ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1986, p. 1–6
0066-4804/86/010001-06$02.00/0

Vol. 29, No. 1

Effects of Norfloxacin on DNA Metabolism in *Pseudomonas aeruginosa*

DORIS M. BENBROOK AND ROBERT V. MILLER*

Department of Biochemistry and Biophysics, and The Program in Molecular Biology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153

Received 10 June 1985/Accepted 15 October 1985

Norfloxacin is a quinolone (pyridonecarboxylic acid derivative) effective against *Pseudomonas aeruginosa* infections. We studied the effects of this drug on DNA metabolism in *P. aeruginosa*. Norfloxacin inhibits DNA replication immediately on its addition to a logarithmically growing culture of *P. aeruginosa*. It inhibits the ability of *P. aeruginosa* DNA gyrase to supercoil relaxed, closed circular DNA in vitro. At intermediate concentrations of the drug, inhibition of DNA replication in vivo is followed by secondary (recovery) synthesis. Both recovery synthesis and the bactericidal effects of norfloxacin are dependent on continued protein synthesis, suggesting that these are inducible functions. Neither norfloxacin nor nalidixic acid induces Weigle-reactivation (inducible DNA repair) or mutagenesis in *P. aeruginosa*.

Quinolones (pyridonecarboxylic acid derivatives) are antimicrobial agents that are effective against many gram-negative bacteria. Norfloxacin has been shown to be a potent antimicrobial agent which acts against a broad spectrum of bacteria (10, 11). Quinolones appear to inhibit DNA synthesis by inhibiting the activity of DNA gyrase (topoisomerase II), an enzyme responsible for ATP-dependent negative supercoiling of DNA (8, 16). Superhelicity of DNA has been shown to be required for DNA replication, RNA transcription, recombination, and DNA repair (for a review, see reference 7). Engle et al. (6) have suggested that these drugs inhibit DNA replication in *Escherichia coli* by forming a tight complex between DNA gyrase and DNA which inhibits replication fork migration. On treatment with the drug, there is an immediate inhibition of DNA synthesis. Secondary DNA synthesis following inhibition is possible in Rec^+ strains of *E. coli* but not in Rec^- strains (6). RNA and protein synthesis are inhibited by higher concentrations of quinolones (19). In *E. coli* cell death occurs at concentrations of these drugs that inhibit DNA synthesis but have little effect on RNA and protein synthesis (19).

Pseudomonas aeruginosa is not inhibited by most quinolones (11), and DNA gyrase from *P. aeruginosa* is more resistant to inhibition by nalidixic acid than is gyrase from *E. coli* (16). Norfloxacin, however, has been shown to be an effective antimicrobial agent against *P. aeruginosa* (10, 11). The study we report here was designed to measure the effects of norfloxacin on in vivo DNA replication and growth in *P. aeruginosa*.

(Portions of this work will be submitted by D.M.B. to the Graduate College, Loyola University of Chicago, in partial fulfillment of the requirements of the degree of Doctor of Philosophy.)

MATERIALS AND METHODS

Strains and media. All strains used in this study (Table 1) were derived from the prototrophic strain PAO1 (17) and were maintained in Luria broth (LB) (15). All experiments were carried out in *Pseudomonas* minimal medium (15) containing 0.4% glucose and 0.01% arginine. Mueller-Hinton medium was used to determine the MICs and MBCs for norfloxacin (gift of Merck Sharp & Dohme, West Point, Pa.) and nalidixic acid for the strains described above (2, 22). These drugs were dissolved in 0.1 N NaOH.

Rate of DNA synthesis. The rate of DNA synthesis in a logarithmically growing culture was determined by a modification of the method of Engle et al. (6). Nucleic acid was pulse-labeled with 1 μCi of [2,8-^{3}H]adenine (29 Ci/mmol). At various times, 0.2 ml of the culture was added to 0.8 ml of prewarmed *Pseudomonas* minimal medium containing glucose, arginine, the appropriate drug(s), and the radioactive label and incubated for 4 min at 37°C. The reaction was terminated with NaOH (final concentration, 0.3 N) and EDTA (final concentration, 0.1%), and RNA was hydrolyzed by incubating the samples at 37°C overnight. Forty micrograms of bovine serum albumin was added to each sample; and the DNA was precipitated with 5% (final concentration) trichloroacetic acid, collected onto cellulose nitrate filters, and counted in a Beckman LS 7500 scintillation counter.

Rate of protein synthesis. A growing culture of bacteria in the early log phase was treated with drug(s) as described below, exposed to 1 μCi of L-[4,5-^{3}H]leucine (6 Ci/mmol), and incubated with shaking at 37°C. At various times, 0.2-ml samples were removed, 40 μg of bovine serum albumin was added, and the samples were precipitated with ice-cold trichloroacetic acid (final concentration, 5%). The precipitate was collected onto nitrocellulose filters and counted.

Effect of antibiotics on growth of *P. aeruginosa*. A logarithmically growing culture in LB was divided into several fractions and added to flasks containing various combinations of antibiotics (nalidixic acid, norfloxacin, or chloramphenicol). These were incubated with shaking at 37°C; and 0.1-ml samples were removed at various times, diluted, and plated in triplicate on plates with LB containing 1.5% agar. These were incubated overnight at 37°C and the number of CFU/ml was determined.

Weigle-reactivation and Weigle-mutagenesis. A logarithmically growing culture of bacteria was treated with antibiotic. At various times 0.2-ml samples were removed and infected with phage D3 (15) which had been exposed to UV radiation (30 J/m^2). After 7 min at room temperature, 2.5 ml of lambda top agar (15) was added, and the mixture was plated onto plates with LB containing 1.5% agar. After incubation at

* Corresponding author.

TABLE 1. Strains used this study

Strain	Genotype[a]	Nalidixic acid		Norfloxacin		Reference or sourec
		MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	
PAO303	*argB21*	350	>700	0.4	16	15
RM8	*argB21, les-908*	700	>700	1.6	16	15
RM168	*argB21, nor-901*	3,000	ND[b]	8.0	20	This study

[a] Abbreviations: *arg*, arginine; *les*, lysogeny establishment; *nor*, resistance to norfloxacin.
[b] ND, Not determined.

37°C overnight, the number of PFU was determined. The plaques were scored for clear plaque morphology as a measure of the frequency of mutagenesis.

Purification of *P. aeruginosa* DNA gyrase. DNA gyrase was purified from PAO303 by a modification of the methods of Miller and Scurlock (16) and Staudenbauer and Orr (18). Pans (40 by 24 cm) with LB containing 1.5% agar were inoculated and incubated overnight at 37°C. Cells were harvested, washed in 100 ml of 0.85% NaCl, and suspended in a 10% sucrose solution prepared in 50 mM Tris hydrochloride (pH 8.0) at a concentration of 0.5 g/ml. The suspension was frozen in dry ice-ethanol and thawed at 25°C, and 0.1 ml of a freshly prepared solution of lysozyme (2 mg/ml of water) was added for each milliliter of cell suspension. This mixture was incubated at 0°C for 60 min and centrifuged at 35,000 × *g* for 30 min. Magnesium acetate was added to the supernatant fluid to a final concentration of 5 mM, and the extract was centrifuged at 30,000 rpm for 4 h in a Beckman 50 Ti rotor. The supernatant fluid was loaded onto a novobiocin-Sepharose column and washed with buffer A (0.2 M KCl, 5 mM dithiothreitol, 10% glycerol, 50 mM Tris hydrochloride [pH 8.0]) until the optical density at 280 nm

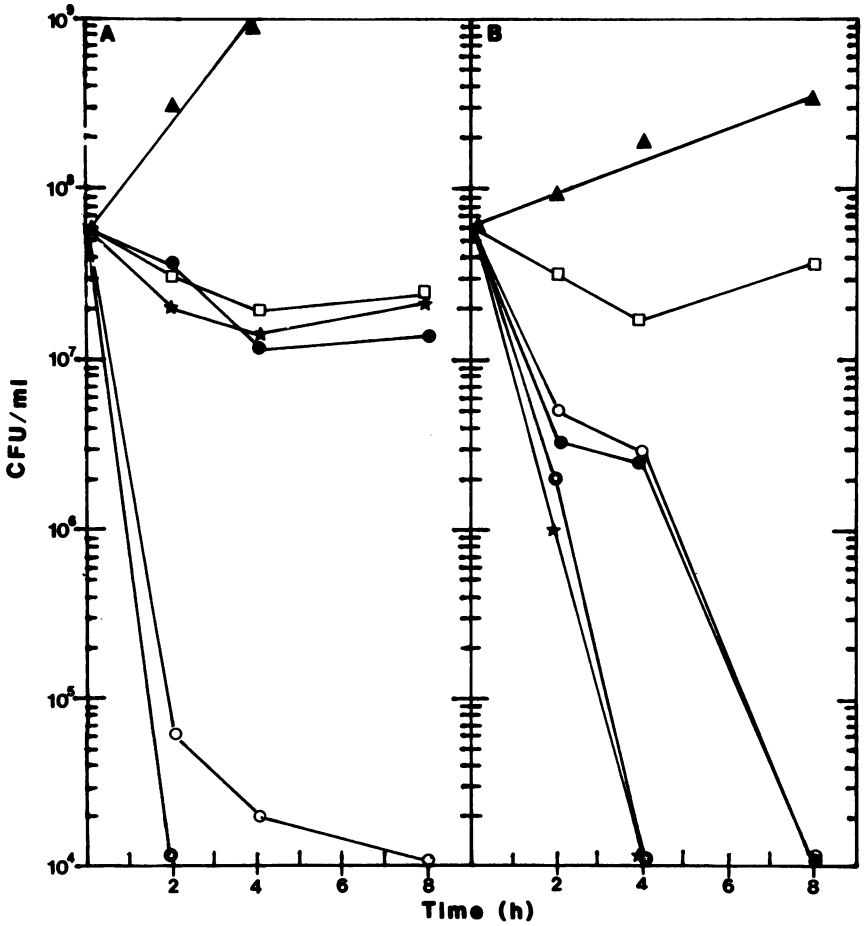

FIG. 1. Logarithmically growing cultures of PAO303 (A) and RM8 (B) were divided. Portions were treated with 700 μg of nalidixic acid per ml (✪), 16 μg of norfloxacin per ml (○), 500 μg of chloramphenicol per ml (□), nalidixic acid and chloramphenicol (★), norfloxacin and chloramphenicol (●), or buffer without added drug (▲), as described in the text. At various times, samples were taken and the number of CFU per milliliter of culture was determined.

reached a minimum. DNA gyrase was eluted from the column with 5 M urea in buffer A. Fractions were diazlyzed against a solution of 1 mM EDTA–5 mM dithiothreitol–10% glycerol–50 mM Tris hydrochloride (pH 7.5) and assayed for supercoiling activity.

DNA gyrase supercoiling assay. One unit of *P. aeruginosa* DNA gyrase (16) was incubated with 0.5 μg of relaxed closed circular φX174 DNA at 37°C in a reaction mixture (40 μl) containing 20 mM KCl, 10 mM $MgCl_2$, 2 mM dithiothreitol, 1.5 mM ATP, 5 mM supermidine chloride, and 50 mM Tris hydrochloride (pH 7.6). After 30 min, 7 μl of a mixture of 20% Ficoll and 40 μg of bromophenol blue per ml of water was added. Samples were loaded onto a 0.8% agarose gel, electrophoresed, stained, and photographed as previously described (16).

RESULTS

Inhibitory effects of norfloxacin on *P. aeruginosa*. The potency of norfloxacin and nalidixic acid as bacteriostatic and bactericidal agents for the strains to be used in this study was determined by measuring the MIC and MBC of both drugs (Table 1). These MICs and MBCs were consistent with those determined for other strains of *P. aeruginosa* (10, 11). Norfloxacin was several hundred- to a thousand-fold more potent than nalidixic acid. A spontaneous norfloxacin-resistant mutant of PAO303 (RM168) was found to have also acquired resistance to nalidixic acid.

Bactericidal action. When *P. aeruginosa* PAO303 was exposed to nalidixic acid or norfloxacin at a concentration equal to the MBC for these drugs, the number of CFU recovered was drastically reduced (Fig. 1A). When chloramphenicol was also present in the culture medium at a concentration which completely inhibited protein synthesis (data not shown), it eliminated the bactericidal effects of nalidixic acid and norfloxacin. Any reduction in the number of CFU per milliliter could be attributed to the inhibitory effect of chloramphenicol alone. Similar results were obtained when RNA synthesis was inhibited with rifampin (data not shown).

Periodically during this experiment, a sample was removed from each of the cultures and examined under a light microscope. Even though the viability of quinolone-treated cultures was decreased by a factor of 10^4 after 8 h, both filamentous and unit-length cells were observed. Some septation of nonfilamented cells was observed even after 8 h of treatment.

Effects of exposure to norfloxacin on DNA replication. The initial inhibition of DNA synthesis by various concentrations of norfloxacin was measured. The amount of DNA synthesis taking place immediately on the addition of the drug was measured by pulse-labeling the culture for 4 min with [^{3}H]adenine. Label was added concurrently with the drug, and incorporation into acid-insoluble, alkali-resistant material was determined as described above. With increasing concentrations of norfloxacin, there was a dramatic increase in the inhibition of DNA synthesis up to a plateau (Fig. 2). Similar dose-response characteristics were observed with nalidixic acid (data not shown).

The kinetics of inihibition of DNA synthesis by norfloxacin and nalidixic acid were determined as described above. Intermediate concentrations of the drugs led to partial inhibition of DNA synthesis (Fig. 3A and B). This inhibition was followed by a secondary increase in DNA synthesis (recovery synthesis). Complete inhibition was observed at higher concentrations (the MBC) of norfloxacin, and no recovery synthesis was observed (Fig. 3B).

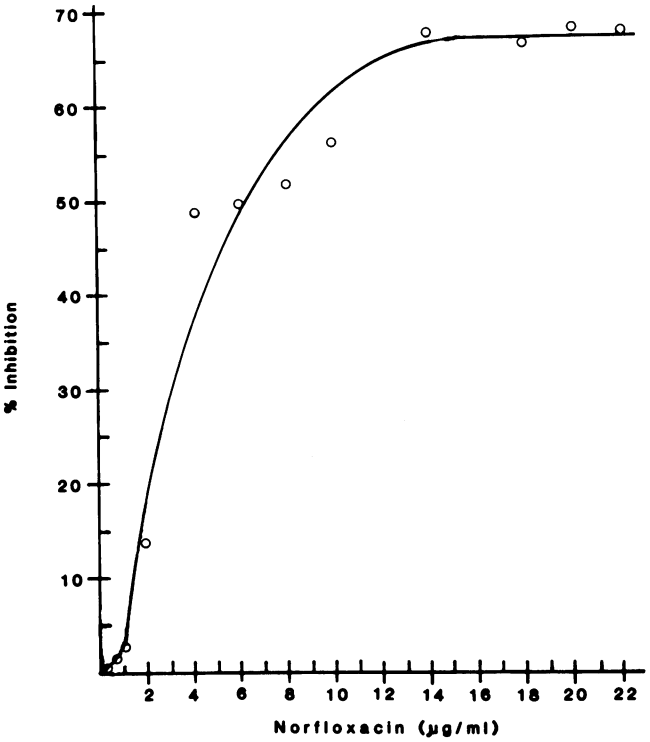

FIG. 2. A logarithmically growing culture of PAO303 was divided and exposed to various concentrations of norfloxacin. DNA synthesis was measured by pulse-labeling with [^{3}H]adenine for 4 min. The label was added to the culture medium concurrently with the drug.

The effects on protein synthesis of nalidixic acid and norfloxacin at four times the MIC were tested. Nalidixic acid was found to completely (>99%) inhibit protein synthesis after 10 min of exposure, while norfloxacin only caused a 40 to 50% inhibition (data not shown). When a combination of chloramphenicol (500 μg/ml) and norfloxacin was added to a logarithmically growing culture, protein synthesis was immediately inhibited and recovery DNA synthesis was not observed (Fig. 4A).

Induction of DNA repair and mutagenesis mechanisms. Quinolones are known to induce the SOS response in *E. coli* (21, 23). The ability of norfloxacin and nalidixic acid to induce an error-prone DNA repair system in *P. aeruginosa* was investigated. The capacity of cells exposed to these drugs to repair UV-induced damage to bacteriophage D3 was measured as described above. The rate of mutagenesis was scored by the frequency of clear-plaque mutants among the repaired phages. Neither increased capacity to repair DNA damage nor mutagenic potential was observed on treatment with either of the drugs (Fig. 5 and Table 2). During the course of the experiment, the phage titer remained constant when phage were plated on untreated cells.

In vitro effects of norfloxacin on isolated *P. aeruginosa* DNA gyrase. DNA gyrase was purified from PAO303, and the ability of norfloxacin and nalidixic acid to inhibit the supercoiling reaction was assessed in vitro. Both drugs inhibited this reaction significantly under the conditions tested (Fig. 6, lanes 4 and 5, respectively).

Effects of norfloxacin in a Rec$^-$ mutant. RecA− strains of *E. coli* are unable to undergo recovery synthesis after treatment with quinolones (6). *lesB* mutations of *P. aeruginosa* have many characteristics which are similar to muta-

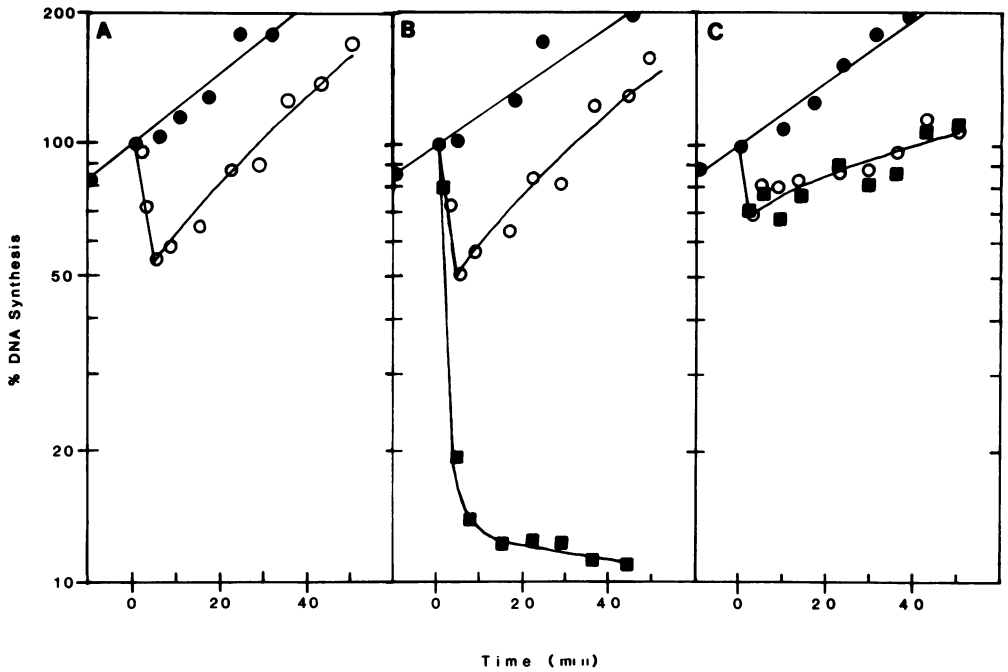

FIG. 3. Logarithmically growing cultures of PAO303 (A and B) and RM8 (C) were divided. One portion of the culture was treated with either four times the MIC (○) or the MBC (■) concentration of nalidixic acid (A) or norfloxacin (B and C). The remaining portion was not treated (●). At various times after introduction of the drug, samples were taken, and the rate of DNA synthesis was measured as described in the text.

tions in the *recA* gene of *E. coli* (15). When the experiments to determine the bactericidal effects of norfloxacin and nalidixic acid were repeated with the *lesB908* strain RM8, this strain was killed by the drugs. However, chloramphenicol did not alleviate the bactericidal effects of nalidixic acid or norfloxacin (Fig. 1B). Microscopic examination of cells from these treated cultures revealed that some cells had filamented, while others were of unit length and showed signs of septation. Simultaneous treatment with either norfloxacin or nalidixic acid-chloramphenicol did not prevent this lethal unbalanced growth.

When the effects of norfloxacin on the rate of DNA synthesis was tested in the *lesB908* strain, only partial inhibition of DNA synthesis followed by recovery synthesis was observed at both the low (four times the MIC) and high (MBC) concentrations of norfloxacin (Fig. 3C). When chloramphenicol was added concurrently with norfloxacin, recovery synthesis was initiated but was not sustained (Fig. 4B).

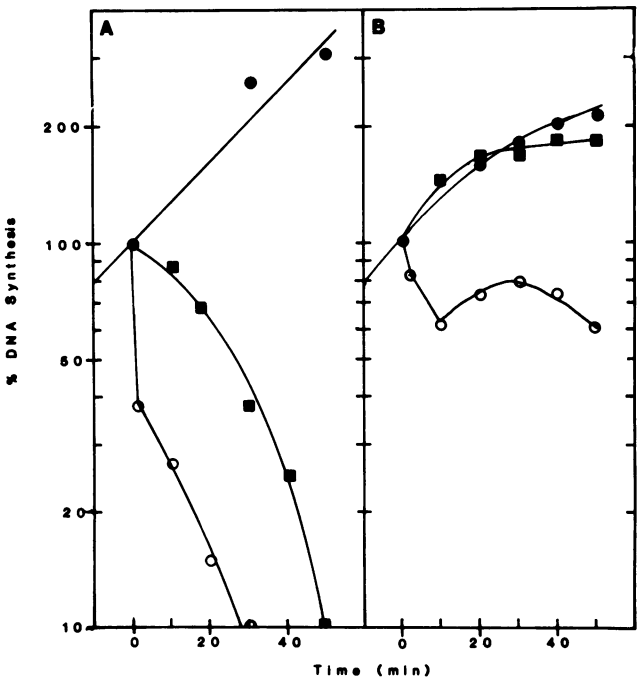

FIG. 4. Logarithmically growing cultures of PAO303 (A) and RM8 (B) were divided, and portions were exposed to chloramphenicol (■), chloramphenicol and norfloxacin (○), or buffer without added drugs (●). At various times after introduction of the drug(s), samples were taken, and the rate of DNA synthesis was measured as described in the text.

DISCUSSION

Treatment with norfloxacin (at MBC concentrations) caused dramatic reductions in viable cells (CFU per milliliter) when protein synthesis was allowed to continue. These killing effects were eliminated in Les$^+$ srains when protein synthesis was inhibited. These results are similar to the findings of Stevens (19), who suggested that the killing of *E. coli* by quinolones is caused by an increase in cell volume without cell division. Such irreversible filamentation is known to cause death in *lon* (1, 9) and *tif* (3, 4, 9) mutants of *E. coli*. When protein synthesis is inhibited, there is no filamentation and, hence, no cell death. Quinolone-treated *P. aeruginosa* cultures contain filamentous and unit-length cells, even though these cells are no longer viable. This

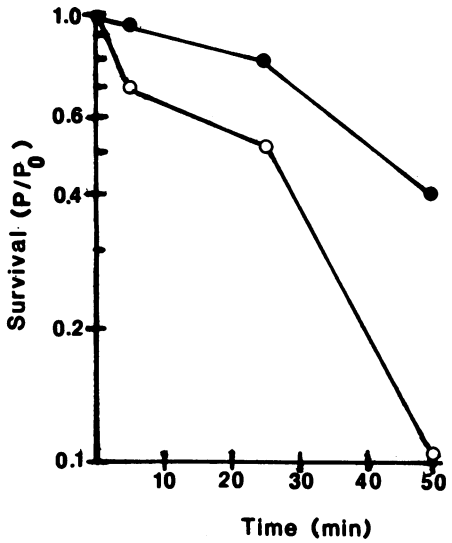

FIG. 5. At time zero, a culture of PAO303 was treated with either nalidixic acid (●) or norfloxacin (○) at a concentration of four times the MIC. At various times after introduction of the drug, samples were taken and infected with UV-irradiated (30 J/m^2) phage D3. The titer of phage (PFU per milliliter) relative to the titer at time zero (P/P_0) was determined.

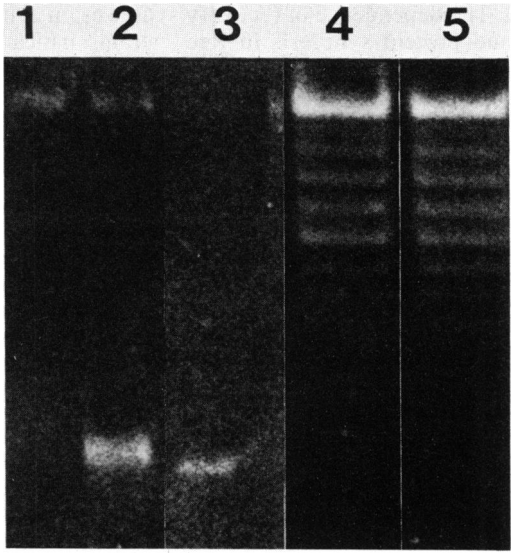

FIG. 6. The effect of norfloxacin on *P. aeruginosa* DNA gyrase supercoiling activity was measured. (Lane 2) Supercoiled ϕX174 (RF1) DNA (lower band) containing some open circular DNA (upper band). (Lanes 1 and 3 through 5) Relaxed, closed circular ϕ174 DNA incubated under the following conditions: lane 1, no added enzyme; lane 3, in the presence of purified *P. aeruginosa* DNA gyrase under standard conditions; lanes 4 and 5, in the presence of enzyme with norfloxacin (16 μg/ml; lane 4) or nalidixic acid (700 μg/ml; lane 5) added. All reactions (lanes 3 through 5) contained one unit of *P. aeruginosa* DNA gyrase (16) and 0.5 μg of DNA. They were incubated at 37°C for 30 min as described in the text. The intermediate bands in lanes 4 and 5 have been shown to be partially supercoiled topoisomers (7).

lethal unbalanced growth is also observed in norfloxacin-treated cultures of *E. coli* (5). As quinolones are known to have either inhibitory or stimulatory effects on the expression of specific genes (5), the actual mechanism of drug-induced cell death may be the deregulation of the normal growth cycle.

Engle et al. (6) have proposed that inhibition of DNA synthesis by quinolones is due to drug-induced covalent attachment of DNA gyrase to DNA, forming a complex through which DNA polymerase cannot proceed. Such complexes have been identified in vitro (7). Recovery from inhibition of DNA synthesis by oxolinic acid at a low concentration (four times the MIC) is hypothesized to be due to removal of these complexes by a DNA damage-inducible repair pathway (6). Results of the in vitro studies reported here suggest that the site of action of norfloxacin in *P. aeruginosa* is DNA gyrase. Initial inhibition of DNA synthesis followed by recovery synthesis is observed in Les$^+$ strains of *P. aeruginosa* treated with norfloxacin. Recovery synthesis in these strains is inhibited by chloramphenicol, indicating that the induction of recovery synthesis requires continued protein synthesis. Les$^-$ strains of *P. aeruginosa* could not sustain recovery synthesis after treatment with nalidixic acid, which led to a complete but delayed inhibition of protein synthesis. These strains were able to recover and sustain their ability to synthesize DNA after exposure to norfloxacin, which only partially inhibited protein synthesis. The immediate and complete inhibiton of protein synthesis by chloramphenicol led to a recovery synthesis that could not be sustained in these strains. The characteristics of the Les$^-$ strain RM8 reported here suggest that this mutation may be constitutive for both recovery synthesis and some unmodulated lethal function. This is reminiscent of several *E. coli recA* alleles recently isolated by Tessman and Peterson (20) which are recombinationally deficient (Rec$^-$) but constitutive for inductive (protease) functions (Prt^c).

TABLE 2. Frequency of mutagenesis in the presence of norfloxacin or nalidixic acid[a]

Drug	Time of exposure (min)	Mutation frequency (mutants per 10^5 PFU)
Norfloxacin	0	10
	5	11
	30	11
	50	8
Nalidixic	0	17
	5	9
	30	11
	50	6

[a] Drugs were used at concentrations of four times the MIC for each strain.

The *recA* and *lexA* genes of *E. coli* control the induction of several functions collectively called SOS functions (12, 21, 23). These functions are inducible by nalidixic acid in *E. coli* (21) and include damage-inducible DNA repair and mutagenesis functions. Engle et al. (6) have suggested that SOS-associated repair mechanisms may be responsible for the recovery synthesis observed after treatment with quinolones. Some of these functions, if not modulated, can be lethal to the cell (1, 3, 4, 9). Even though we have shown that *P. aeruginosa* contains a genetic analog to the *E. coli recA* gene (12), neither nalidixic acid nor norfloxacin induces SOS-associated DNA repair or mutagenesis in *P. aeruginosa*. These processes also are not induced by UV irradiation (C. S. Simonson and R. V. Miller, manuscript in preparation). This suggests that recovery synthesis in *P. aeruginosa* is not dependent on a UV-inducible DNA repair

system. The dependence of recovery synthesis on continued RNA and protein synthesis in Rec^+ strains indicates the requirement for some inducible phenomenon. This phenomenon may be the induction of DNA gyrase itself. Menzel and Gellert (14) have shown that transcription of the structural genes for DNA gyrase are induced by relaxed DNA in *E. coli*.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grant AI-12759 from the National Institute of Allergy and Infectious Disease and by a grant from the Merck Institute of Therapeutic Research. R.V.M. is the recipient of Research Career Development Award AI-00449 from the Public Health Service.

LITERATURE CITED

1. **Adler, H. I., and A. A. Hardigree.** 1964. Analysis of a gene controlling cell division and sensitivity to radiation in *Escherichia coli*. J. Bacteriol. **87**:720–726.
2. **Anhalt, J. P., L. D. Sabath, and A. L. Barry.** 1980. Special tests: bactericidal activity, activity of antimicrobics in combination, and detection of β-lactamase production, p. 478–490. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
3. **Castellazzi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. I. Further characterization of the thermosensitive mutation *tif-1* whose expression mimics the effect of UV irradiation. Mol. Gen. Genet. **119**:139–152.
4. **Castellazzi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. II. Linked (*recA*, *zab*) and unlinked (*lex*) suppressors of *tif-1* mediated induction of filamentation. Mol. Gen. Genet. **119**:153–174.
5. **Crumplin, G. C., M. Kenwright, and T. Hirst.** 1984. Investigations into the mechanism of action of the antibacterial agent norfloxacin. J. Antimicrob. Chemother. **13**(Suppl. B):9–13.
6. **Engle, E. C., S. H. Manes, and K. Drlica.** 1982. Differential effects of antibiotics inhibiting gyrase. J. Bacteriol. **149**:92–98.
7. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
8. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA **73**:3872–3876.
9. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
10. **Hirai, K., A. Ito, Y. Abe, S. Suzue, T. Irikura, M. Inoue, and S. Mitsuhashi.** 1981. Comparative activity of AM-715 and pycemidic and nalidixic acids against experimentally induced systemic urinary tract infections. Antimicrob. Agents Chemother. **19**:188–189.
11. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suzue, T. Irikura, and S. Mitsuhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrob. Agents Chemother. **17**:103–108.
12. **Kokjohn, T. A., and R. V. Miller.** 1985. Molecular cloning and characterization of the *recA* gene of *Pseudomonas aeruginosa* PAO. J. Bacteriol. **163**:568–572.
13. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of Escherichia coli. Cell **29**:11–22.
14. **Menzel, R., and M. Gellert.** 1983. Regulation of the genes for E. coli gyrase: homeostatic control of DNA supercoiling. Cell **34**:105–113.
15. **Miller, R. V., and C.-M. C. Ku.** 1978. Characterization of *Pseudomonas aeruginosa* mutants deficient in the establishment of lysogeny. J. Bacteriol. **134**:875–883.
16. **Miller, R. V., and T. R. Scurlock.** 1983. DNA gyrase (topoisomerase II) from *Pseudomonas aeruginosa*. Biochem. Biophys. Res. Commun. **110**:694–700.
17. **Royle, P. L., H. Matsumoto, and B. W. Holloway.** 1981. Genetic circularity of the *Pseudomonas aeruginosa* PAO chromosome. J. Bacteriol. **145**:145–155.
18. **Staudenbauer, W. L., and E. Orr.** 1981. DNA gyrase: affinity chromatography on novobiocin-Sepharous and catalytic properties. Nucleic Acid. Res. **9**:3589–3603.
19. **Stevens, P. J. E.** 1980. Bactericidal effect against *Escherichia coli* of nalidixic acid and four structurally related compounds. J. Antimicrob. Chemother. **6**:535–542.
20. **Tessman, E. S., and P. K. Peterson.** 1985. Isolation of protease-proficient, recombinase-deficient *recA* mutants of *Escherichia coli* K-12. J. Bacteriol. **163**:688–695.
21. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
22. **Washington, J. A. II, and V. L. Sutter.** 1980. Dilution susceptibility test: agar and macro-broth dilution procedures, p. 453–458. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
23. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.