The Sensitivity of Suppressed and Unsuppressed lon Strains of Escherichia coli to Chemical Agents which Induce Filamentation

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SUMMARY

The sensitivities of lon strains of Escherichia coli to the filament inducing agents nalidixic acid, gentian violet, crystal violet and penicillin were examined. The strains used included those in which the lon gene is suppressed by sul, exr and rec as well as strains in which the Lon phenotype is expressed. The sensitivity of the bacteria to agents which act on DNA was reduced when lon was suppressed, but the extent of the decrease in sensitivity was dependent on the mechanism by which suppression was effected. Sensitivity to penicillin, on the other hand, was reduced only in the sul strain and by a mechanism which appears to be independent of the direct suppression of lon. A model to account for these results indicates possible sites at which the lon, sul, exrA and recA genes are expressed.

INTRODUCTION

Many factors control the radiation sensitivity of strains of Escherichia coli. Apart from the well-established enzymatically controlled repair processes which reduce the expression of radiation damage (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964; Rupp & Howard-Flanders, 1968), a major factor in determining radiation response is the sensitivity of processes directly involved in mechanisms controlling division of the bacteria. In some strains, septum formation, and therefore complete division of irradiated bacteria, is inhibited without significant concomitant effects on increase in mass, so that they grow into non-septate multinucleated filaments which eventually lyse (Witkin, 1947; Alper, 1957; Van de Putte, Westenbroek, & Rösch, 1963; Adler & Hardigree, 1965; Brown & Gillies, 1972). This defect, the expression of a gene originally called fil+ in E. coli B and lon in strains of E. coli K12 but now generally referred to as lon, is mainly responsible for the high radiosensitivity of such strains. In several strains of E. coli the Lon phenotype is suppressed by other genes such as sul, exr and rec (Donch, Green & Greenberg, 1968; Donch, Chung & Greenberg, 1969; Green, Greenberg & Donch, 1969), so that after irradiation little or no filamentation is observed. In the sul strain E. coli B/r the suppression of lon confers radiation resistance (Donch et al., 1969), but the same effect is not observed in exrA and recA strains and this is presumably because of the marked radiation sensitivity arising directly from these mutations (Howard-Flanders & Boyce, 1966; Mattern, Zwenk & Rösch, 1966). The mechanisms by which expression of the Lon phenotype is suppressed are unknown at present.

Several chemical agents also cause filamentation in unsuppressed lon strains, e.g. nalidixic acid (Kantor & Deering, 1968; Walker & Pardee, 1968), gentian violet (Normark & Westling, 1971), crystal violet (Walker, Shafiq & Allen, 1971), and penicillin (Leighton &
Table 1. Relevant markers and source of Escherichia coli strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>B</td>
<td>lon sul+</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>Bp-2</td>
<td>lon sul+ exrA</td>
<td>M.H.L. Green</td>
</tr>
<tr>
<td>Bp-11</td>
<td>lon sul+ recA</td>
<td>M.H.L. Green</td>
</tr>
<tr>
<td>Bp+uv</td>
<td>lon sul+ uvr</td>
<td>M.H.L. Green</td>
</tr>
<tr>
<td>Bp/F+</td>
<td>lon sul+ F+ lon+</td>
<td>Laboratory stock (Farnsworth &amp; James, 1972)</td>
</tr>
<tr>
<td>B/F</td>
<td>lon sul</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>SB3100</td>
<td>lon sul/F' ara+</td>
<td>E. Englesberg</td>
</tr>
</tbody>
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Donachie, 1970). The sites of action of some of these agents are known. Nalidixic acid and gentian violet cause filamentation by inhibiting DNA synthesis, whereas the activity of penicillin is restricted to the bacterial envelope. The mechanism of the action of crystal violet is less well understood.

This paper describes the effect of these agents on the colony-forming ability of several lon mutants of Escherichia coli and derivatives which carry mutations at sul, recA, exrA and uvr loci. A model is put forward to account for the observations and to suggest sites in the bacterium at which these genes are expressed.

METHODS

Bacterial strains. The relevant genotypes and sources of the strains of Escherichia coli used are indicated in Table 1. Stock cultures of these strains were maintained on slopes of Oxoid Blood Agar Base (BAB) at 4 °C.

Growth conditions. Strains were grown overnight in Oxoid nutrient broth at 37 °C. They were then diluted 1:10 into fresh broth and incubation continued for a further 2 h. For treatment with gentian violet, crystal violet and benzyl penicillin, it was convenient to incubate the bacteria, at suitable dilution, for 24 h on plates of BAB containing different concentrations of the agents. Colony counts were made at this time. For treatment with nalidixic acid, a few experiments only were made in this way because growth on BAB containing as little as 5 µg of the drug/ml was too slow to allow colony counts to be made after 24 h of incubation. Instead, the bacteria were diluted 1:5 into Oxoid nutrient broth containing nalidixic acid (100 µg/ml) and incubation continued at 37 °C. Samples of the suspension were removed at intervals, diluted in 0.067 M-phosphate buffer and plated on BAB. The conclusions drawn concerning the relative sensitivity of the bacteria to nalidixic acid were unaffected by the particular method used.

RESULTS

Sensitivity to nalidixic acid. The effect on survival of incubating different strains of Escherichia coli in nutrient broth containing nalidixic acid (100 µg/ml) is shown in Fig. 1. The rate of killing of E. coli B was much more rapid than that of E. coli B/F because of the marked filamentation induced in the unsuppressed lon strain by nalidixic acid (Green, Donch, Chung & Greenberg, 1969). That this is the main cause of the difference in sensitivity of these strains was supported by the finding that F-duction of lon+ into E. coli B by the method of Farnsworth & James (1972) made this strain almost as resistant to nalidixic acid as E. coli B/F. Escherichia coli Bp-14, which lacks the repair enzyme system controlled by one of the uvr genes, was as sensitive to nalidixic acid as E. coli B, but sensitivity to this agent was markedly reduced in the recA strain (E. coli Bp-11) and in the exrA strain (E. coli Bp-2). Similar
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Fig. 1. Survival of *Escherichia coli* strains incubated with nalidixic acid. Bacteria were incubated at 37 °C with shaking in nutrient broth containing 100 μg nalidixic acid/ml for the times indicated before plating on BAB. ○, *E. coli B*; ●, *E. coli B/r*; ◇, *E. coli B/F` lon*; ■, *E. coli B`lon*; □, *E. coli B`n+*; ■, *E. coli B`n-12*.

Fig. 2. Survival of *Escherichia coli* strains incubated with gentian violet. Bacteria were plated at suitable dilutions on BAB plates containing various concentrations of gentian violet and incubated overnight at 37 °C. Symbols as for Fig. 1.

observations on the *rec* and *exr* strains were made by Green, Donch, Chung & Greenberg (1969).

*Sensitivity to gentian violet.* Gentian violet produced a response similar to that caused by nalidixic acid in the different strains tested (see Fig. 2).

*Sensitivity to crystal violet.* The effect of this compound on viability was examined in three strains only (Fig. 3). Again *Escherichia coli B/r* was much more resistant to killing than was *E. coli B*, confirming the observations of Rörsch, Edelman, Van Der Kamp & Cohen (1962), but *E. coli B`n+* was almost as resistant as *E. coli B/r* to this agent.

*Sensitivity to benzylpenicillin.* *Escherichia coli B/r* was more resistant to inactivation by penicillin than was *E. coli B*, as was observed previously by Rörsch et al. (1962). However, *E. coli B/F`lon+* and the suppressed *lon* strains *E. coli B`n+2* and *E. coli B`n+11* as well as the *uvr* strain *E. coli B`n+12* were even more sensitive to the action of penicillin than was *E. coli B* (Fig. 4). *Escherichia coli SB3100*, an *F*‘ derivative of *E. coli B/r* was almost as resistant as the parent strain, indicating that the *F*‘ factor was not responsible for the high sensitivity of *E. coli B/F`lon+* (Fig. 4).
Fig. 3. Survival of *Escherichia coli* strains incubated with crystal violet. Bacteria were plated at suitable dilution on BAB plates containing various concentrations of crystal violet and incubated overnight at 37 °C. ○, *E. coli* B; ●, *E. coli* B/r; ◆, *E. coli* Bφ2.

Fig. 4. Survival of *Escherichia coli* strains incubated with benzylpenicillin. Bacteria were plated at suitable dilutions on BAB plates containing various concentrations of benzylpenicillin and incubated overnight at 37 °C. ○, *E. coli* B; ●, *E. coli* B/r; ◆, *E. coli* B/F­lon+. ●, *E. coli* Bφ2; □, *E. coli* Bφ2-11; ■, *E. coli* Bφ2-12; ▼, *E. coli* B/r/F’ ara+.

**Discussion**

The results show that there are marked differences in the relative sensitivities of *lon* strains of *Escherichia coli* to agents which induce filament formation.

Unsuppressed *lon* strains were more sensitive to agents which appear to induce filamentation by inhibition of DNA synthesis than were those in which the Lon phenotype is suppressed. Thus *Escherichia coli* B and *E. coli* Bφ12 were much more sensitive to inactivation by nalidixic acid and gentian violet than was *E. coli* B/r (in which *lon* is suppressed by *sul*) or *E. coli* B/F­lon+ (in which *lon*+ is dominant). However, the degree of sensitivity to these agents appears to be dependent on the mechanism by which *lon* is suppressed. *Escherichia coli* Bφ2 and *E. coli* Bφ12 in which *lon* is suppressed by *exrA* and *recA* respectively were both more resistant to nalidixic acid and gentian violet than was *E. coli* B but they still were more sensitive to these compounds than was *E. coli* B/r.

The sensitivities of the strains to penicillin, however, reveal a rather different picture. Only suppression of *lon* by *sul* reduced sensitivity to penicillin. Suppression of *lon* by *exrA* or *recA* slightly increased rather than reduced sensitivity to penicillin. Therefore sensitivity to penicillin, which at low concentration induces filamentation by an effect on polymer murein biosynthesis (Schwarz, Asmus & Frank, 1969; Hartmann, Holtje & Schwarz, 1972),
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was again highly dependent on the mechanism by which lon is suppressed. Moreover the introduction of F' lon+ into Escherichia coli B did not reduce sensitivity. Although the F' factor might have been responsible for this, this is unlikely, because the presence of F' in E. coli B/F' had little effect on its sensitivity to penicillin. Therefore the resistance of E. coli B to penicillin is most likely the result of the expression of sul producing some effect in addition to direct suppression of lon. It is interesting that ten radiation-resistant mutants of the same u.v. sensitivity as E. coli B/F' which we have isolated from E. coli B by the method of Witkin (1947) were all much more resistant to penicillin than was the parent strain (unpublished results).

Because of the differences shown between agents which induce filamentation in lon strains by a specific action on DNA and on the bacterial envelope, it may be possible to deduce the site of action of other filament inducing compounds. For example, the observed sensitivities of Escherichia coli B, E. coli B~ and E. coli B/F' to crystal violet (Fig. 3) suggest that this agent induces filamentation in a manner analogous to nalidixic acid, i.e. by affecting DNA. This agrees with the conclusion of Witkin (1961) rather than that of Walker et al. (1971), who suggested that it acted on the bacterial envelope.

The detailed processes involved in bacterial division are poorly understood but many authors (Clark, 1968; Helmstetter & Pierucci, 1968; Donachie, 1969; Inouye, 1969) are agreed that there is probably a close relationship between completion of DNA synthesis and septum formation. Zusman, Inouye, & Pardee (1972) have suggested that a specific protein produced at the end of a round of DNA replication may be involved in the coordination of these processes, possibly by binding to the membrane site at which septation is initiated. Although the mechanism by which lon interferes with the formation of a normal septum is unknown, Farnsworth & James (1972) proposed that lon caused a defect in the bacterial envelope – a suggestion supported by the recent finding by Leighton (1972) that there is a deficiency in a protein component of the envelope of a lon strain of Escherichia coli K12.

We propose that the lon-controlled envelope defect reduces the affinity of the septum-initiation site to bind the septum-promoting protein. This results effectively in an increase in the amount of the protein required to trigger septum formation, an increase which is normally met. However, because of the sensitivity of the binding site to reductions in the production of the protein, prevention of septum initiation readily occurs following inhibition of DNA synthesis.

Because of the marked effect of sul in reducing sensitivity to penicillin, which acts directly on the bacterial envelope, we propose that sul is also expressed as an alteration in the envelope. Sul may act, therefore, by countering the higher concentration of septum-promoting protein required in lon mutants, and thereby suppressing the Lon phenotype.

The mechanism of suppression of lon by the DNA repair mutations recA and exrA is unknown. Farnsworth & James (1972) found that these mutations affect the permeability properties of Escherichia coli B, thus suggesting some interaction with the bacterial envelope. However, our results show that although these mutations reduce the lethal effect of filament inducing agents which act directly on the DNA (Fig. 1, 2) they do not modify the penicillin sensitivity of E. coli B. This suggests that their site of action may also involve the coupling between DNA replication and septum formation, but is of a completely different nature from that involving sul.

The sensitivities of Escherichia coli B and E. coli B~ to each of the agents used were very similar, indicating that the uvr gene had no effect on modifying survival under conditions of filament induction. This is as expected from our knowledge of the reactions controlled by
the uvr genes and is consistent with the observation by Green et al. (1969) that uvr does not suppress filament formation.

These conclusions are summarized in the model presented in Fig. 5, in which we indicate proposed sites of action of the filament inducing agents, the location of the lon lesion and the points at which suppression of lon by sul, exrA and recA may occur. This model contains features of similarity to that proposed by Zusman et al. (1972). Whereas these authors postulated that in filamentous strains the lon mutation was expressed as a reduction in the amount of the septum-promoting protein produced following treatments which inhibit DNA synthesis, we suggest that the reduction in the amount of septum-initiation protein made is a consequence of the inhibition of DNA synthesis in both the Lon⁻ and Lon⁺ strains. However, this leads to filamentation only in Lon⁻ strains because of the reduced affinity of the septum site for the binding protein.

Nothing is known concerning the nature of the expression of the sul gene, but if, as we suggest, it interacts with lon by producing further alterations in the bacterial envelope, then it may be possible to detect these. This possibility is being tested by an examination of the
envelope proteins of Escherichia coli and one of the radiation-resistant mutants derived by us from Escherichia coli.

The mechanisms by which exr and rec suppress filamentation are also unknown, but our model predicts that these are not the same as those involving sul. Certainly, Holland & Tuckett (1972) found that recA did not alter the envelope protein profile of Escherichia coli. RecA and exrA suppress production of bacteriophage in E. coli (Donch et al. 1968; Green, Greenberg & Donch, 1969) whereas sul does not, but the reverse is true for suppression of mucoidy in E. coli K12 1899 (Donch et al. 1968; Donch et al. 1971), adding support to the view that rec and exr have no direct effect on the envelope but are involved in modifying effects on the bacterial genome.

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REFERENCES


