Enhancement by Nalidixic Acid of the Thermal Susceptibility of the Ts-7 Mutant of *Escherichia Coli* TAU-Bar

MIKIO NISHIDA, YUKIO MISHIMA, JUN KAWADA, AND K. LEMONE YIELDING*

Laboratory of Molecular Biology, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294

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Nalidixic acid at $5 \times 10^{-8}$ M produced a substantial increase in thermal susceptibility of Ts-7, suggesting either that the thermal and nalidixic acid targets are identical or closely interdependent.

Nalidixic acid, a derivative of naphthyridine, is a potent bactericidal compound whose precise mode of action is unknown. The drug was reported to be a specific inhibitor of bacterial deoxyribonucleic acid (DNA) synthesis and replication (3, 4, 5), and of DNA repair synthesis as shown by the accumulation of small-molecular-weight DNA after extensive ultraviolet (UV) irradiation (4). Recent experiments with toluene-induced cells, however, have shown only inhibition at the growing fork, with no effect on repair synthesis (5). Various enzymes involved in DNA metabolism were found not to be affected by the drug (2, 11). When assayed in vitro no evidence could be found for direct binding of nalidixic acid to DNA (1). Nalidixic acid resistance loci have been mapped for *Escherichia coli* K-12 (8), and a specific protein factor has been proposed for conferring nalidixic acid susceptibility (1).

Our studies have shown decreased post-UV survival of *E. coli* plated on nalidixic acid, without a corresponding inhibition of liquid holding recovery after UV, but with complete photorecovery (M. Nishida, N. Nakamura, and K. L. Yielding, manuscript in preparation). In an attempt to localize this presumed drug effect on UV repair, we have found that Ts-7, a mutant of *E. coli* TAU-bar (9, 10) displaying impaired DNA ligase function and UV susceptibility at elevated temperature, also shows an increased thermal susceptibility in the presence of nalidixic acid. These results are reported here with the hope they will provide an additional clue to the locus of drug action.

The temperature-sensitive mutant Ts-7 derived from *E. coli* TAU-bar grows normally at 25 C but shows a time-dependent loss of viability with initial growth above 40 C (9, 10). Both strains were kindly supplied to us by Roy Curtiss of this institution. Cells were grown to late log phase in tryptone broth (10 g of tryptone [Difco], 5 g of NaCl) with shaking at 25 C, diluted to appropriate density with 0.9% NaCl, and plated on tryptone-NaCl agar (1.5% agar) with and without added nalidixic acid. An initial period of incubation at 40 C followed by continued culture at 25 C was employed to express the temperature sensitivity. The sequences of incubation temperatures and drug concentrations are indicated in the legends.

The dose response curve for Ts-7 (Fig. 1) showed that survival at 25 C was unaffected by drug concentrations below $5 \times 10^{-8}$ M. However, this concentration of drug was found to produce a sharp increase in the time-dependent loss of viability resulting from an initial period of growth at 40 C in the mutant, but not in the parent strain (Fig. 2). This difference in susceptibility was also reflected in a change in the apparent dose response curve of Ts-7 for nalidixic acid when plates were incubated initially at 40 C prior to culture at 25 C (Fig. 3). Strain N-1635, a temperature-sensitive ligase mutant studied in Gellert’s laboratory (6), was obtained from Rolf Stemgalz and, unlike Ts-7, did not show any increase in susceptibility to nalidixic acid at 40 C (data not shown). These results suggested that the target of action for nalidixic acid corresponds to the temperature-sensitive target of Ts-7. An alternate explanation would be that the thermal insult has made the organism more susceptible to nalidixic acid. This possibility was tested by applying filter paper disks containing the drug at $10^{-8}$ M on inoculated plates immediately and after preliminary inoculation at 25 or 40 C. These results indicated equal susceptibility as measured by the size of the killing circles, whether drug application followed exposure at 25 or 40 C. In contrast, application of the drug prior to incubation at 40 C produced a substantially large zone of killing.

A specific replicating protein other than the classic enzymes of replication has been pro-
FIG. 1. Effect of nalidixic acid on temperature-sensitive mutant of Escherichia coli, Ts-7. Late log-phase cells were diluted and plated on tryptone-NaCl agar containing the indicated amounts of the drug (see text). Plates were incubated at 25°C for 24 to 36 h to produce colonies large enough to count. Dotted line shows the survival fractions at the concentration of $5 \times 10^{-5} M$ nalidixic acid.

FIG. 2. Effects of nalidixic acid on the temperature sensitivities of Escherichia coli Ts-7 and TAU-bar. Ts-7 and its parent strain TAU-bar were cultured as in Fig. 1. Cells were diluted appropriately and plated on agar without or with nalidixic acid at $5 \times 10^{-5} M$. Half of the plates were incubated at 25°C and half were incubated at 40°C for the indicated periods in the figure and then continued at 25°C. Each point on the curves is an average number of two identical plates. Symbols: O, Ts-7 without nalidixic acid; •, Ts-7 with nalidixic acid; △, TAU-bar without nalidixic acid; ▲, TAU-bar with nalidixic acid.

FIG. 3. Enhanced killing effect of nalidixic acid on temperature-sensitive mutant of Escherichia coli at higher temperatures. Ts-7 and the parent strain, TAU-bar, were cultured as in Fig. 2 and plated on tryptone-NaCl agar without and with nalidixic acid as indicated. Plates of both strains were incubated at 25°C with or without an initial period of 2 h at 40°C. Each point represents an average of two duplicate experiments. Surviving fractions for each curve are expressed relative to survival in the absence of nalidixic acid. Symbols: O, Ts-7; •, Ts-7, 40°C for 2 h, then 25°C; △, TAU-bar, 25°C; ▲, TAU-bar 40°C for 2 h, then 25°C.

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