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Effects of Quinolones on Nucleoid Segregation in *Escherichia coli*

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The effects of quinolone antibiotics on nucleoid segregation in growing *Escherichia coli* were examined by using fleroxacin (Ro 23-6240, AM 833) as a prototype compound. At levels that were close to its MIC and induced growth arrest and filamentation, fleroxacin caused large nucleoids to appear in midcell, suggesting inhibition of nucleoid segregation. With increasing fleroxacin concentrations, nucleoids became progressively smaller, suggesting inhibition of DNA replication. Removal of fleroxacin restored normal cell and nucleoid morphology in filaments with large nucleoids but not in filaments with small nucleoids. The results are consistent with inhibition of chromosome decatenation at low quinolone concentrations (bacteriostatic effect) and DNA supercoiling at high concentrations (bactericidal effect).

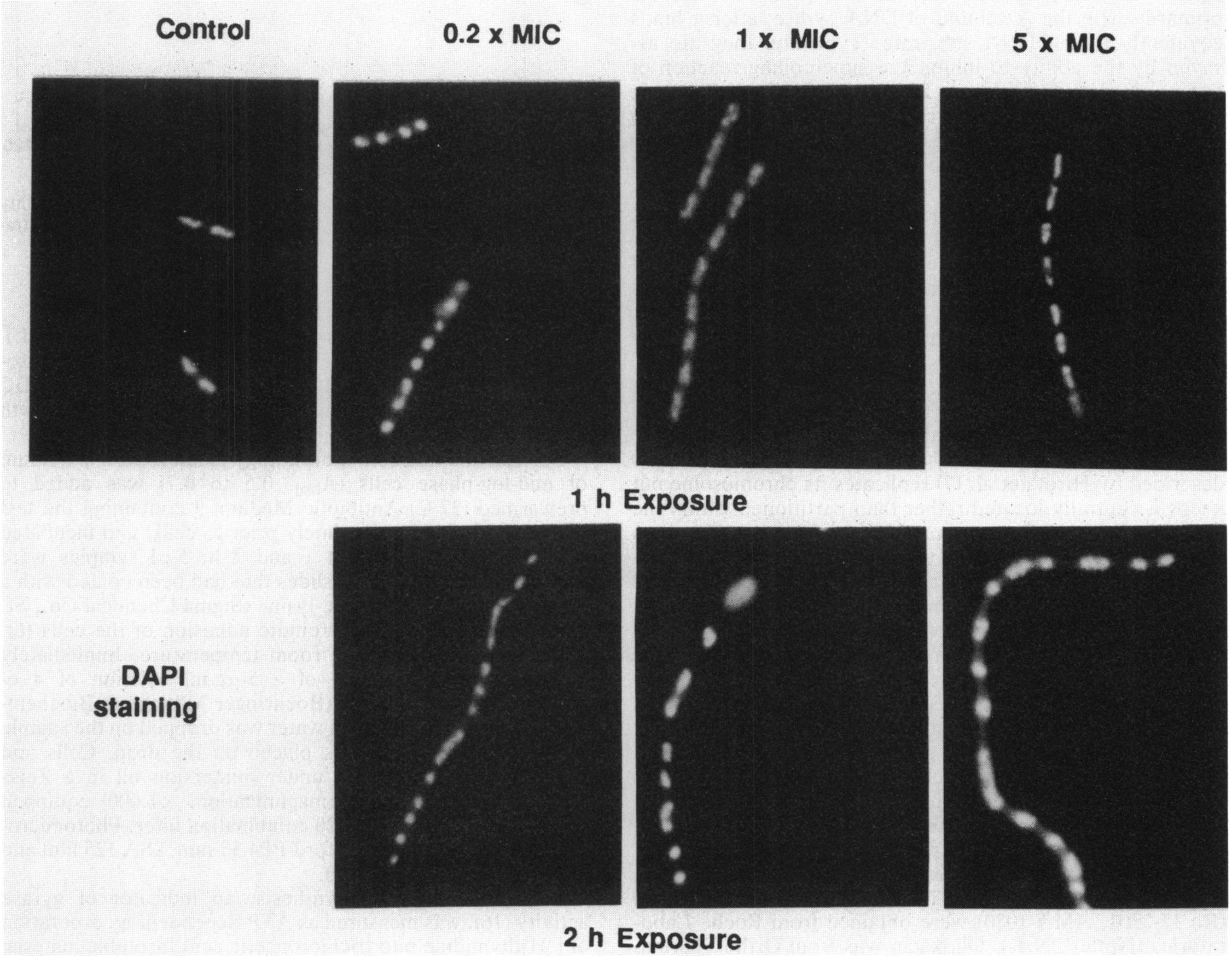

FIG. 1. Photomicrographs of *E. coli* ATCC 25922 grown for 1 or 2 h in different carumonam concentrations (shown as MIC multiples). Cells were stained with 4′,6-diamidino-2-phenylindole, and fluorescence images were taken as described in the text.

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TABLE 1. In vitro activities of quinolones and carumonam against *E. coli* and effects on replicative DNA biosynthesis

Antibacterial agent	MIC (μg/ml) for *E. coli* 25922	IC_{50}[a] (μg/ml)
Fleroxacin	0.1	0.3
Pefloxacin	0.1	0.3
Norfloxacin	0.1	0.15
Ciprofloxacin	≤0.05	0.04
Ofloxacin	0.1	0.15
Carumonam	0.2	

[a] Concentration that inhibited replicative DNA biosynthesis by 50%.

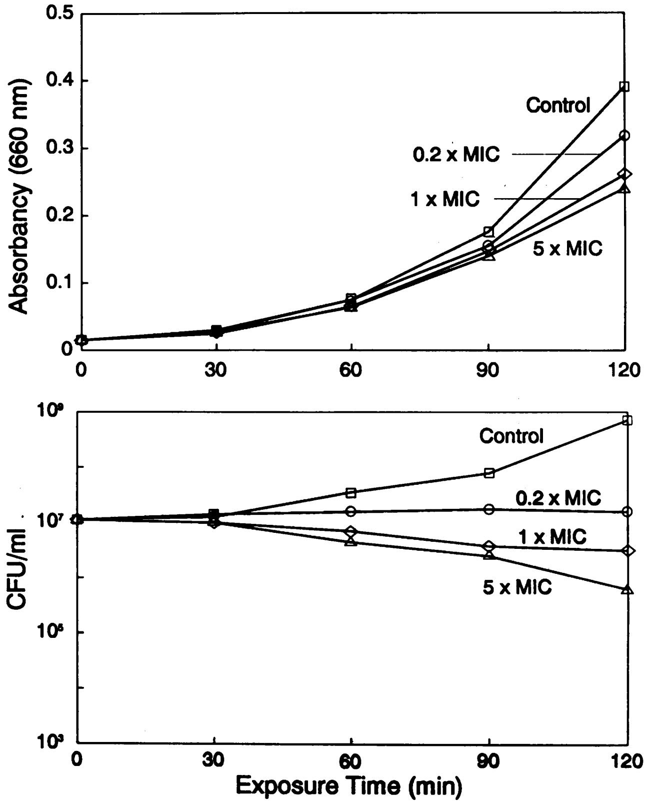

FIG. 2. Effects of carumonam on the growth (A_{660}) and viability (CFU per milliliter) of *E. coli* ATCC 25922. Carumonam concentrations are as in Fig. 1.

Quinolones are synthetic antibacterial agents with potent, broad-spectrum bactericidal activity and favorable pharmacokinetics (8, 14, 23, 24). For the past 10 years, they have been the subject of intense research and clinical interest. Their molecular target is DNA gyrase, a unique and essential bacterial enzyme involved in DNA replication, transcription, recombination, and other activities that require packaging or unpackaging of DNA (5, 20–22). Quinolones interact primarily with the A subunit of DNA gyrase, after it binds covalently to the DNA substrate. Typically, they are assayed by the ability to inhibit the supercoiling reaction of gyrase on circular relaxed DNA (supercoiling assay) or freeze the covalent DNA-gyrase complex (cleavage assay) (1). The relative activities of quinolones obtained by either assay have been used to establish structure-activity relationships (4, 19). Discrepancies between gyrase- and growth-inhibitory activities exist, the latter being generally lower than the former, sometimes by orders of magnitude (4, 25). Another complication is the recent finding of a second gyraselike enzyme (topoisomerase IV), also essential and composed of two subunits, A (75 kDa) and B (70 kDa), whose exact physiological function and quinolone sensitivity are unknown (10, 11).

In addition to supercoiling, DNA gyrase has been shown to be the major decatenating activity in *Escherichia coli* (2) and to participate in nucleoid segregation (17). One of the three classes of conditional filamenting *E. coli* mutants described by Hirota et al. (7) replicates its chromosome but keeps it centrally located rather than partitioned within the filaments, hence the name *par* mutants. Four such mutants are defective in subunits A (*parD*) and B (*parA*) of DNA gyrase or subunits A (*parC*) and B (*parE*) of topoisomerase IV (9–11). Thus, DNA gyrase may have two essential activities: supercoiling, involved in chromosome replication, and decatenation, involved in chromosome partitioning. The present study examined the effects of fleroxacin on (i) chromosome partitioning in *E. coli* by using a DNA-specific dye to visualize the chromosome and (ii) cell viability.

E. coli ATCC 25922 was purchased from the American Type Culture Collection (Rockville, Md.). *E. coli* JF568 (15) was a gift from J. Foulds of the National Institutes of Health (Bethesda, Md.); its fleroxacin-resistant mutants JSC100 and JSC101 were previously described (3). Strains were grown at 37°C in Antibiotic Medium 3 (Difco Laboratories, Detroit, Mich.). Fleroxacin (Ro 23-6240, AM 833) and carumonam (Ro 17-2301, AMA-1080) were obtained from Roche Laboratories (Nutley, N.J.). Ofloxacin was from Ortho Pharmaceutical Corp. (Raritan, N.J.); ciprofloxacin was from Miles Inc., Pharmaceuticals Division (West Haven, Conn.); norfloxacin was from Merck Sharp and Dohme Research Laboratories (Rahway, N.J.); and pefloxacin was from Rhone-Poulenc Pharmaceuticals (Monmouth Junction, N.J.). Antibiotic susceptibility was determined by the broth microdilution method (300 μl [10^5 CFU/ml] per well). The MIC was the lowest concentration that inhibited visible growth after 18 h of incubation at 37°C.

For cell and nucleoid morphology studies, a 1% inoculum of mid-log-phase cells (A_{660}, 0.5 to 0.7) was added to prewarmed (37°C) Antibiotic Medium 3 containing the test compound (added immediately prior to cells) and incubated at 37°C with shaking. At 1 and 2 h, 5-μl samples were removed, spread on glass slides that had been coated with a 10-μg/ml solution of poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.) in water to promote adhesion of the cells (6), and allowed to air dry at room temperature. Immediately before observation, 5 μl of a 5-μg/ml solution of 4′,6-diamidino-2-phenylindole (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) in water was dropped on the sample and a glass coverslip was placed on the drop. Cells and nucleoids were observed under immersion oil in a Zeiss Standard 16 microscope (magnification, ×1,000) equipped with a G365, FT 395, LP420 combination filter. Photomicrographs were taken using Ilford FP4 35-mm ASA 125 film and a camera speed of ASA 50.

Replicative DNA biosynthesis, an indicator of gyrase activity (16), was measured as ATP-dependent incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material by toluene-treated cells (13). The drug concentration that reduced label incorporation to half of that of the drug-free control was defined as the 50% inhibitory concentration.

Antibiotic susceptibility of *E. coli* ATCC 25922 and 50%

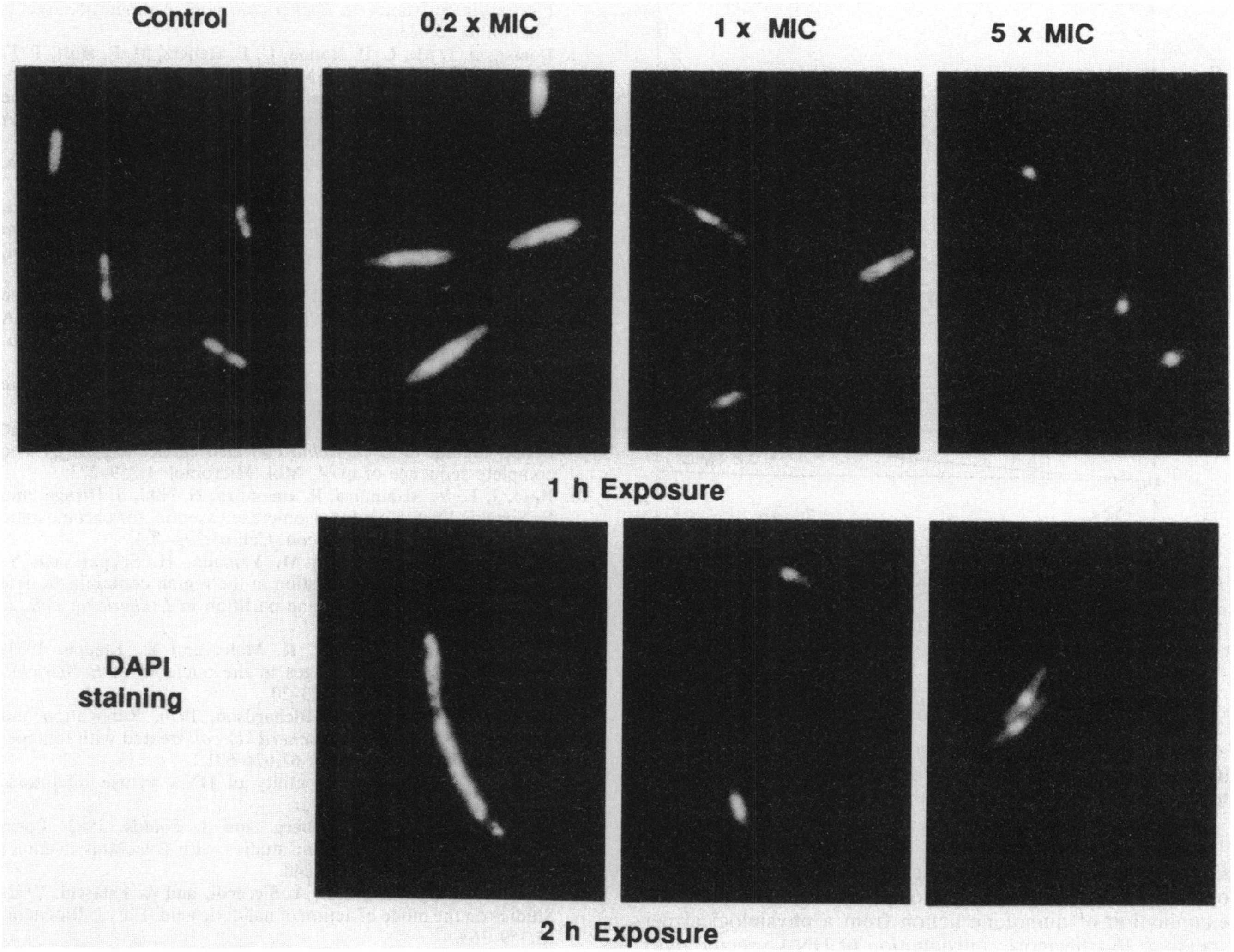

FIG. 3. Photomicrographs of *E. coli* ATCC 25922 grown for 1 or 2 h in different fleroxacin concentrations (shown as MIC multiples). Cells were stained with 4′,6-diamidino-2-phenylindole, and fluorescence images were taken as described in the text.

inhibitory concentrations for replicative DNA biosynthesis are shown in Table 1. The 50% inhibitory concentrations were similar to those previously reported for inhibition of DNA gyrase (cleavage assay) (4).

For the effects of carumonam and fleroxacin on nucleoid segregation, see Fig. 1 and 3, respectively. Carumonam, a monocyclic β-lactam that specifically inhibits septation (18), was used as a control for induction of filaments without affecting chromosome replication or partitioning. Nucleoids appeared regularly spaced within filaments at several carumonam concentrations, ranging from subinhibitory (0.2 times the MIC) to suprainhibitory (5 times the MIC) (Fig. 1). Subinhibitory concentrations of carumonam did not affect cell growth or viability, while suprainhibitory concentrations affected both (Fig. 2). Fleroxacin, on the other hand, produced shorter filaments with large nucleoids in the midcell which became progressively smaller at increasing concentrations (Fig. 3). Accordingly, the amount of DNA per cell, measured fluorometrically, decreased (data not shown). Fleroxacin concentrations that produced large nucleoids had little effect on cell mass or viability (Fig. 4). Since nucleoid segregation involves decatenation of replicated chromosomes and appropriate positioning of daughter chromosomes, fleroxacin most likely inhibits the former process at bacteriostatic concentrations. *E. coli* exposed for 2 h to 0.02 μg of fleroxacin per ml, a concentration that produced large nucleoids, followed by removal of the antibiotic and regrowth in Antibiotic Medium 3, exhibited normal nucleoid morphology after 3 h. However, in *E. coli* exposed to 0.1 μg of fleroxacin per ml (the MIC), which caused very small chromosomes to form in midcell, normal nucleoid morphology was not restored (data not shown). Pefloxacin, norfloxacin, ciprofloxacin, and ofloxacin produced similar concentration-dependent effects; filaments with large nucleoids at subinhibitory concentrations (0.2 times the MIC) became progressively smaller with increasing concentrations (data not shown).

E. coli JF568, a K-12 strain for which the fleroxacin MIC is the same as that for *E. coli* ATCC 25922, exhibited the same aberrant nucleoid morphology at the same fleroxacin concentrations as *E. coli* ATCC 25922. *E. coli* JSC100 and JSC101, two JF568-derived strains with fleroxacin-resistant DNA gyrase (3), exhibited the aberrant nucleoid morphology at 10-fold higher fleroxacin concentrations, although they were the same in relationship to MICs (data not shown).

Hirota's milestone discovery of chromosome-partitioning

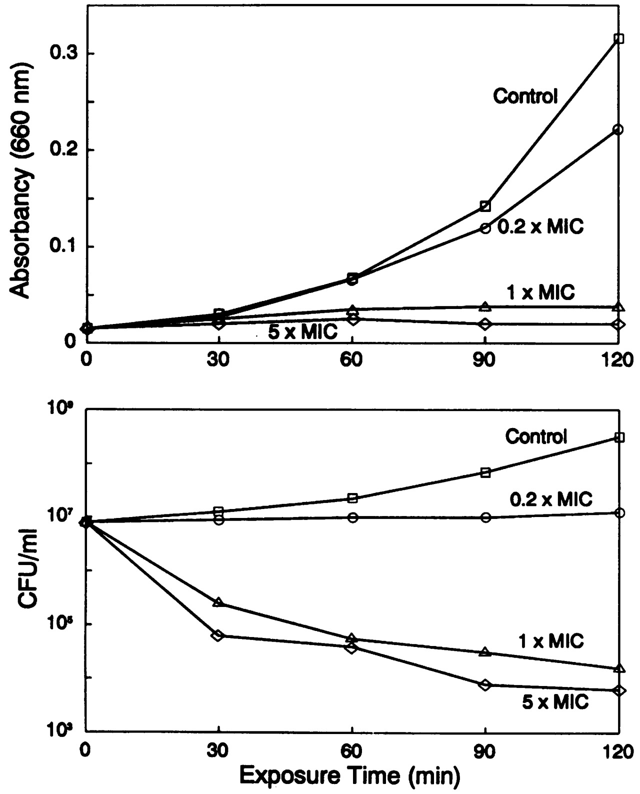

FIG. 4. Effects of fleroxacin on the growth (A_{660}) and viability (CFU per milliliter) of *E. coli* ATCC 25922. Antibiotic concentrations are as in Fig. 3.

E. coli mutants (7) and subsequent studies showing that most of them were gyrase or topoisomerase IV mutants allowed examination of quinolone action from a physiological perspective. Furthermore, introduction of DNA-specific dyes, such as 4′,6-diamidino-2-phenylindole, greatly simplified the methodology for visualizing chromosomes (6). The present study of quinolone effects on chromosome replication and partitioning in growing *E. coli* is thus a logical extension of these earlier studies. It suggests that quinolones inhibit both nucleoid segregation and DNA replication, although the possibility that inhibition of the former process is secondary to the SOS response cannot be excluded (12). Further studies are needed to determine whether two distinct enzymes, such as gyrase and topoisomerase IV, are involved in the mechanism of action of quinolones.

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