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Quinolone Resistance-Determining Region in the DNA Gyrase *gyrA* Gene of *Escherichia coli*

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Nucleotide sequence analysis of the *gyrA* genes of 10 spontaneous quinolone-resistant *gyrA* mutants of *Escherichia coli* KL16, including four mutants examined previously, disclosed that quinolone resistance was caused by a point mutation within the region between amino acids 67 and 106, especially in the vicinity of amino acid 83, of the GyrA protein.

Quinolones are considered to exert antibacterial activity by inhibiting DNA gyrase (EC 5.99.1.3), which catalyzes topological changes of DNA (4, 11). DNA gyrase of *Escherichia coli* consists of subunits A and B, which are the products of the *gyrA* and *gyrB* genes, respectively. Mutations in either gene can cause quinolone resistance (4, 15–17). Mutations in the *gyrA* and *gyrB* genes are equally frequent in spontaneous quinolone-resistant mutants of *E. coli* KL16, and mutations in the *gyrA* gene are more common in quinolone-resistant clinical isolates of *E. coli* (12). DNA sequence analysis previously done has disclosed four- and two-point mutations causing quinolone resistance in the *gyrA* and *gyrB* genes of mutants of *E. coli* KL16. All of the four *gyrA* mutations resulted in an amino acid change within a small region near the N terminus of the A subunit and close to Tyr at amino acid 122, which is the binding site of transiently cleaved DNA (7). The two *gyrB* mutations caused an amino acid change in a small region at the center of the B subunit and near the N terminus of the processed B subunit called ν (16). An unexpected finding with the *gyrA* mutations was that two independently isolated mutants that were highly resistant to nalidixic acid had a mutation at the same amino acid 83 of the A subunit, although the amino acid alterations differed (17). This finding suggests that the site may have special importance with respect to inducing quinolone resistance. To gain more information on this point, six additional quinolone-resistant *gyrA* mutants were analyzed.

Spontaneous quinolone-resistant mutants of *E. coli* KL16 were isolated on LB agar (10) containing nalidixic acid or pipemidic acid (8) and established as *gyrA* mutations by transformation with the wild-type *gyrA* gene (12). Nalidixic acid, pipemidic acid, norfloxacin, enoxacin, ofloxacin, and ciprofloxacin were synthesized in our laboratories. Plasmid vector pBR322, restriction endonucleases, T4 DNA ligase, the sequencing kit, and phage M13mp18 and M13mp19 vectors were purchased from Takara Shuzo Co., Ltd., [α-^{32}P]dCTP (>400 Ci/mmol) was from Amersham International, and other reagents from Nacalai Tesque, Inc. Chromosomal DNA was prepared by the method of Cosloy and Oishi (2). Small-scale plasmid DNA isolation was carried out by the rapid boiling method of Holmes and Quigley (5), and large-scale plasmid isolation was by the method of Wilkie et al. (14). DNA fragments of 8.5 kilobases containing the *gyrA* gene were cloned from spontaneous *gyrA* mutants of *E. coli* KL16 as described previously (17). Nucleotide sequences were determined by dideoxy-chain termination (9) with phage M13mp18 and M13mp19 vectors.

Table 1 shows the sites and types of mutations and the levels of resistance to quinolones of 10 quinolone-resistant *gyrA* mutants of *E. coli* KL16. Four mutants (N-51, P-18, P-10, and N-89) were analyzed previously (17). All 10 point mutations were considered to be solely responsible for quinolone resistance, because replacement of the 0.6-kilobase *Sac*I-*Sma*I fragment containing the mutations by the corresponding fragment from wild-type *gyrA* gene resulted in complete loss of quinolone resistance (data not shown). Sequencing of the 0.6-kilobase *Sac*I-*Sma*I fragments of the mutant *gyrA* genes revealed that these mutations were located within a relatively small region (amino acids 67 through 106) of the A subunit, which we call a quinolone resistance-determining region. There were no other mutations in all of the sequenced fragments. Eight of the 10 mutations were in a limited area (amino acids 81 through 87) of the region; surprisingly, five mutations were situated at the same site of amino acid 83. The levels of resistance to quinolones seemed to be related to the mutation sites, because quinolone MICs were high in the decreasing order of MICs for mutants with mutations at amino acids 83, 87, 81, 84, 67, and 106. This result suggests the importance of an area around amino acid 83 of the gyrase A subunit for determining quinolone resistance.

Amino acid changes detected at amino acid 83 were Ser to Leu in four mutants and Ser to Trp in one mutant. Since point mutations in the codon TCG for Ser-83 can induce amino acid changes to Leu, Trp, Pro, Ala, and Thr, this result may mean that only the mutant A subunit with Leu or Trp at amino acid 83 causes marked resistance to quinolones and that mutants with such a mutation may have a selective advantage. It has been reported that a clinical isolate of *E. coli* (from urine) that is highly resistant to nalidixic acid has a mutation causing an amino acid change from Ser to Trp at amino acid 83 (3). The area around amino acid 83 of the wild-type A subunit with Ser-83 is likely to form a turn as a local secondary structure, but that of the mutant A subunit with Leu-83 or Trp-83 can form an α-helix as predicted by computer analysis by the method of Chou and Fasman (1). In addition, the hydrophobic profile of this area calculated by the algorithm of Hopp and Woods (6) is changed significantly by the mutation from Ser-83 to Leu or Trp. Therefore, the mutations are likely to induce a local conformation change of the A subunit. The bulkiness of Leu-83 or Trp-83 compared with that of Ser-83 may contribute to conformation of this area.

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TABLE 1. Quinolone resistance mutations in the *gyrA* gene of *E. coli* KL16

Strain[a]	MIC (μg/ml)[b]						Mutation
	NA	PPA	NFLX	ENX	OFLX	CPFX	
KL16	3.13	1.56	0.05	0.1	0.05	0.0125	Wild type
N-112	400	25	0.78	1.56	0.78	0.39	Ser-83 (TCG) → Leu (TTG)
N-118	400	25	0.78	1.56	0.78	0.39	Ser-83 (TCG) → Leu (TTG)
N-119	400	25	0.78	1.56	0.78	0.39	Ser-83 (TCG) → Leu (TTG)
N-51	400	25	0.78	1.56	0.78	0.39	Ser-83 (TCG) → Leu (TTG)
P-18	400	25	0.78	1.56	0.78	0.39	Ser-83 (TCG) → Trp (TGG)
N-113	200	25	0.39	1.56	0.78	0.2	Asp-87 (GAC) → Asn (AAC)
N-97	50	12.5	0.39	0.78	0.39	0.1	Gly-81 (GGT) → Cys (TGT)
P-5	25	12.5	0.39	0.78	0.39	0.1	Ala-84 (GCG) → Pro (CCG)
P-10	25	6.25	0.2	0.39	0.2	0.05	Ala-67 (GCC) → Ser (TCC)
N-89	12.5	3.13	0.1	0.2	0.1	0.05	Gln-106 (CAG) → His (CAT)

[a] N-112, N-118, N-119, N-51, N-113, N-97, and N-89 were selected with nalidixic acid, and P-18, P-5, and P-10 were selected with pipemidic acid.
[b] NA, Nalidixic acid; PPA, pipemidic acid; NFLX, norfloxacin; ENX, enoxacin; OFLX, ofloxacin; and CPFX, ciprofloxacin.

It has been reported that the inhibition of DNA gyrase by norfloxacin occurs as a result of its binding to a site that appears after gyrase-DNA complex formation (13). Since oxolinic acid, one of quinolones, inhibits the reunion of DNA transiently cleaved by DNA gyrase and each 5′-phosphate end of cleaved DNA covalently binds to Tyr-122 of the gyrase A subunit when a detergent is added, it is likely that the quinolone-binding site is located near Tyr-122 and therefore is near or involves the quinolone resistance-determining region. The quinolone resistance-determining region of the gyrase B subunit may also participate in constituting the quinolone-binding site. Quinolone binding experiments with DNA gyrase reconstituted of the wild-type and mutant subunits A and B will clarify this point.

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