexes, UvrD convert the ternary complex to a nonreversible protein-DNA adduct, which can be subsequently repaired by the postreplicative repair system. In contrast, when UvrD collides with the Gyr-Norf-DNA ternary complexes, the ternary complexes remain reversible and eventually fall off the DNA. The possibility exists that collisions between UvrD and the Gyr-Norf-DNA ternary complex reverse the ternary complex formation and force Gyr to RELIGATE the DNA strands. The reversal of Gyr (A59)-Norf-DNA ternary complexes upon their collisions with UvrD (Figs. 7 and 8) supports this possibility. In any case, in addition to the postreplicative repair of the ternary complexes formed with either Gyr or Topo IV relative to the replication forks, the different responses between Topo IV-Norf-DNA and Gyr-Norf-DNA ternary complexes to the UvrD helicase might contribute to the Topo IV-specific repair of DNA damage by the postreplicative repair system.

After the collisions between UvrD and the ternary complexes, the majority of Gyr-Norf-DNA ternary complexes remained reversible, whereas the majority of Topo IV-Norf-DNA ternary complexes was converted to a non-reversible form (Fig. 6). It is not clear what makes the difference between the ternary complexes formed with Gyr and those formed with Topo IV. The fact that the ternary complexes formed with Gyr (A59) remained reversible (Fig. 7) suggests that the Gyr-mediated wrapping of the DNA strand does not make the Gyr-Norf-DNA ternary complex different from the Topo IV-Norf-DNA ternary complex. It is interesting to speculate that there is a specific protein-protein interaction between UvrD and the topoisomerase in the ternary complex. Thus, the UvrD helicase can interact with some topoisomerases but not others. This protein-protein interaction determines the fate of the ternary complex after the collision of UvrD with the ternary complex. The observation that UvrD disrupts the ternary complexes formed with phage T4 topoisomerase (27), but this helicase had no effect on the reversibility of the ternary complexes formed with yeast topoisomerase II, supports this possibility.

How does the collision between the UvrD helicase and a topoisomerase-quinolone-DNA ternary complex take place on the chromosome? Unlike the replication fork, UvrD does not translocate throughout the chromosome. One likely explanation is that some tracking and scanning system for DNA damage recognizes the topoisomerase-quinolone-DNA ternary complex as a protein-DNA adduct and recruits the UvrD helicase as a part of the repair machinery. MutS protein, which scans for mismatches in the replicated region, seems to be a good candidate. A recent demonstration (33) of the stimulation of the UvrD activity by MutS and MutL proteins supports this model.

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REFERENCES

1. Wang, J. C. (1996) Annu. Rev. Biochem. 65, 635–692
2. Smith, J. T. (1984) Fortschr. Antimikrob. Antineoplast. Chemother. 493, 3–5
3. Higgins, N. P., Peebles, C. L., Sugino, A., and Cozzarelli, N. R. (1978) Proc. Natl. Acad. Sci. U. S. A. 74, 4767–4771
4. Mizuuchi, K., O’Dea, M. H., and Gellert, M. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5960–5963
5. Higgins, N. P., Peebles, C. L., Sugino, A., and Cozzarelli, N. R. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 1773–1777
6. Gellert, M., Mizuuchi, K., O’Dea, M. H., Itoh, T., and Tomizawa, J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4772–4776
7. Kreuzer, K. N., and Cozzarelli, N. R. (1979) J. Bacteriol. 130, 424–435
8. Maxwell, A. (1992) J. Antimicrob. Chemother. 30, 191–218
9. Chen, A. Y., and Liu, L. F. (1994) Annu. Rev. Pharmacol. Toxicol. 34, 219–238
10. Frolich-Ammon, S. J., and Osheroff, N. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11801–11805
11. Hiasa, H., Yousef, D. O., and Marians, K. J. (1996) J. Biol. Chem. 271, 26424–26429
12. Khodursky, A. B., and Cozzarelli, N. R. (1998) J. Biol. Chem. 273, 27668–27677
13. Khodursky, A. B., Zechiedrich, E. L., and Cozzarelli, N. R. (1998) Proc. Natl. Acad. Sci. U. S. A. 92, 11806–11809
14. Hiasa, H., and Marians, K. J. (1996) J. Biol. Chem. 271, 21529–21535
15. Sheu, M. E., and Hiasa, H. (1999) J. Biol. Chem. 274, 22747–22754
16. Kampranis, S. C., and Maxwell, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14164–14167
17. Franke, B., and Ray, D. S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 186–187
18. Model, P., and Zinder, N. D. (1974) J. Bacteriol. 140, 21429–21432
19. Liu, L. F., and Wang, J. C. (1981) J. Bacteriol. 142, 435–441
20. Liu, L. F., and Wang, J. C. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2098–2102
21. Liu, L. F., and Wang, J. C. (1978) Cell 15, 979–984
22. Kirkegaard, K., and Wang, J. C. (1981) Cell 23, 721–729
23. Fisher, I., Mizuuchi, K., O’Dea, M. H., Ohmori, H., and Gellert, M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4165–4169
24. Peng, H., and Marians, K. J. (1995) J. Biol. Chem. 270, 25286–25290
25. Marians, K. J., and Hiasa, H. (1997) J. Biol. Chem. 272, 9401–9409
26. Peng, H., and Marians, K. J. (1993) J. Biol. Chem. 268, 24481–24490
27. Howard, M. T., Neece, S. H., Matson, S. W., and Kreuzer, K. N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12031–12035
28. Champoux, J. J., and Been, M. D. (1980) in Mechanistic Studies of DNA Replication and Genetic Recombination (Alberts, B., ed) pp. 809–815, Academic Press, Inc., New York
29. Ullsperger, J. C., Vologodskii, A. A., and Cozzarelli, N. R. (1995) in Nucleic Acids and Molecular Biology (Lilley, D. M. J., and Eckstein, F., eds) pp. 115–142, Springer-Verlag Inc., Berlin
30. Peter, B. J., Ullsperger, J. C., Hiasa, H., Marians, K. J., and Cozzarelli, N. R. (1998) Cell 94, 819–827
31. Ferrero, L., Cameron, B., Manse, B., Lagneaux, D., Crouzet, J., Famechon, A., and Blanche, F. (1994) Mol. Microbiol. 13, 641–653
32. Strumberg, D., Nitis, J. L., Rose, A., Nicklaus, M. C., and Pommier, Y. (1999) J. Biol. Chem. 274, 7292–7301
33. Yamaguchi, M., Dao, V., and Modrich, P. (1998) J. Biol. Chem. 273, 9197–9201
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