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# Lon Protease Is Essential for Paradoxical Survival of *Escherichia coli* Exposed to High Concentrations of Quinolone▽

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**A deficiency of the *Escherichia coli* Lon protease blocked paradoxical survival occurring at very high nalidixic acid concentrations. The absence of Lon also blocked a parallel increase in cell lysate viscosity likely to reflect DNA size. Thus, Lon may participate in repairing quinolone-mediated DNA lesions formed at high drug concentrations.**

The quinolones are lethal antibacterials that form drug-gyrase/topoisomerase IV-DNA complexes in which DNA is broken. Release of DNA ends from protein-mediated constraint allows chromosome fragmentation (1, 9). An unexplained aspect of quinolone action is a paradoxical increase in survival occurring at very high drug concentrations (2). To explore protease involvement in paradoxical survival, we treated *Escherichia coli* with nalidixic acid and examined effects of *lon* mutations on survival and on the presence of chromosomal DNA lesions, the latter assayed by an empirical measure of cell lysate viscosity. The Lon protease is known to degrade damaged proteins and proteins produced in excess (15); it also serves as a chaperone (6, 15). However, a role in chromosome maintenance has not been reported. In the present work a deficiency of Lon protease activity lowered survival at high concentrations of nalidixic acid with little effect at maximal bactericidal concentration.

*E. coli* K-12 strains, listed in Table 1, were grown in Luria-Bertani (LB) liquid medium and on LB agar plates (10) at 30°C; nalidixic acid was obtained from Sigma Chemical (St. Louis, MO). The effect of nalidixic acid was monitored by measuring lethal action and cell lysate viscosity (9). Lethal action was assayed by incubating exponentially growing cultures at 30°C for 180 min with nalidixic acid followed by drug removal using brief centrifugation, dilution, and enumeration of CFU by incubation on drug-free agar. Data were expressed relative to an untreated control taken at the time of drug addition. For viscosity, cell lysates were prepared as for isolation of bacterial nucleoids (cells were harvested by centrifugation, treated with lysozyme, and incubated for several minutes at 20°C with nonionic detergents [9]), and then sodium dodecyl sulfate was added to 0.8% to release DNA breaks from constraint by protein. Viscosity was estimated by measuring the time required to fill a 25-μl glass capillary (9) at several DNA concentrations to estimate specific viscosity.

We began by confirming general features of paradoxical survival: it was observed with a variety of quinolones (nalidixic acid, norfloxacin, and ciprofloxacin), several incubation temperatures (30, 37, and 42°C), and two growth media (LB and nutrient broth [not shown]). We also found that paradoxical survival associated with nalidixic acid treatment was unaffected by a noninducible *lexA3* mutation (not shown) or by concurrent

TABLE 1. Bacterial strains

| Strain | Relevant genotype | Source or reference |
|---|---|---|
| DM4100 | Wild type | 13 |
| KD2140 | DM4100 *gyrA67* (A67S) *gyrB225* | This work by transduction (17) from strain KD1911 (9) and *gyrB225* from strain SD104-20 (3); large-colony variant[a] |
| KD2372 | DM4100 *lon-100* | This work by transduction (17) from strain SG20252 (14) |
| KD2377 | KD2372 (*lon-100*) *sulA3* | This work by transduction (17) from strain KL789 (11) (CGSC7699[b]) |
| KD2510 | DM4100 *sulA3* | This work by transduction (17) from strain KL789 (11) (CGSC7699[b]) |
| KD3037 | KD2377 pBAD24 | 16[c] |
| KD3039 | KD2377 pBAD24*lon* S679A | 16[c] |
| KD3041 | KD2377 pBAD24*lon* K362Q | 16[c] |
| KD3045 | KD2377 pBAD24*lon*$^+$ | 16[c] |

[a] Chloramphenicol blocks lethal action of nalidixic acid with wild-type cells but not with *gyrA67* mutants; large-colony variants show a strong paradoxical effect.
[b] *E. coli* Stock Center collection.
[c] Plasmids were introduced by bacterial transformation with selection for ampicillin resistance. In these plasmids *lon* was under the control of the pBAD arabinose promoter. The Lon S679A variant was deficient in protease activity; the K362Q variant was deficient in ATPase activity (16).

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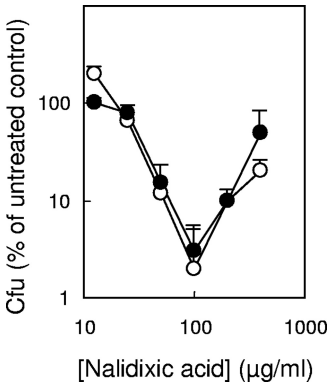


FIG. 1. Paradoxical survival in the presence of chloramphenicol. An exponentially growing culture of strain KD2140 (*gyrA67 gyrB225*) was treated with 20 μg/ml chloramphenicol for 10 min prior to addition of the indicated concentrations of nalidixic acid for an additional 180 min, at which time samples were diluted, applied to drug-free agar, and incubated to determine bacterial survival. Symbols: filled circles, cultures with chloramphenicol; open circles, cultures without chloramphenicol. Similar results were obtained in a replicate experiment.

chloramphenicol treatment of a *gyrA67 gyrB225* mutant (Fig. 1) (the two gyrase mutations separately interfere with the ability of chloramphenicol to block quinolone-mediated killing [7, 9]; together they show strong paradoxical survival). Thus, neither the SOS response (8) nor protein synthesis is required for observation of paradoxical survival, a phenomenon that is common to several different quinolones and growth conditions.

To examine the involvement of Lon protease, we treated a *lon*-deficient strain (KD2372) with nalidixic acid at a variety of concentrations. At maximal bactericidal concentrations, survival was similar to that of wild-type cells, but at very high concentrations, wild-type survival was about 3 orders of magnitude greater (Fig. 2A). Since accumulation of SulA (SfiA) causes *lon*-deficient mutants to be sensitive to DNA-damaging agents (4, 5), we also examined lethal action in a *lon sulA* double mutant. Such a mutant behaved similarly to a Lon-deficient strain, and a *sulA* mutant exhibited paradoxical survival similar to the wild-type strain (Fig. 2B). Thus, paradoxical survival requires Lon activity but not through its effect on SulA stability.

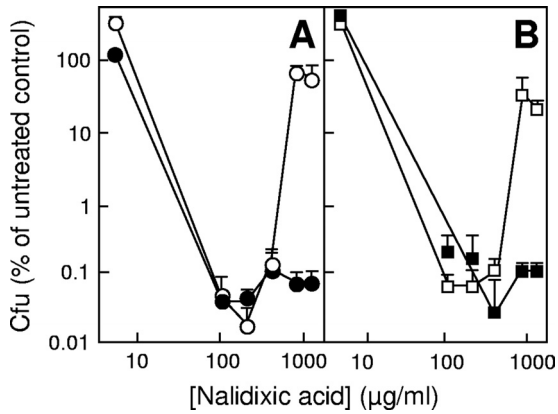


FIG. 2. Effect of *lon-100* and *sulA3* mutations on survival during treatment with nalidixic acid. Exponentially growing cultures were treated with the indicated concentrations of nalidixic acid for 180 min. The cells were then concentrated by centrifugation, resuspended in medium, diluted, and plated. (A) Lon deficiency. Open circles, wild-type strain DM4100; filled circles, *lon-100* strain KD2372. (B) SulA deficiency. Open squares, *sulA3* strain KD2510; filled squares, *lon-100 sulA3* strain KD2377. Similar results were observed in three replicate experiments.

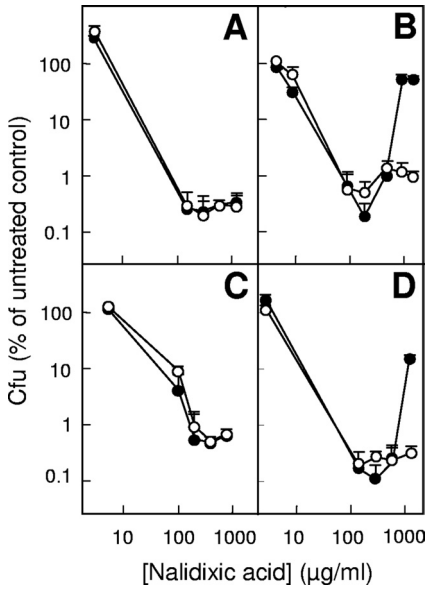


FIG. 3. Effect of expression of Lon variants on survival during treatment with nalidixic acid. Exponentially growing cells deficient in chromosomal *lon* and containing pBAD24 vector (A) (strain KD3037), pBAD24*lon*$^+$ (B) (strain KD3045), pBAD24*lon* S679A (C) (strain KD3039), or pBAD24*lon* K362Q (D) (strain KD3041) were cultured with 0.1% arabinose (filled circles) or without arabinose (open circles) and treated with nalidixic acid as described in the legend to Fig. 1.

Lon protease has multiple domains, one functioning as an ATPase and another as a protease (16). We asked whether both or only one Lon activity is required for paradoxical survival by using mutant *lon* genes expressed from plasmids. A *lon*-deficient strain (KD3037) containing the pBAD24 vector-only control showed little paradoxical survival in the presence or absence of 0.1% arabinose, the inducer used for this expression vector (Fig. 3A). These data demonstrated that no plasmid component contributed to survival at high quinolone concentration. When the plasmid contained wild-type *lon* (strain KD3045), paradoxical survival was observed when arabinose was present but not when arabinose was absent (Fig. 3B). A strain (KD3039) containing pBAD24*lon* S679A that lacked protease activity failed to show the paradoxical effect (Fig. 3C), while a strain (KD3041) having pBAD24*lon* K362Q deficient in ATPase activity did (Fig. 3D). Neither strain showed paradoxical survival in the absence of arabinose (Fig. 3C and D). These data confirmed that Lon is necessary for paradoxical survival and indicated that activity of its ATPase is dispensable. A comparable experiment using complementation of UV sensitivity and capsule overproduction as assays led to the conclusion that protease activity rather than ATPase activity is dispensable (16). The reason for this difference is currently not understood.

An empirical viscometric assay was used to examine the effect of nalidixic acid concentration on chromosomal quino-

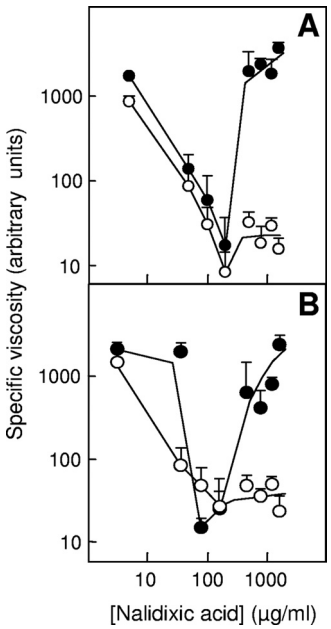


FIG. 4. Viscosity of nucleoids from wild type and *lon* mutant exposed to nalidixic acid. Wild-type (filled circles) and *lon* mutant (KD2372, open circles) cultures were incubated with nalidixic acid for 180 min (A) or 15 min (B). Cells were lysed gently, and sodium dodecyl sulfate was added to denature proteins. Similar results were obtained with five replicate experiments.

lone-gyrase-DNA complexes. Cells were exposed to various concentrations of nalidixic acid, gently lysed, and treated with sodium dodecyl sulfate to unfold chromosomes and release broken DNA from cleaved complexes. Viscosity of lysates from cells exposed to various nalidixic acid concentrations for 180 min exhibited a response similar to that seen when survival was measured with both wild-type and *lon*-deficient cells (Fig. 4A). These data are consistent with chromosomal DNA breaks being more prevalent when cells are treated with bactericidal concentrations of nalidixic acid than with much higher concentrations and with Lon playing a role in reducing the number of DNA breaks.

Since quinolone-gyrase-DNA complexes form quickly after addition of quinolone (12), while cell death is a slower process (9), we also measured lysate viscosity after a brief, 15-min treatment with nalidixic acid. Wild-type bacterial lysates exhibited a minimum in viscosity that occurred at the same nalidixic acid concentration as that with drug treatment for 180 min; when drug concentration was very high, lysates from a *lon*-deficient mutant were less viscous than those from wild-type cells (Fig. 4B). Thus, cell death is not required for observation of *lon*-dependent changes in lysate viscosity.

In eukaryotic systems proteosome activity facilitates processing of DNA lesions generated by trapping of DNA topoisomerase II (18). Lon may serve a similar role for an undefined repair process with bacterial gyrase trapped on DNA by quinolones. In the present case, we observed the Lon effect only at very high concentrations of nalidixic acid, suggesting that complexes formed at high drug concentrations differ from those formed at optimal bactericidal concentrations. An understanding of the structural differences between complexes formed at optimal and at very high quinolone concentrations should help solve the paradox of increased survival at high quinolone concentrations. We are now examining Lon action biochemically to determine whether it acts directly on the complexes.

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