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Induction of DnaK and GroEL Heat Shock Proteins by Fluoroquinolones in *Escherichia coli*

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Various fluoroquinolones (norfloxacin, enoxacin, ofloxacin, levofloxacin, and sparfloxacin) induce DnaK and GroEL heat shock proteins in *Escherichia coli*. The induction is transient, consistent with the kinetics of cellular DNA relaxation. The concentrations of fluoroquinolones required for the induction are similar to those required for DNA relaxation and much higher than those required for cell death.

When cells are exposed to high temperatures or chemicals, heat shock proteins are induced (12, 18, 19, 25, 27). These proteins act as molecular chaperones to reactivate denatured proteins (3, 4, 6, 31) and are considered necessary to maintain homeostasis in cells under stress conditions. Genetic analysis of the heat shock response in *Escherichia coli* has revealed that σ^{32}, encoded by the *rpoH* gene, is essential for the heat shock response (7, 8, 11, 17, 19, 26, 29, 30). The common sensor which recognizes the various stresses inducing the heat shock response, however, remains to be identified.

We previously reported that cellular plasmid DNA relaxes in association with induction of heat shock proteins by various stresses (10, 14). The DNA relaxation was observed in an *rpoH* mutant in which heat shock proteins were not induced by the stress, thereby indicating that DNA relaxation is not the result of induction of heat shock proteins (14). Based on these results, we proposed that cells recognize various stresses through DNA relaxation, which leads to the heat shock response.

Fluoroquinolones have broad-spectrum activity against gram-positive and -negative bacteria (28) and are used clinically to treat infections. Biochemical and genetic studies have revealed that DNA gyrase is a target of fluoroquinolones (2, 5, 9, 22). Recently, we found that fluoroquinolones transiently relax DNA in *E. coli* at concentrations higher than those required to kill (13). These results prompted us to examine whether fluoroquinolones induce the heat shock response. Here, we report induction of heat shock proteins by fluoroquinolones. Our results show that this induction correlates well with DNA relaxation.

Norfloxacin was provided by Kyorin Pharmaceutical Co. Enoxacin and sparfloxacin were provided by Dainippon Pharmaceutical Co. Ofloxacin and levofloxacin were provided by Daiichi Pharmaceutical Co. The bacterial strains used were derivatives of *E. coli* K-12 (1). W3110 was from our laboratory stock. KY1429 (*rpoH6*) (26) was kindly provided by T. Yura (HSP Research Institute). P1 transduction of the *rpoH6* mutation to W3110 was performed as described in our previous reports (15, 16, 23). Concentrations of drugs necessary for 50% inhibition of colony formation (IC_{50}) were determined as described in reference 13). Protein pulse-labeling experiments and identification of DnaK and GroEL proteins were performed as described previously (25). Analysis of plasmid DNA supercoiling in cells was also done as described previously (14, 15, 20, 21).

We first examined the influence of fluoroquinolones on the pattern of protein synthesis by protein pulse-labeling experiments. Figure 1A shows that sparfloxacin induces DnaK and GroEL proteins, the major heat shock proteins of *E. coli*, in a dose-dependent manner. A densitometric scanning analysis revealed that synthesis of DnaK and GroEL proteins was stimulated two- and sixfold, respectively, by 10 μg of sparfloxacin per ml (Fig. 1B). The concentration of sparfloxacin required for induction of heat shock proteins was much higher than the IC_{50} of the drug (0.04 μg/ml). On the other hand, more than 1 μg of sparfloxacin per ml was necessary to cause DNA relaxation (Fig. 1C). Thus, similar concentrations of this drug were needed for both induction of heat shock proteins and DNA relaxation. As shown in Fig. 1A, 10 μg of sparfloxacin per ml did not induce DnaK and GroEL proteins in an *rpoH* mutant, indicating that the heat shock response induced by sparfloxacin is dependent on the function of σ^{32}. Because DNA relaxation induced by sparfloxacin occurred in the *rpoH* mutant (Fig. 1C), we concluded that it was not due to the heat shock response. DNA relaxation by sparfloxacin is transient: relaxed DNA supercoils again to its original state within 60 min after the addition of sparfloxacin (13). In this study, we examined the time course of the heat shock response caused by fluoroquinolones. As shown in Fig. 2A, the rate of synthesis of heat shock proteins reached a maximum 10 min after exposure to the drug and decreased gradually. After 60 min, the rate of synthesis of DnaK and GroEL proteins was similar to that before the addition of the drug. We confirmed that DNA relaxation by sparfloxacin was transient under the same condition as that for the protein pulse-labeling experiments (Fig. 2B). Thus, the kinetics of the induction of heat shock proteins and of DNA relaxation by sparfloxacin were similar. Similar results were obtained for fluoroquinolones other than sparfloxacin (norfloxacin, ofloxacin, levofloxacin, and enoxacin) (data not shown).

Heat shock proteins help maintain cellular homeostasis under stress conditions. By using the *rpoH* mutant, which cannot produce heat shock proteins, we examined whether the heat shock response contributes to bacterial survival in the presence of fluoroquinolones. The IC_{50} of sparfloxacin, enoxacin, norfloxacin, ofloxacin, and levofloxacin in the *rpoH* mutant were similar to those in wild-type cells (data not shown). It is known that the dose-response curve of the bactericidal activity of fluoroquinolones shows a reversal pattern: the bactericidal ac-

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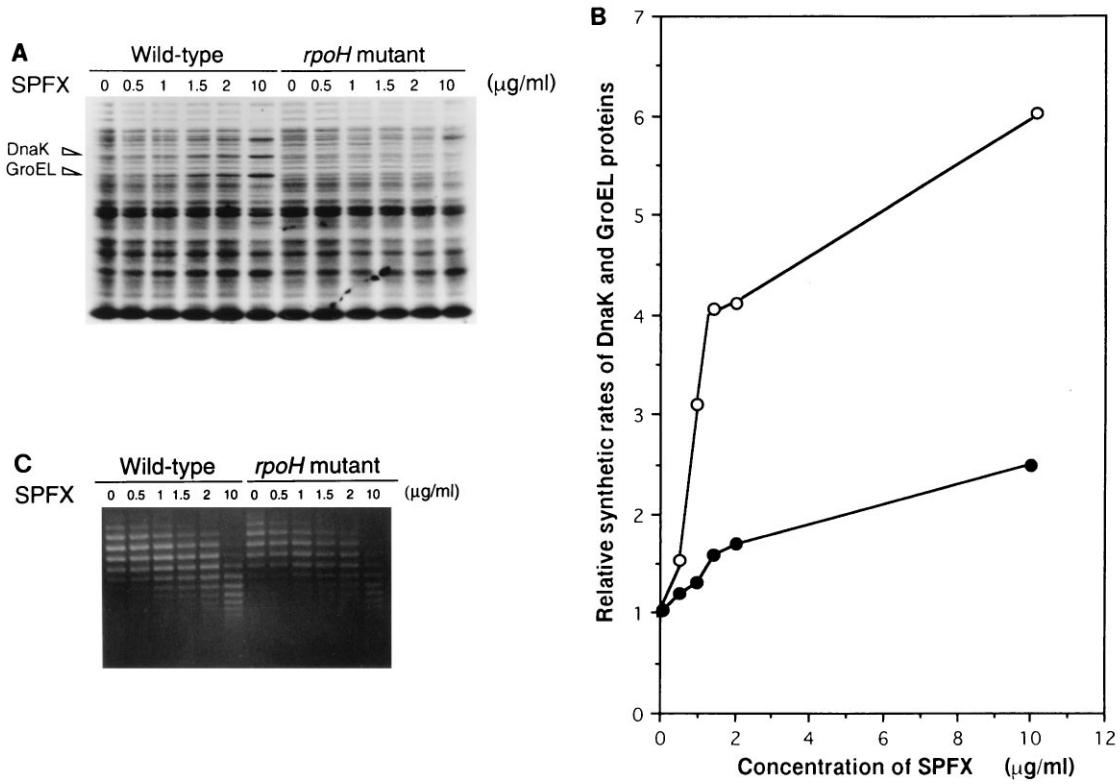

FIG. 1. Effects of sparfloxacin (SPFX) on induction of heat shock proteins and DNA relaxation. (A) *E. coli* cells harboring pUC118 were incubated at 30°C with the indicated concentrations of sparfloxacin for 10 min. Proteins were pulse-labeled with [^{35}S]methionine-cysteine. Cells were harvested by centrifugation, and proteins were separated by sodium dodecyl sulfate-polyacrylamide (10%) gel electrophoresis followed by fluorography. (B) Protein bands in the fluorograms were scanned with a densitometer, and the relative rate of synthesis of each protein was determined (●, DnaK; ○, GroEL). (C) pUC118 was extracted and analyzed by 1% agarose gel electrophoresis in the presence of 15 μg of chloroquine per ml. Under this condition, more relaxed DNA migrates faster.

tivity of these drugs decreases with increasing concentrations at doses higher than the IC_{50} (24). Because the concentrations of fluoroquinolones at which this phenomenon is observed are much the same as those required for the heat shock response, we considered the possibility that the heat shock response is involved in the reversal phenomenon. However, as shown in Fig. 3, the dose-response curves of sparfloxacin and norfloxacin showed the reversal phenomenon in the *rpoH* mutant to the same degree as in the wild-type strain. The results indicate that the heat shock response induced by fluoroquinolones does not affect the reversal phenomenon of these drugs. We thus conclude that induction of heat shock proteins does not affect the bactericidal action of fluoroquinolones.

In this study, we demonstrated the induction of heat shock proteins by fluoroquinolones in *E. coli*. We focused on the concentrations of fluoroquinolones required for the induction of heat shock proteins. Considering the close relationship between DNA relaxation and the heat shock response in *E. coli* cells (10, 14), induction of heat shock proteins was predicted to require much the same concentrations of fluoroquinolones as DNA relaxation, which are much higher than the IC_{50} of these drugs. On the other hand, given that heat shock proteins are required for cell survival under stress conditions, it was reasonable to assume that induction of heat shock proteins requires fluoroquinolone concentrations close to the IC_{50} for these drugs. The results of our study clearly demonstrated that induction of heat shock proteins by fluoroquinolones correlates well with DNA relaxation but not with cell death. There-

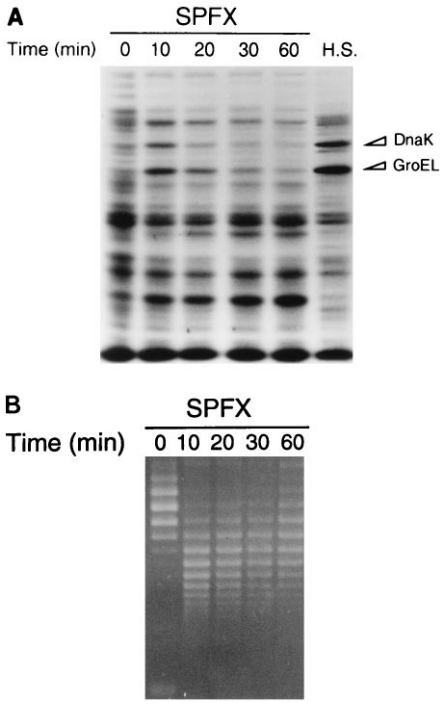

FIG. 2. Time course of induction of heat shock proteins and DNA relaxation by sparfloxacin (SPFX). Exponentially growing cells harboring pUC118 were incubated at 30°C with 10 μg of sparfloxacin per ml for the indicated periods. Protein pulse-labeling experiments (A) and analysis of DNA supercoiling (B) were performed as described in the legend to Fig. 1.

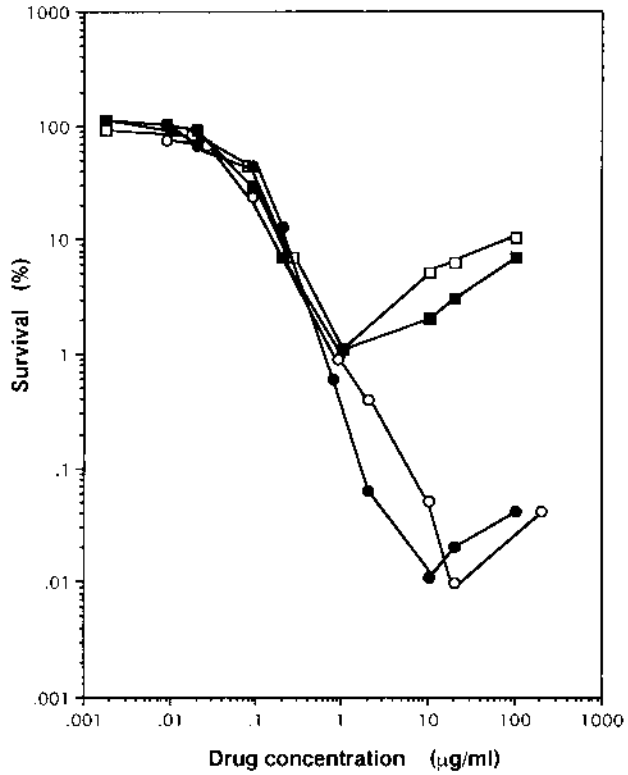

FIG. 3. Bactericidal activities of sparfloxacin and norfloxacin against an *rpoH* mutant and the wild-type strain. Exponentially growing cells (● and ■, wild type; ○ and □, *rpoH* mutant) were incubated with the indicated concentrations of sparfloxacin (● and ○) or norfloxacin (■ and □) for 60 min. The cultures were appropriately diluted, spread on Luria-Bertani agar plates, and incubated at 28°C for 24 h. Cell survival was determined by counting the number of colonies.

fore, the induction of heat shock proteins by fluoroquinolones does not contribute to cell survival in the presence of drugs.

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