Type II topoisomerases bind to DNA at the catalytic domain across the DNA gate. DNA gyrase also bind to DNA at the non-homologous C-terminal domain of the GyrA subunit, which causes the wrapping of DNA about itself. This unique mode of DNA binding allows gyrase to introduce the negative supercoils into DNA molecules. We have investigated the biochemical characteristics of *Staphylococcus aureus* (S. aureus) gyrase. S. aureus gyrase is known to require high concentrations of potassium glutamate (K-Glu) for its supercoiling activity. However, high concentrations of K-Glu are not required for its relaxation and decatenation activities. This is due to the requirement of high concentrations of K-Glu for S. aureus gyrase-mediated wrapping of DNA. These results suggest that *S. aureus* gyrase can bind to DNA at the catalytic domain independent of K-Glu concentration, but high concentrations of K-Glu are required for the binding of the C-terminal domain of GyrA to DNA and the wrapping of DNA. Thus, salt modulates the DNA binding mode and the catalytic activity of *S. aureus* gyrase. Quinolone drugs can stimulate the formation of covalent *S. aureus* gyrase-DNA complexes, but high concentrations of K-Glu inhibit the formation of *S. aureus* gyrase-quinolone-DNA ternary complexes. In the absence of K-Glu, ternary complexes formed with *S. aureus* gyrase cannot arrest replication fork progression in *vitro*, demonstrating that the formation of a wrapped ternary complex is required for replication fork arrest by a *S. aureus* gyrase-quinolone-DNA ternary complex.

Because of the helical structure of DNA, DNA unlinking is an essential issue in many aspects of DNA metabolism. DNA topoisomerases are the enzymes responsible for unlinking the parental strands during DNA replication (1, 2). Topoisomerase function is essential for removal of the topological constraint to maintain replication fork progression. Biochemical studies have revealed two distinct modes of DNA unlinking during DNA replication (3). DNA gyrase removes the positive super-coils in front of the advancing replication forks, whereas topoisomerase IV (Topo IV) decatenates the precatenanes behind the replication forks. Recent studies have demonstrated that both gyrase and Topo IV can support replication fork progression during chromosome replication in *Escherichia coli* (E. coli) (4). Thus, the combined efforts of gyrase and Topo IV ensure the completion of DNA unlinking during DNA replication and chromosome segregation.

Both DNA gyrase and Topo IV are the cellular targets of the quinolone antibacterial drugs (5–7). It has been shown that quinolone drugs block DNA replication not by depriving the cell of gyrase but by converting gyrase into a poison of DNA replication (8). The poisoning of topoisomerases is mediated by trapping of a covalent topoisomerase-DNA complex as a topoisomerase-drug-DNA ternary complex, which leads to the inhibition of DNA replication and the generation of double-strand breaks (9–11). The cytotoxicity of quinolone drugs correlates with the inhibition of DNA replication (7, 12). Some anticancer drugs that target human topoisomerases also convert their targets into poisons in a similar manner (11, 13).

Interestingly, DNA gyrase is shown to be the primary target of quinolone drugs in *E. coli* (8, 9, 14, 15), whereas Topo IV becomes the primary target in other bacteria, such as *Staphylococcus aureus* (S. aureus) (16–18) and *Streptococcus pneumoniae* (S. pneumoniae) (19, 20). These observations have suggested that DNA gyrase and Topo IV are the primary targets in Gram-negative and Gram-positive bacteria, respectively. However, more recent studies run counter to this view. It has been demonstrated that each quinolone drug has a preferred target and the target selection can be altered by changes in quinolone structure (21, 22). Thus, it is not clear what determines the primary target in cells. One hypothesis, proposed based on the action of quinolone drugs in *E. coli*, is that the locations of gyrase and Topo IV, relative to advancing replication forks, may affect the effectiveness of cell killing (3, 7, 23). In *E. coli*, gyrase is thought to act in front of replication forks, whereas Topo IV acts behind forks. Thus, ternary complexes formed with gyrase more frequently collide with replication forks than those formed with Topo IV.

Despite of the clinical importance of Gram-positive bacteria, the majority of biochemical studies on topoisomerases and quinolone antibacterial drugs have been carried out with *E. coli* topoisomerases (1, 2, 9–11). It is not clear if other topoisomerases act in the same manner as *E. coli* topoisomerases and if...
the findings made in the *E. coli* system can be generalized. Some studies have suggested that quinolone drugs affect *S. aureus* gyrase in a distinct manner and *S. aureus* gyrase-quinolone-DNA ternary complexes contain no broken DNA strand (24).

Here, we investigated the biochemical characteristics of *S. aureus* gyrase and compare them with those of *E. coli* gyrase. We found that catalytic activities of *S. aureus* gyrase were, in general, more resistant to salt than those of *E. coli* gyrase. *S. aureus* gyrase required high concentrations of potassium glutamate (K-Glu) for its supercoiling activity but not for its relaxation and decatenation activities. We also found that high concentrations of K-Glu were required for *S. aureus* gyrase-mediated wrapping of DNA. Thus, the unique requirement of high concentrations of K-Glu for *S. aureus*-catalyzed supercoiling reaction was due to the requirement of high concentrations of K-Glu for *S. aureus* gyrase-mediated wrapping of DNA. These results demonstrated that salt could modulate the DNA binding mode and thus the catalytic activity of *S. aureus* gyrase. Quinolone drugs stimulated the formation of covalent gyrase-DNA formed with either *E. coli* or *S. aureus* gyrase. Both *E. coli* gyrase-quinolone-DNA and *S. aureus* gyrase-quinolone-DNA ternary complexes were sensitive to high concentrations of K-Glu. Interestingly, in the absence of K-Glu, ternary complexes formed with *S. aureus* gyrase failed to arrest replication fork progression in vitro. In contrast, ternary complexes formed with *S. aureus* Topo IV could arrest replication fork progression. These results suggested that collisions between replication forks and *S. aureus* gyrase-quinolone-DNA ternary complexes would not cause the inhibition of DNA replication and trigger the cytotoxic events. This may explain why Topo IV is the primary target of quinolone drugs in *S. aureus*.

**MATERIALS AND METHODS**

**DNAs and Proteins**—An orci plasmid, pBROT8535 type I, was prepared according to Hiasa and Marians (25). pBR322 form I (negatively supercoiled) DNA and kinetoplast DNA (kDNA) were purchased from New England Biolabs and Topogen, respectively. *E. coli* and *S. aureus* gyrA and gyrB genes were generated by PCR using *E. coli* C600 and *S. aureus* WC1H9294 genomic DNA as a template, respectively, and cloned into pET vectors (Novagen). Overproduction and purification of *E. coli* GyrA and GyrB proteins were done as described previously (25–27). *S. aureus* GyrA and GyrB were overexpressed in *E. coli* Rosetta(DE3) (Novagen) and purified according to unpublished protocols, similar to those described previously (24, 28). Hydroxynaphthyl (BioRad) and phenyl-Sepharose (Amersham Biosciences) columns, and heparin-Sepharose (Amersham Biosciences) and phenyl-Sepharose columns were used for the purification of *S. aureus* GyrA and GyrB, respectively. The final preparations of *S. aureus* GyrA and GyrB were greater than 90% homogeneous for a single band on SDS-PAGE (data not shown). Purified *E. coli* GyrA and GyrB and *S. aureus* GyrA and GyrB were mixed at a molar ratio of 1:1 (addition of a 5-fold excess of either subunit did not change the specific activity) to reconstitute *E. coli* and *S. aureus* gyrase, respectively. Mixtures of either *E. coli* GyrA and *S. aureus* GyrB or *S. aureus* GyrA and *E. coli* GyrB did not yield an active gyrase (data not shown). This indicated that the preparations of *S. aureus* GyrA and GyrB were not contaminated significantly with *E. coli* GyrA and GyrB, respectively. *S. aureus* grrA (parC) and grkB (parE) genes were generated by PCR using *S. aureus* RN4220 genomic DNA as a template and cloned into pET vectors (Novagen). *S. aureus* GrrA (ParC) and GrrB (ParE) proteins were overexpressed in *E. coli* BL21(DE3) (Novagen) and purified according to protocols used for purification of *E. coli* ParC and ParE proteins, respectively (6, 29). The final preparations of *S. aureus* GrrA and GrrB were greater than 90% homogeneous for a single band on SDS-PAGE (data not shown). Purified *S. aureus* GrrA and GrrB were mixed, at a molar ratio of 1:1 to reconstitute *S. aureus* Topo IV. Mixtures of either *E. coli* ParC and *S. aureus* GrrB or *S. aureus* GrrA and *E. coli* ParE did not yield an active gyrase (data not shown), indicating that the preparations of *S. aureus* GrrA and GrrB were not contaminated significantly with *E. coli* ParC and ParE, respectively.

*E. coli* replication proteins, generous gifts of Kenneth Marians (Memorial Sloan-Kettering Cancer Center), were as described previously (30–32). Calf thymus Topo I was obtained from Invitrogen. **Supercoiling Reaction**—pBR322 form I (relaxed) DNA was prepared by incubating pBR322 form I DNA with *E. coli* Topo I and used as a substrate in the supercoiling reaction. Standard supercoiling reactions (20 μl) contained 40 mM HEPESE-KOH (pH 7.6), 10 mM magnesium acetate (MgOAc2), 10 mM dithiothreitol (DTT), 50 μM bovine serum albumin (BSA), 2 mM ATP, 0.29 μg (molecule) pBR322 form I DNA, the indicated concentrations of K-Glu, and the indicated amounts (as tetramer) of either *E. coli* or *S. aureus* gyrase. Reaction mixtures were incubated at 37 °C for 15 min and terminated by adding EDTA to 25 mM and incubating at 37 °C for 5 min. The DNA products were analyzed by electrophoresis through vertical 1.2% Seakem-agarose (BMA) gels (14 × 10 × 0.3 cm) at 2 V/cm for 12 h in a running buffer of 50 mM Tris-HCl (pH 7.9 at 23 °C), 40 mM sodium acetate, and 1 mM EDTA (TAE buffer). Gels were stained with ethidium bromide and photographed using an Eagle Eye II system (Stratagene).

**ATP-independent Relaxation of Negatively Supercoiled Plasmid DNA**—Reaction mixtures (20 μl) containing 40 mM HEPESE-KOH (pH 7.6), 10 mM MgOAc2, 10 mM DTT, 50 μg/ml BSA, 10 mM ATP, 0.29 μg (molecule) pBR322 form I DNA, the indicated concentrations of K-Glu, and the indicated amounts (as tetramer) of either *E. coli* or *S. aureus* gyrase were incubated at 37 °C for 30 min. Reactions were terminated by adding EDTA to 25 mM and incubating at 37 °C for 5 min. The DNA products were analyzed and photographed as described in the previous section.

**Decatenation of kDNA**—Reaction mixtures (20 μl) contained 40 mM HEPESE-KOH (pH 7.6), 10 mM MgOAc2, 10 mM DTT, 50 μg/ml BSA, 2 mM ATP, 0.29 μg of kDNA, and the indicated concentrations of K-Glu, and the indicated amounts of either *E. coli* or *S. aureus* gyrase (as tetramer). Reaction mixtures were incubated at 37 °C for 30 min and terminated by adding EDTA to 25 mM and incubating at 37 °C for 5 min. The DNA products were analyzed and photographed as described in the previous section.

**DNA Cleavage Reaction**—pBR322 form I DNA was linearized by digestion with EcoRI endonuclease and then 3’-labeled by incorporation of two residues of [32P]dAMP with Klenow enzyme. This DNA fragment was used as the substrate in the DNA cleavage reaction.

**Reaction mixtures (20 μl) containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 10 mM DTT, 50 μg/ml BSA, 1 mM ATP, 20 fmol (as molecule) DNA substrate, 200 fmol (as tetramer) of either *E. coli* or *S. aureus* gyrase, and 50 μM norfloxacin were incubated at 37 °C for 10 min. SDS was added to 1% and the reaction mixtures were further incubated at 37 °C for 5 min. EDTA and proteinase K were then added to 25 mM and 100 μg/ml, respectively, and the incubation was continued for an additional 15 min. The DNA products were purified by extraction of the reaction mixtures with phenol-chloroform (1:1, v/v) and then analyzed by electrophoresis through 1.2% Seakem-agarose (BMA) gels (14 × 10 × 0.3 cm) at 5 V/cm for 2.5 h in TAE buffer. Gels were dried under vacuum onto #3 filter papers (Whatman) and autoradiographed with Hyperfilm MP films (Amersham Biosciences).

**Gyrase-induced Constraint of Supercoils in DNA**—Reaction mixtures (20 μl) containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 100 μg/ml BSA, 10% glycerol, 100 fmol (as molecule) of pBR322 form I DNA, either 100 or 400 mM K-Glu, and the indicated amounts (as tetramer) of either *E. coli* or *S. aureus* gyrase were incubated at 37 °C for 5 min. Calf thymus Topo I, either 2 units (for reaction mixtures containing 100 mM K-Glu) or 20 units (for reaction mixtures containing 400 mM K-Glu), was added to the reaction mixtures, and the incubation was continued at 37 °C for 30 min. SDS was added to 1% to terminate the reaction, and the reaction mixtures were further incubated at 37 °C for 5 min. EDTA and proteinase K were then added to 25 mM and 100 μg/ml, respectively, and the incubation was continued for an additional 15 min. The DNA products were purified by extraction of the reaction mixtures with phenol-chloroform (1:1, v/v) and then analyzed by electrophoresis through 1.2% Seakem-agarose (BMA) gels (14 × 10 × 0.3 cm) at 2 V/cm for 16 h in TAE buffer. Gels were stained with ethidium bromide and photographed using an Eagle Eye II system (Stratagene).

**Staged Nascent Chain Elongation during orci DNA Replication**—The modified pulse-chase protocol was performed, using pBROT8535 type I DNA as the DNA template, as described previously (33).
RESULTS

High Concentrations of K-Glu Are Required for S. aureus Gyrase-catalyzed Supercoiling Activity—Previous studies have shown that high concentrations of K-Glu stimulate S. aureus gyrase-catalyzed supercoiling activity (24, 28). During the initial characterization of S. aureus gyrase, we noticed that the apparent specific activity of this enzyme changed drastically, depending on the concentrations of K-Glu. To determine the effect of K-Glu on the catalytic activity of S. aureus gyrase, the supercoiling assay was performed in the presence of various concentrations of K-Glu. Because the apparent specific activities of E. coli and S. aureus gyrases were different, we performed the assay in the presence of both a subsaturated amount and an excess amount of gyrases (Fig. 1). E. coli gyrase could catalyze the supercoiling reaction in a wide range of K-Glu concentration, although its activity was optimal in the presence of 100–200 mM K-Glu (Fig. 1A, lanes 3 and 4). An inhibitory effect of salt on E. coli gyrase-catalyzed supercoiling reaction was observed when 800 mM K-Glu was present (Fig. 1A, lanes 6 and 11). In contrast, S. aureus gyrase was able to catalyze supercoiling reaction only in the presence of high concentrations (400–800 mM) of K-Glu (Fig. 1B, lanes 5, 6, 10, and 11). Thus, E. coli and S. aureus gyrases exhibited distinct salt sensitivities for their supercoiling activities.

Quinolone Drugs Can Stimulate the Covalent S. aureus Gyrase-DNA Complex Formation—It was possible that high concentrations of K-Glu were required for S. aureus gyrase to bind to DNA and catalyze the supercoiling reaction. To examine the effect of K-Glu on the binding of S. aureus gyrase to DNA, quinolone-stimulated, gyrase-catalyzed cleavage of DNA was measured in the presence of various concentrations of K-Glu (Fig. 2). Either E. coli or S. aureus gyrase-catalyzed cleavage was observed in the absence of K-Glu (Fig. 2, lanes 2 and 7). Both E. coli gyrase-norfloxacin-DNA and S. aureus gyrase-norfloxacin-DNA ternary complexes were sensitive to K-Glu, although ternary complexes formed with S. aureus gyrase were more resistant to salt than those formed with E. coli gyrase. These results demonstrated that S. aureus gyrase could bind to DNA in the presence of low concentrations (0–200 mM) of K-Glu.

S. aureus Gyrase Can Catalyze Relaxation and Decatenation Reactions in the Presence of Low Concentrations of K-Glu—S. aureus gyrase was able to bind to DNA when K-Glu concentrations were 0–200 mM (Fig. 2) but could not catalyze the

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** S. aureus gyrase-catalyzed supercoiling reaction requires high concentrations of K-Glu. The strand supercoiling reaction mixtures containing 100 fmol (as molecule) of pBR322 form I DNA, the indicated amounts (as tetramer) of either E. coli (A) or S. aureus (B) gyrase, and the indicated concentrations of K-Glu were incubated, and the DNA products were analyzed as described under “Materials and Methods.”

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Quinolone drugs can stimulate formation of covalent S. aureus gyrase-DNA complexes. The strand DNA cleavage reaction mixtures containing 20 fmol (as molecule) of 32P-labeled linear pBR322 DNA, 50 μM norfloxacin, 200 fmol (as tetramer) of either E. coli (lanes 2–6) or S. aureus (lanes 7–11) gyrase, and the indicated concentrations of K-Glu were incubated, and the DNA products were processed as described under “Materials and Methods.”
supercoiling reaction under these conditions (Fig. 1B). It was possible that the DNA binding and the catalytic activity of S. aureus gyrase required distinct K-Glu concentrations. In this case, S. aureus gyrase could bind to DNA at low K-Glu concentrations but required high concentrations of K-Glu for its supercoiling, relaxation, and decatenation activities. Alternatively, high K-Glu concentrations might be a unique requirement for S. aureus gyrase-catalyzed supercoiling reaction but not for relaxation and decatenation activities. In this case, S. aureus gyrase could bind to DNA and catalyze relaxation and decatenation reactions in the presence of low concentrations of K-Glu. This might imply that high concentrations of K-Glu were required only for S. aureus gyrase-mediated wrapping of DNA and S. aureus gyrase-catalyzed supercoiling activity. To distinguish between these two possibilities, we assessed the effects of K-Glu on S. aureus gyrase-catalyzed relaxation and decatenation reactions.

Relaxation (Fig. 3) and decatenation (Fig. 4) activities of S. aureus gyrase were measured in the presence of various concentrations of K-Glu. E. coli gyrase catalyzed relaxation and decatenation reactions the most efficiently when K-Glu was absent and the addition of K-Glu in the reaction mixtures resulted in inhibitions of these catalytic activities (Figs. 3 and 4). On the other hand, the optimal K-Glu concentrations for S. aureus gyrase-catalyzed relaxation and decatenation reactions were 200–400 mM and 100–200 mM, respectively (Figs. 3 and 4). Higher concentrations of K-Glu were inhibitory to these activities. These results showed that, in the presence of low concentrations (0–200 mM) of K-Glu, S. aureus gyrase could bind to DNA and catalyze relaxation and decatenation reactions. Thus, requirement of high concentrations of K-Glu was unique to S. aureus gyrase-catalyzed supercoiling activity, indicating that high concentrations of K-Glu might be required for S. aureus gyrase-mediated wrapping of DNA.

S. aureus Gyrase-mediated Wrapping of DNA Requires High Concentrations of K-Glu—DNA gyrase binds to DNA at the catalytic domain across the DNA gate as well as the C-terminal domain of the GyrA subunit. It is the binding of C-terminal domain of GyrA to DNA that causes the wrapping of DNA about itself and enables gyrase to catalyze supercoiling reaction (1). Results described in the previous sections suggested that S. aureus gyrase was able to bind to DNA at the catalytic domain at a wide range of K-Glu concentration. However, the binding of the C-terminal domain of GyrA to DNA and wrapping of DNA could take place only in the presence of high concentrations of K-Glu. Thus, S. aureus gyrase-catalyzed supercoiling reaction required high concentrations of K-Glu.

To directly test this possibility, we measured the constraint of supercoils in DNA induced by gyrase-mediated wrapping of DNA in the presence of various concentrations of K-Glu. When positive supercoils are generated as a result of gyrase-mediated wrapping of DNA, negative supercoils must be generated in other regions of the DNA molecule to maintain the overall linking number (1). Addition of Topo I would result in the relaxation of negative supercoils, and the subsequent removal of proteins from the DNA would leave positive supercoils in the DNA. Thus, by measuring the constraint of supercoils introduced in DNA, we can determine the extent of gyrase-mediated wrapping of DNA. Form I’ DNA was bound by either E. coli or S. aureus gyrase in the absence of ATP and the presence of either 100 or 400 mM K-Glu (Fig. 5). E. coli gyrase could wrap DNA about itself in the presence of either 100 or 400 mM K-Glu.
Salt Modulates S. aureus Gyrase-DNA Interaction

(Fig. 5, lanes 3 and 4), which correlated with its ability to catalyze supercoiling reaction at various K-Glu concentrations (Fig. 1A). In contrast, S. aureus gyrase-mediated wrapping of DNA was detected only when 400 mM K-Glu was present (Fig. 5, lane 6). These results demonstrated that S. aureus gyrase-mediated wrapping required high concentrations of K-Glu. Thus, it was likely that S. aureus gyrase required high concentrations of K-Glu for its supercoiling activity, because its interaction with DNA at the C-terminal domain of GyrA and wrapping of DNA could occur only in the presence of high concentrations of K-Glu.

S. aureus Gyrase-Quinolone-DNA Ternary Complexes Can Not Arrest Replication Fork Progression in Vitro—Our previous studies have demonstrated that E. coli gyrase-mediated wrapping of DNA is required for the formation of gyrase-quinolone-DNA ternary complexes that can arrest replication fork progression in vitro (27). Thus, the formation of a wrapped ternary complex is required for replication fork arrest by a ternary complex formed with E. coli gyrase. We examined if this was also the case with S. aureus gyrase. If S. aureus gyrase-mediated wrapping were required for replication fork arrest by a ternary complex, S. aureus gyrase could not wrap DNA and thus would fail to halt replication fork progression at low K-Glu concentrations. In contrast, wrapped ternary complexes would be formed at high K-Glu concentrations and replication fork progression would be arrested. Unfortunately, high concentrations of K-Glu inhibited oriC replication reaction (roughly 50 and 90% inhibition by 200 and 400 mM K-Glu, respectively (25)) and reversed formation of gyrase-quinolone-DNA ternary complexes (Fig. 2) (8). Thus, the ability of S. aureus gyrase to arrest replication fork progression could not be assessed at high K-Glu concentrations.

The modified oriC pulse-chase protocol (33) was employed to assess the ability of the ternary complex formed with either E. coli or S. aureus gyrase to arrest replication fork progression in vitro (Fig. 6A). Early replicative intermediates (ERI) were formed and labeled. The paused replication forks in the ERI were released by linearizing the DNA template with the SmaI restriction endonuclease, which digested the DNA template once at oriC. Linearization of the DNA template was sufficient to release the paused replication forks and generate the full-length product as a result of the run-off DNA replication (Fig. 6A, lane 1). Because no topoisomerase was required to relieve topological constraint, this reaction was insensitive to the presence of norfloxacin (Fig. 6A, lane 2).

Either E. coli or S. aureus gyrase was added, together with norfloxacin, to the reaction mixtures to form ternary complexes prior to the linearization of the template DNA. The subsequent addition of the SmaI restriction endonuclease released paused replication forks, which collided with ternary complexes. The ternary complexes that arrest replication fork progression would, therefore, manifest themselves in this assay by preventing the appearance of the full-length DNA product (33). In the absence of norfloxacin, neither the presence of E. coli gyrase nor S. aureus gyrase affected elongation of the nascent chains in the ERI to full-length product (Fig. 6A, lanes 3 and 5). When norfloxacin was present, replication fork progression was blocked in the presence of E. coli gyrase (Fig. 6A, lane 4) but not in the presence of S. aureus gyrase (Fig. 6A, lane 6). These results demonstrated that, under the conditions where S. aureus gyrase did not wrap DNA (Fig. 5), S. aureus gyrase-quinolone-DNA ternary complexes could not arrest replication fork progression. Thus, gyrase-mediated wrapping of DNA was required for the formation of gyrase-quinolone-DNA ternary complexes that could block replication fork progression in vitro.

Here, we assessed the effect of S. aureus gyrase-quinolone-DNA ternary complexes on replication fork progression in E. coli replication system. It was possible that the topoisomerase in a ternary complex could interact with the component(s) of the replication fork, such as the replicative helicase, and this interaction would influence the fate of a replication fork upon its collision with a ternary complex. Thus, the inability of S. aureus gyrase-quinolone-DNA ternary complexes to arrest E. coli replication fork progression could be due to the fact that S. aureus gyrase was used in the E. coli replication system. We examined the effect of S. aureus Topo IV-quinolone-DNA ternary complexes on replication fork progression under the same conditions (Fig. 6B). S. aureus Topo IV exhibited similar drug and salt sensitivities to E. coli Topo IV (Ref. 24 and data not shown). In the absence of norfloxacin, S. aureus Topo IV did not affect elongation of the nascent chains in the ERI to full-length product (Fig. 6B, lane 5). When norfloxacin was present, replication fork progression was blocked in the presence of either E. coli or S. aureus Topo IV (Fig. 6B, lanes 4 and 6). These results showed that a ternary complex formed with S. aureus Topo IV could arrest the progression of E. coli replication forks. Thus, it was likely that the inability of S. aureus gyrase to arrest replication fork progression in the absence of K-Glu was due to the lack of the formation of a wrapped ternary complex.

**DISCUSSION**

Among the type II topoisomerases, DNA gyrase is the only enzyme that can introduce negative supercoils into DNA molecules (1, 2). The binding of conventional type II topoisomerases to DNA takes place at the catalytic domain across the DNA gate, whereas gyrase bind to DNA not only at the catalytic domain but also at the non-homologous C-terminal domain of the GyrA subunit. The topoisomerase-DNA interaction at the catalytic domain is sufficient for type II topoisomerases to catalyze relaxation and decatenation reactions. On the other hand, the binding of the C-terminal domain of GyrA to DNA is required for wrapping of DNA about itself and catalyzing the supercoiling reaction (35).
High concentrations of K-Glu were required for *S. aureus* gyrase-catalyzed supercoiling activity (Fig. 1). In contrast, *S. aureus* gyrase was able to catalyze relaxation and decatenation reactions in the presence of low concentrations of K-Glu and higher concentrations of K-Glu were inhibitory to these reactions (Figs. 3 and 4). *S. aureus* gyrase could wrap DNA only when high concentrations of K-Glu were present (Fig. 5). These results demonstrated that *S. aureus* gyrase could bind to DNA at the catalytic domain cross the DNA gate in the presence of either low or high concentrations of K-Glu. However, high concentrations of K-Glu were required for the DNA binding of the C-terminal domain of *S. aureus* GyrA. Thus, salt could modulate the DNA binding mode and the catalytic activity of *S. aureus* gyrase. *E. coli* gyrase did not show any requirement of high concentrations of salt for its wrapping of DNA and supercoiling activity (Figs. 1 and 5). It is not clear why *S. aureus* but not *E. coli* Topo I-catalyzed relaxation activity was inhibited by high concentrations of K-Glu (data not shown). Thus, the supercoiling activity of DNA gyrase and the relaxation activity of Topo I, which determine the superhelicity of DNA in bacterial cells (1, 2), exhibit similar salt sensitivities. These observations indicate that gyrase-catalyzed supercoiling activity and Topo I-catalyzed relaxation activity could keep their balance at various salt concentrations to maintain a certain superhelicity of the genome in *S. aureus*. *S. aureus* is known to contain high concentrations of dicarboxy-

**TABLE I**

| Gyra           | E. coli | S. aureus |
|----------------|---------|-----------|
| Asp + Glu      | 59      | 76        |
| Lys + Arg      | 41      | 42        |
| Histid*        | 3       | 6         |
| Overall charge* | -18     | -34       |

*His was assumed to be neutral.*

concentrations of K-Glu,

4 I. Wildin, personal communication.
lic amino acids (36), and thus S. aureus topoisomerases may have evolved to accommodate this particular environment.

Quinolone antibacterial drugs target both DNA gyrase and Topo IV (5–7). These drugs trap covalent topoisomerase-DNA complexes by forming topoisomerase-quinolone-DNA ternary complexes and collisions between replication forks and ternary complexes result in the inhibition of DNA replication (9–11). Although quinolone drugs seem to affect both gyrase and Topo IV in the same manner (23) and these two topoisomerases are highly homologous with each other (1, 2), quinolone drugs select one of these topoisomerases as the primary target in cells. Interestingly, DNA gyrase is the primary target in some bacteria (8, 9, 14, 15) and Topo IV becomes primary target in others (16–20). In addition, each quinolone drug has a preferred target (21, 22). Fisher and his coworkers (21) have conducted extensive studies on S. pneumoniae topoisomerases. Sparfloxacin and ciprofloxacin select Topo IV and gyrase as the primary target in vivo, respectively. In contrast, both of these quinolone drugs form the covalent topoisomerase-DNA complex with S. pneumoniae Topo IV much more efficiently than with S. pneumoniae gyrase in vitro (37). Fournier et al. (22) have demonstrated that norfloxacin and nalidixic acid target Topo IV and gyrase, respectively, in S. aureus. Thus, it is not clear how the primary target is selected between two type II topoisomerases in bacterial cells. Furthermore, it has been shown that only a subset of quinolone-induced covalent topoisomerase-DNA complexes are physiologically relevant for the drug action in S. aureus (22). It is likely that the frequency of the formation of topoisomerase-quinolone-DNA ternary complexes on the genome, the cytotoxicity of each ternary complex, and the efficiency of the repair of ternary complexes affect the target selection in vivo (38).

Blanche et al. (24) have reported that quinolone drugs affect S. aureus gyrase in a distinct manner. It is proposed that quinolone drugs interfere with S. aureus gyrase prior to its strand scission and S. aureus gyrase-quinolone-DNA ternary complexes do not contain any broken DNA strands. This conclusion is based on their observations that quinolone drugs do not stimulate S. aureus gyrase-catalyzed cleavage, although both E. coli gyrase- and S. aureus Topo IV-catalyzed cleavages are stimulated by quinolone drugs. The apparent unique effect of quinolone drugs on S. aureus gyrase is likely to be due to the salt concentrations used in the assays. Blanche et al. (24) have performed the cleavage assay for S. aureus gyrase in the presence of high concentrations of K-Glu and that for either E. coli gyrase or S. aureus Topo IV in the presence of low concentrations of salt. As described here, quinolone drugs stimulated the formation of either S. aureus gyrase-DNA or E. coli gyrase-DNA covalent complexes at low K-Glu concentrations (Fig. 2). However, both S. aureus gyrase-quinolone-DNA and E. coli gyrase-quinolone-DNA ternary complexes were sensitive to salt and no cleavage of DNA was observed when high concentrations of K-Glu were present. Saiki et al. (39) have reported similar results. Thus, quinolone drugs can affect S. aureus gyrase and other bacterial type II topoisomerases in the same manner and trap S. aureus gyrase as a covalent enzyme-DNA complex.

Differences between E. coli and S. aureus gyrase described here do not explain why Topo IV becomes the primary target in S. aureus. It is reasonable to assume that drug sensitivities of gyrase and Topo IV play a critical role in determining the primary target in vivo. Apparent quinolone resistance of S. aureus gyrase observed in the supercoiling assay (24) may contribute to the target selection. However, the cytotoxicity of quinolone drugs is mediated by poisoning of topoisomerases not by depriving the cell of gyrase (8). Thus, the potency of quinolone drugs should correlate better with quinolone-stimulated, topoisomerase-catalyzed cleavage of DNA than with the inhibitory effect of quinolones on the catalytic activity of topoisomerases. The difference between the stimulatory effect of quinolones on E. coli gyrase-catalyzed cleavage and that on S. aureus gyrase-catalyzed cleavage (Fig. 2) was much less than the difference between the inhibition of E. coli gyrase-catalyzed supercoiling reaction by quinolones and that of S. aureus gyrase-catalyzed reaction (24). It is likely that other factors may be involved in the selection of the primary target by quinolone drugs in vivo. One possible factor is the locations of topoisomerase complexes relative to advancing replication forks. In E. coli, the locations of gyrase and Topo IV relative to replication forks are critical for the efficiency of gyrase and Topo IV-mediated cell killings (23). Further studies are necessary to determine functional activities of S. aureus topoisomerases during DNA replication and mechanisms of cell killing by quinolone drugs.

We found distinct effects of S. aureus gyrase-quinolone-DNA and S. aureus Topo IV-quinolone-DNA ternary complexes on replication fork progression. In the absence of K-Glu, S. aureus Topo IV-quinolone-DNA, but not S. aureus gyrase-quinolone-DNA, ternary complexes could arrest the progression of replication forks in vitro (Fig. 6). It is likely that, as it is the case with E. coli gyrase (20), S. aureus gyrase-mediated wrapping of DNA is required for the formation of a wrapped ternary complex that can arrest replication fork progression. At low K-Glu concentrations, S. aureus gyrase does not wrap DNA to form a wrapped ternary complex that can arrest replication fork progression. High concentrations of K-Glu allow S. aureus gyrase to wrap DNA but also reverse the ternary complex formation. Thus, ternary complexes formed with Topo IV could be more cytotoxic than those formed with gyrase in S. aureus.

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