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Inhibitory Activities of Quinolones against DNA Gyrase and Topoisomerase IV Purified from *Staphylococcus aureus*

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In order to clarify the mechanism of action of quinolones against *Staphylococcus aureus*, GrlA and GrlB proteins of topoisomerase IV encoded by genes with or without mutations were purified separately as fusion proteins with maltose-binding protein in *Escherichia coli*. The reconstituted enzymes showed ATP-dependent decatenation and relaxing activities but had no supercoiling activity. The inhibitory effects of quinolones on the decatenation activity of topoisomerase IV were determined by quantitative electrophoresis with kinetoplast DNA as a substrate. The 50% inhibitory concentrations (IC_{50}s) of levofloxacin, DR-3354, DU-6859a, DV-7751a, ciprofloxacin, sparfloxacin, and tosufloxacin against topoisomerase IV of *S. aureus* FDA 209-P were 2.3, 97, 0.45, 1.5, 2.5, 7.4, and 1.8 μg/ml, respectively, and were correlated well with their MICs. The IC_{50}s of these drugs were from 2 to 20 times lower than those for the DNA gyrase. These results support genetic evidence that the primary target of new quinolones is topoisomerase IV in quinolone-susceptible strains of *S. aureus*. Three altered proteins of topoisomerase IV containing Ser→Phe changes at codon 80 or Glu→Lys changes at codon 84 of *grlA*, or both, were also purified. The inhibitory activities of quinolones against the topoisomerase IV which contained a single amino acid change were from 8 to 95 times weaker than those against the nonaltered enzyme. These results suggest that the mutations in the corresponding genes confer quinolone resistance.

DNA gyrase has been reported to be a major target of quinolones in *Escherichia coli*, and topoisomerase IV was shown to be less sensitive than DNA gyrase to quinolone inhibition (8). DNA gyrase has been purified from many bacterial species, and the inhibitory effects of quinolones against these enzymes have been measured (6). A good correlation has been found, and while the 50% inhibitory concentrations (IC_{50}s) of quinolones for DNA gyrase from *E. coli* were similar to the MICs, the IC_{50}s for the enzyme from *Staphylococcus aureus* were almost 100 times the MICs (15).

Recently, Ferrero et al. (3) reported that topoisomerase IV in *S. aureus* is a primary target of quinolones. The two genes that code for the subunits of topoisomerase IV, *grlA* and *grlB*, have been cloned, and changes in the serine-80 codon of *grlA* were found in isolates with low and high levels of quinolone resistance, although no mutations were observed in the quinolone resistance-determining region (QRDR) of *gyrA* in the strains with low levels of quinolone resistance. Blanche et al. (1) reported on the inhibitory activities of ciprofloxacin, sparfloxacin, norfloxacin, and ofloxacin against topoisomerase IV activity; however, they determined IC_{50}s for enzymes which contained no or a single amino acid change (Ser-80→Tyr). In this study, we determined (i) mutations in the genes which encode topoisomerase IV and DNA gyrase using laboratory strains of *S. aureus* FDA 209-P and clinical isolates, (ii) the inhibitory concentrations of quinolones for the two topoisomerases purified by the same method, and (iii) the inhibitory concentrations of quinolones for three altered topoisomerases.

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MATERIALS AND METHODS

Antibacterial agents and bacterial strains. All quinolones used in this study were synthesized at the New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan. The bacterial strains used in this study were *S. aureus* FDA 209-P, nine quinolone-resistant mutants of FDA 209-P, six clinical isolates (16), and *E. coli* MC1061 (9). Five of nine mutants (DU-6859a-2-1 to DU-6859a-2-5) were obtained after 48 h of incubation on Mueller-Hinton agar plates containing 0.025 μg of DU-6859a per ml (twice the MIC) (12), and four mutants were obtained after 10 serial exposures to DU-6859a, levofloxacin, ciprofloxacin, and sparfloxacin at sub-MICs.

Determination of MICs. The MICs were determined by standard agar dilution methods (11) with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). Drug-containing agar plates were inoculated with one loopful (5 μl) of an inoculum corresponding to about 10^4 CFU per spot and were incubated for 18 h at 37°C. The MIC was defined as the lowest drug concentration which prevented visible growth of bacteria.

Nucleotide sequence analysis. PCR amplification of part of the *gyrA* gene from quinolone-resistant strains was performed by the same method described by Hori et al. (7). PCR-amplified DNA was sequenced by the cycle sequencing method (10) with a commercially available kit (New England Biolabs, Beverly, Mass.). Thirty-five cycles were performed for each reaction, as follows: 1 min a 93°C, 1 min at 94°C, 1 min at 66°C, and 1.5 min at 74°C. Parts of the *gyrB*, *grlA*, and *grlB* genes were amplified by PCR with primers 1 and 2, 3 and 4, and 5 and 6, respectively (Table 1), and the subsequent cloning and DNA sequence analysis were performed with an Original TA Cloning Kit (Invitrogen) and a fluorescence sequencer (Pharmacia Biotech, Tokyo, Japan).

Construction of fusion plasmids and cloning. PCR was performed with genomic DNA from *S. aureus* FDA 209-P by using *Taq* polymerase (Takara Syuzo, Shiga, Japan) with primers 7 and 8 for *grlA*, primers 9 and 10 for *grlB*, primers 11 and 12 for *gyrA*, and primers 13 and 14 for *gyrB* (Table 1). DNA was amplified for 30 cycles, in which the conditions were 0.5 min at 95°C for denaturation, 1 min at 55°C for annealing, and 5 min at 72°C for polymerization. The DNA fragments and pMAL-c2 (New England Biolabs) were digested with *Bam*HI for *grlA*, *Eco*RI and *Pst*I for *grlB* and *gyrA*, and *Bam*HI and *Hin*dIII for *gyrB*, ligated, and transformed into competent cells of *E. coli* MC1061. Mutated *grlA* genes prepared by site-directed mutagenesis with Mutan-K (Takara) were also amplified and ligated to pMAL-c2.

Purification of topoisomerase IV and DNA gyrase from the transformants. GrlA and GrlB proteins of topoisomerase IV and GyrA and GyrB of DNA gyrase were purified separately as fusion proteins with maltose-binding protein (MBP) from overproducing strains of *E. coli*. *E. coli* MC1061/pMAL-c2 containing each gene was incubated in Luria-Bertani broth until the late log phase, and isopropyl-β-D-thiogalactopyranoside was added to the culture at a final concentration of 0.3 mM to induce protein synthesis. After a 2-h incubation, the cells

TABLE 1. Primers used in this study

Primer no.	Sequence
1	5′-CAGCGTTAGATGTAGCAAGTCTTC-3′
2	5′-CGATTTTGTGATATCTTGCTTTCG-3′
3	5′-TTCCGTAAAAGTGCGAAAACAG-3′
4	5′-AATACCATTGGTTCGAGTGGTC-3′
5	5′-CAAGCGTAAAGACACTTTGC-3′
6	5′-CCCGATTGTGTGGATAATTG-3′
7	5′-GATTTGGATCCGTTTGGTCAAAGTCTATCGC-3′
8	5′-TGAGGATCCAATCTAGTGAGTGAAATAATTC-3′
9	5′-ACGTACGTTTGCAGGAGGCGGAATTCTTGGC-3′
10	5′-GATTTGGATCCGTTTGGTCAAAGTCTATCGC-3′
11	5′-GAAGGAGGAATTCTTGATGGC-3′
12	5′-CTCCCCTGCAGATAGGGAAGT-3′
13	5′-TTATAGGATCCCAGAAAGCGATGG-3′
14	5′-CTTCAAAAGCTTCAGTTCACAG-3′

were harvested and stored at −20°C until use. The cells were suspended in TGED buffer (50 mM Tris-HCl [pH 8.0], 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol [DTT]) supplemented with 0.5 mg of lysozyme per ml and were incubated at 4°C for 30 min. The suspension was centrifuged at 100,000 × *g* for 40 min, and the supernatant was loaded onto an amylose resin column previously equilibrated with TGED buffer. The column was washed with 10 volumes of TGED buffer, and the fusion proteins were eluted with 10 mM maltose. The eluted fractions were dialyzed twice against TGED buffer at 4°C for 6 h, concentrated by dialysis against 50 mM Tris-HCl (pH 8.0)–50% glycerol–1 mM EDTA–1 mM DTT, and stored at −80°C. A portion of the purified proteins (120 μg of protein) was incubated with 0.5 μg of factor Xa at 37°C for 1 h and then loaded onto sodium dodecyl sulfate (SDS)-polyacrylamide gels.

Decatenation of kDNA by topoisomerase IV. The decatenation activity of topoisomerase IV was measured electrophoretically. The reaction mixture contained 78 mM Tris-HCl (pH 7.5), 120 mM KCl, 10 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 2 mM DTT, 1 mM ATP, 0.5 μg of factor Xa, and appropriate amounts of GrlA and GrlB proteins (to give 1 U of decatenated activity); the mixture was incubated at 37°C for 1 h, and then 0.4 μg of kinetoplast DNA (kDNA; Nippon Gene, Toyama, Japan) and various concentrations of antibacterial agents were added. After 1-h incubation of 20-μl samples of this mixture at 37°C, the reactions were terminated by adding 3-μl portions of a solution containing 5% Sarkosyl, 0.025% bromophenol blue, and 25% glycerol. The reaction results were analyzed by 0.7% agarose gel electrophoresis. The gel was stained with ethidium bromide and photographed under UV light with Polaroid type 665 film. The brightness of the bands, which corresponded to decatenated monomers of kDNA, was examined with an Image Analyzer (Bio Image Co., Ann Arbor, Mich.). One unit of decatenating activity was defined as the amount of GrlA and GrlB proteins required to fully decatenate 0.4 μg of kDNA. The IC_{50}s were determined as the drug concentrations that reduced the decatenation activity seen with drug-free controls by 50%.

Determination of supercoiling activity. The supercoiling activity of type II topoisomerases, that is, the conversion of relaxed pBR322 DNA to the supercoiled form, was measured by the method described previously (15) after cleaving with factor Xa.

Relaxation of supercoiled pBR322 plasmid DNA. The reaction mixture for measuring relaxation activity contained 39 mM Tris-HCl (pH 7.5), 60 mM KCl, 5 mM $MgCl_2$, 50 μg of bovine serum albumin per ml, 1 mM DTT, 0.5 mM ATP, 0.5 μg of factor Xa, 0.2 μg of pBR322 DNA (Boehringer Mannheim GmbH, Mannheim, Germany), appropriate amounts of GrlA and GrlB proteins (to give 1 U of relaxing activity), and various concentrations of antibacterial agents. The reactions were carried out and analyzed in the same manner as described above for the decatenation assays, except that the IC_{50}s were calculated from the decrease in the amount of supercoiled pBR322 DNA. One unit of relaxing activity was defined as the amount of GrlA and GrlB protein required to fully relax 0.2 μg of supercoiled pBR322 DNA.

RESULTS

Mutations in QRDRs of the genes encoding DNA gyrase and topoisomerase IV. The partial nucleotide sequences of the *gyrA* and *gyrB* genes of the parent and resistant strains were determined and are presented in Table 2 along with MICs. The mutations in the clinical isolates reported previously (15) are included in Table 2. The strain in which sparfloxacin resistance was selected for by treatment with sub-MICs of sparfloxacin had a mutation at codon 84 [TCA(Ser)→TTA(Leu)], and the strain selected for resistance to DU-6859a had a mutation at codon 88 [GAA(Glu)→AAA(Lys)]. All six clinical isolates also had a single mutation at codon 84 or 88. No strains had mutations in the QRDR of *gyrB*. In contrast, no colonies selected for resistance to DU-6859a at a concentration of two times the MIC had any mutation in this portion of *gyrA* and *gyrB*.

The mutations found in *grlA* are presented in Table 3. One of the strains selected for resistance to DU-6859a (strain DU-6859a-2-4) conferred a Ser→Phe change at codon 80, and the sparfloxacin-resistant strain conferred a Glu→Gln change at codon 84. Among the clinical isolates, isolate 87-21 contained double mutations in codons 80 and 84 and the other five strains had a single mutation in codon 80 or 84 of *grlA*. Strain 891185 also contained a GAT (Asp)-432→AAT (Asn) mutation in

TABLE 2. Antimicrobial activities of quinolones and mutations in *gyrA* and *gyrB* in *S. aureus*[a]

Organism	MIC (μg/ml)				*gyrA* mutations			*gyrB* mutations
	DU-6859a	LVFX	CPFX	SPFX	Codon no.	Nucleotide mutation	Amino acid change	
FDA 209-P	0.012	0.20	0.10	0.05	—[b]	—	—	—
DU-6859a-2-1	0.10	0.78	0.78	0.20	—	—	—	—
DU-6859a-2-2	0.05	0.78	0.78	0.20	—	—	—	—
DU-6859a-2-3	0.05	0.39	0.39	0.20	—	—	—	—
DU-6859a-2-4	0.10	0.78	1.56	0.39	—	—	—	—
DU-6859a-2-5	0.05	0.78	0.78	0.39	—	—	—	—
DU-6859a-r[c]	0.05	0.39	0.39	0.20	88	GAA→AAA	Glu→Lys	—
LVFX-r	0.05	0.78	0.78	0.20	—	—	—	—
CPFX-r	0.05	0.39	0.78	0.20	—	—	—	—
SPFX-r	0.10	6.25	6.25	1.56	84	TCA→TTA	Ser→Leu	—
87-20	0.39	6.25	25	6.25	84	TCA→TTA	Ser→Leu	—
87-21	0.78	12.5	50	3.13	88	GAA→AAA	Glu→Lys	—
90-37	0.78	12.5	50	6.25	84	TCA→TTA	Ser→Leu	—
890958	3.13	25	800	6.25	88	GAA→AAA	Glu→Lys	—
891185	12.5	100	>800	12.5	84	TCA→TTA	Ser→Leu	—
900165	3.13	50	400	12.5	84	TCA→TTA	Ser→Leu	—

[a] LVFX, levofloxacin; CPFX, ciprofloxacin; SPFX, sparfloxacin.
[b] —, no change.
[c] r, resistant strain.

TABLE 3. Mutations in *grlA* in *S. aureus*

Organism[a]	Codon no.	Nucleotide mutation	Amino acid change
FDA 209-P	—	—	—
DU-6859a-2-1	—	—	—
DU-6859a-2-2	—	—	—
DU-6859a-2-3	—	—	—
DU-6859a-2-4	80	TCC→TTC	Ser→Phe
DU-6859a-2-5	—	—	—
DU-6859a-r	—	—	—
LVFX-r	—	—	—
CPFX-r	—	—	—
SPFX-r	84	GAA→CAA	Glu→Gln
87-20	80	TCC→TTC	Ser→Phe
87-21	80, 84	TCC→TTC, GAA→AAA	Ser→Phe, Glu→Lys
90-37	80	TCC→TTC	Ser→Phe
890958	80	TCC→TTC	Ser→Phe
891185	80	TCC→TTC	Ser→Phe
900165	84	GAA→AAA	Glu→Lys

[a] LVFX-r, CPFX-r, and SPFX-r, strains resistant to levofloxacin, ciprofloxacin, and sparfloxacin, respectively.

grlB. Six laboratory strains possessed no mutation in the QRDRs of *grlA*, *grlB*, *gyrA*, and *gyrB*. It is possible that a mutation in the other region of these genes or the quinolone efflux system, or both, might confer quinolone resistance.

Purification and characterization of topoisomerase IV and DNA gyrase. The GrlA and GrlB proteins of topoisomerase IV of *S. aureus* FDA 209-P were separately purified with a protein fusion and purification system (New England Biolabs). The bands of each protein on an SDS-polyacrylamide gel with Coomassie brilliant blue staining were about 90 and 79 kDa for GrlA and GrlB, respectively (Fig. 1). Reconstituted enzyme showed ATP-dependent decatenation and relaxing activity but no supercoiling activity (Fig. 2). The optimum concentration range for the potassium cation was 60 to 80 mM, and that for the magnesium cation was greater than 2 mM (data not shown). From these results, the conditions for the decatenation assay were determined as described in Materials and Methods. GyrA and GyrB of DNA gyrase were purified by the same method, and the reconstituted enzyme showed the same characteristics as the enzyme purified directly from *S. aureus* cells (14). The specific activities of GrlA, GrlB, GyrA, and GyrB were about 8×10^2, 1×10^2, 1×10^2, and 2×10^2 units per mg of protein, respectively.

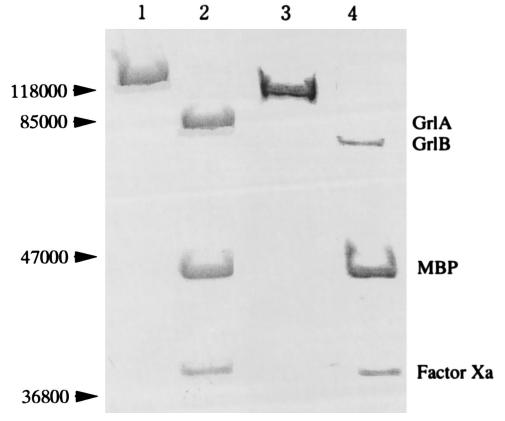

FIG. 1. SDS-polyacrylamide gel electrophoresis of purified proteins stained with Coomassie brilliant blue. Lane 1, purified MBP-GrlA fusion protein; lane 2, MBP-GrlA fusion protein after factor Xa cleavage; lane 3, purified MBP-GrlB fusion protein; lane 4, MBP-GrlB fusion protein after factor Xa cleavage. Numbers on the left are in daltons.

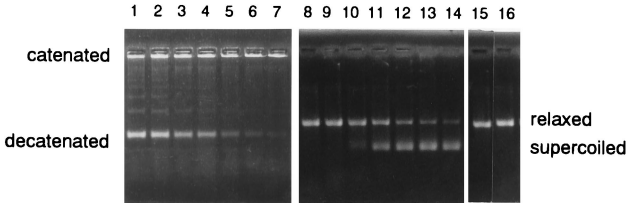

FIG. 2. Decatenation, relaxing, and supercoiling activities of topoisomerase IV from *S. aureus* FDA 209-P. Lanes 1 to 7, decatenation activity; lanes 9 to 14, relaxing activity; lanes 15 to 17, supercoiling activity. Standard reaction mixtures contained 2 U (as a decatenation enzyme; lanes 1 and 8), 1 U (lanes 2 and 9), 1/2 U (lanes 3 and 10), 1/4 U (lanes 4 and 11), 1/8 U (lanes 5 and 12), 1/16 U (lanes 6 and 13), or 1/32 U (lanes 7 and 14). Standard reaction mixtures containing 50 U (as a decatenation enzyme) were incubated with relaxed pBR322 with or without ATP (lanes 15 and 16, respectively).

Comparison of inhibitory activities of antibacterial agents against topoisomerase IV and DNA gyrase. The effects of the quinolones tested on the decatenation activity of topoisomerase IV and the supercoiling activity of DNA gyrase are presented in Fig. 3. The IC_{50}s of the quinolones and novobiocin were calculated from the quantitation of bands which corresponded to fully decatenated substrate or supercoiled DNA (Table 4). The ratio of the IC_{50}s of quinolones for the type II enzymes varied between 1.7 and 15. Among the quinolones tested, DU-6859a showed the highest level of inhibitory activity against both enzymes. The correlation between inhibition of topoisomerase IV and DNA gyrase is presented in Fig. 4. The inhibitory activities of quinolones against type II topoisomerases were correlated well: the correlation coefficients were 0.998 for decatenation and 0.997 for relaxation.

Inhibitory activities of antibacterial agents against altered topoisomerase IV. The altered GrlA proteins with a substitution of Phe (TCC) for Ser-80 (TCC) or of Lys (AAA) for Glu-84 (GAA) and both of the altered proteins were also purified as fusion proteins with MBP. The IC_{50}s for these enzymes reconstituted with altered GrlA and GrlB of *S. aureus* FDA 209-P are presented in Table 5. The IC_{50}s of novobiocin for the three enzymes were almost the same. Those of the quinolones tested, except nalidixic acid, for topoisomerase IV containing a single amino acid change were 8 to 95 times higher than those for the enzyme from *S. aureus* FDA 209-P. The IC_{50}s of the new quinolones for the enzyme which contained a double amino acid change were 110 to 530 times the IC_{50}s for the enzyme from *S. aureus* FDA 209-P. These results

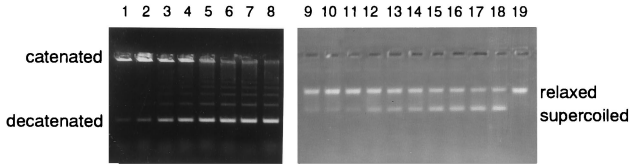

FIG. 3. Inhibitory activity of levofloxacin against decatenation activity of topoisomerase IV and supercoiling activity of DNA gyrase from *S. aureus* FDA 209-P. Lanes 1 to 8, decatenation activity; lanes 9 to 19, supercoiling activity; lanes 8 and 18, no inhibitor; lanes 1 to 7, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 μg of levofloxacin per ml, respectively; lanes 9 to 17, 800, 400, 200, 100, 50, 25, 12.5, 6.25, and 3.13 μg of levofloxacin per ml, respectively; lane 19, no enzyme.

TABLE 4. Inhibitory activities of quinolones and novobiocin against topoisomerase IV and DNA gyrase

Drug[a]	MIC (μg/ml)	IC_{50} (μg/ml)		
		Topoisomerase IV		DNA gyrase, supercoiling
		Decatenation	Relaxation	
LVFX	0.20	2.3	0.69	31
DR-3354	6.25	97	43	750
DU-6859a	0.012	0.45	0.11	1.5
DV-7751a	0.025	1.5	0.29	2.6
CPFX	0.10	2.5	0.60	52
SPFX	0.05	7.4	3.1	30
TFLX	0.025	1.8	0.47	25
NA	50	280	430	350
NOVO	0.05	16	8.4	0.20

[a] LVFX, levofloxacin; CPFX, ciprofloxacin; SPFX, sparfloxacin; TFLX, tosufloxacin; NA, nalidixic acid; NOVO, novobiocin.

TABLE 5. Inhibitory activities of quinolones and novobiocin against topoisomerase IV

Drug[a]	IC_{50} (μg/ml) for enzymes with a mutation at the following amino acid position(s):			
	None	80	84	80 and 84
LVFX	2.3	180	130	1,160
DR-3354	97	>1,600	770	>1,600
OFLX	3.9	260	285	1,250
DU-6859a	0.45	4.2	13	170
DV-7751a	1.5	14	21	800
CPFX	2.5	75	110	530
SPFX	7.4	270	570	1,190
TFLX	1.8	90	170	>200
NA	280	635	370	650
NOVO	16	14	10	11

[a] See footnote *a* to Table 4.

suggest that mutations at codons Ser-80 and Glu-84 may have conferred quinolone resistance.

DISCUSSION

Topoisomerase IV in *S. aureus* was reported by Ferrero et al. (3) to be a primary target of quinolones on the basis of genetic evidence. In this study, we purified topoisomerase IV and DNA gyrase of *S. aureus* FDA 209-P in the same manner and compared the inhibitory activities of quinolones against the purified enzymes. The inhibitory activities against the decatenation activity were higher than those against the supercoiling activity of DNA gyrase. Our results confirm that the primary target of new quinolones in *S. aureus* is topoisomerase IV in susceptible strains. In the case of *E. coli*, the ratio of IC_{50}s of the new quinolones for type II topoisomerases (IC_{50} for topoisomerase IV/IC_{50} for DNA gyrase) ranged from 15.3 to 27.1 (8), while the ratio (IC_{50} for DNA gyrase/IC_{50} for topoisomerase IV) for enzymes from *S. aureus* varied from 1.7 to 20.8. DU-6859a, DV-7751a (14), and sparfloxacin had lower values than the other compounds, which may explain the low frequency of appearance of resistant strains.

Genetically, three point mutations in *gyrA*, two in *gyrB*, and three in *grlA* have been found (2, 4–6, 13, 17, 19) in quinolone-resistant *S. aureus* strains. We also found two mutations in *gyrA*, two mutations in *grlA*, and one novel mutation in *grlB* of the single-step and multistep mutants from *S. aureus* FDA 209-P and clinical isolates. A novel alteration (Asp-432→Asn) in GrlB is considered to correspond to the Asp-437→Asn change in GyrB. A multistep mutant selected by DU-6859a possessed a mutation only in *gyrA*. This phenomenon is the first of its kind to be reported in the absence of a *grlA* mutation. Whether this is specific to DU-6859a or not, we speculated that this might result from the similar IC_{50}s of DU-6859a for topoisomerase IV and DNA gyrase.

Blanche et al. (1) reported the inhibitory activities of ciprofloxacin, sparfloxacin, norfloxacin, and ofloxacin for the altered topoisomerase IV (GrlA Ser-80→Tyr). In our study, we found a substitution of Phe (TCC) for Ser-80 (TCC) or Lys (AAA) for Glu-84 (GAA), or both, in GrlA proteins, so that these three types of GrlA proteins were constructed and purified. From the IC_{50}s of the quinolones for the altered topoisomerase IV, a mutation at position 80 or 84 conferred quinolone resistance. The IC_{50}s of drugs for the enzyme containing double amino acid substitutions were higher than those for enzymes with single amino acid substitutions. The QRDR and DNA-binding site of DNA gyrase have been clarified in *E. coli* (20). The QRDR was considered part of the quinolone-binding pocket which is the binding site of quinolones to the DNA-DNA gyrase complex, and Willmott and Maxwell (18) have found that an altered GyrA protein can greatly reduce the level of binding to the DNA-DNA gyrase complex. Alteration of the electric charge or the conformation of the binding pocket after mutation might be the cause of the reduction. The sequences of the regions of DNA gyrase and topoisomerase IV were well conserved in *E. coli* and *S. aureus*; thus, quinolones might bind to the DNA-topoisomerase IV complex in a similar manner.

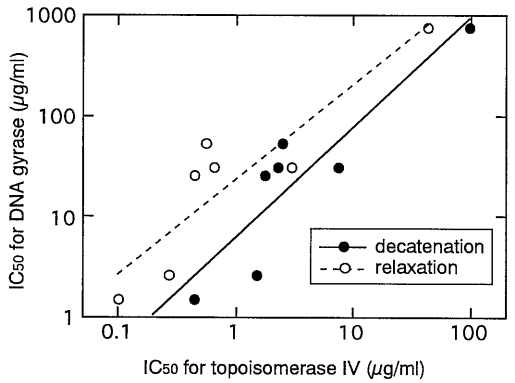

FIG. 4. Correlation between inhibition of topoisomerase IV and DNA gyrase. ●, decatenation activity of topoisomerase IV versus supercoiling activity of DNA gyrase; ○, relaxation activity of topoisomerase IV versus supercoiling activity of DNA gyrase.

TABLE 6. Summary of MICs and IC_{50}s of levofloxacin

Strain	Levofloxacin MIC (μg/ml)	IC_{50} (μg/ml) for the following:		Amino acid alteration in the following protein:			Quinolone efflux pump
		Topoisomerase IV	DNA gyrase	GrlA	GrlB	GyrA	
FDA 209-P	0.20	2.3	31	—[a]	—	–	
900165	50	130	>1,600	Lys-84	—	Leu-84	+
891185	100	180	1,590	Phe-80	Asn-432	Leu-84	+
87-21	12.5	1,160	30	Phe-80, Lys-84	—	Lys-88	–

[a] —, no change.

The MICs of quinolones might be influenced by two factors: inhibition of topoisomerase IV and DNA gyrase. The MICs and IC_{50}s for two topoisomerases are presented in Table 6. The IC_{50}s for DNA gyrase and the association of *norA* were from our previous data (16). In a resistant strain (strain 891185 or 900165) which possesses two changes at Ser-80 of GrlA and Ser-84 of GyrA or at Glu-84 of GrlA and Ser-84 of GyrA, the primary target appears to be topoisomerase IV, and the primary target appears to be DNA gyrase in the strain (strain 87-21) which had three alterations, at Ser-80 and Glu-84 of GrlA and at Glu-84 of GyrA. The relationship between GrlB alterations and quinolone resistance will be clarified by further study.

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