Effects of \( p \)-Fluorophenylalanine and Chloramphenicol on Chemotaxis in \textit{Escherichia coli} \\

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The effects of chloramphenicol and \( p \)-fluorophenylalanine (\( p \)-FPA) on growth, proportion of motile cells, average rate of motility, and the chemotactic response of a methionine auxotroph of \textit{Escherichia coli} K-12 were studied. Kinetic studies revealed that the inhibition of chemotaxis by \( p \)-FPA can be explained by the effect on growth, proportion of motile cells, and average rate of motility rather than a selective inhibition of chemotaxis per se. The effect of chloramphenicol on chemotaxis could not be explained in terms of these characteristics. It is concluded that low concentrations of chloramphenicol, unlike \( p \)-FPA, selectively inhibit chemotaxis.

Coetzee (4) indicated the independent nature of motility and swarming in \textit{Proteus} when he reported the successful transduction of the characteristic for swarming from a motile swarming culture of \textit{Proteus} to a motile nonswarming culture. Kopp et al. (7) reported that \( p \)-nitrophenyl-glycerol abolished swarming in \textit{Proteus} with no apparent change in growth or motility. The findings of Lominski and Lendrum (8) with \textit{Proteus} and of Adler (2) with \textit{Escherichia coli} suggest that swarming in \textit{Proteus} and chemotaxis (a coordinated bandlike movement of cells in a semisolid or liquid medium) in \textit{E. coli} are similar phenomena resulting from a cellular response to a concentration gradient. Armstrong et al. (3) isolated 40 mutants from a chemotactic strain of \textit{E. coli} which, although unchanged in morphology and motility, would not demonstrate the chemotactic response. In our work we have attempted to separate chemotaxis and motility in \textit{E. coli} by selective inhibition with chloramphenicol and \( p \)-fluorophenylalanine (\( p \)-FPA).

\textbf{MATERIALS AND METHODS}

\textit{Organism}. An Hfr methionine auxotroph of \textit{E. coli} K-12, carried in our culture collection as number 503 and originally obtained from J. Lederberg, was used in this study. A prototrophic strain of this organism was isolated by selection in methionine-free medium and was used, in addition to the auxotroph, for the chloramphenicol experiments.

\textit{Growth medium}. The growth medium was the H medium of Kaiser and Hoggness (5) as used by Adler (2), with the addition of 0.030 g of ethylenediaminetetraacetic acid (EDTA) and 0.001 g of thiamine per liter. \( \beta \)-Galactose (autoclaved separately) was added at 5.0 g per liter as the energy source, and \( L \)-methionine (20 mg per liter) was added to satisfy the methionine requirement. \( L \)-Threonine, \( L \)-leucine, and \( L \)-asparagine (20 mg each per liter) were added to increase the growth rate of the organism. No amino acids were used when the prototroph was grown. The growth procedure was that of Adler (2). However, cells to be used as the inoculum for the above growth medium were originally taken from a Nutrient Agar slant and grown in Nutrient Broth to an optical density (OD) of 0.8 at 540 \( \mu \)m. Growth was at room temperature on a gyratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.). Cells were harvested and washed according to the procedure of Adler (2)—but increasing centrifugation time to 15 min. After washing, the cells were resuspended in fresh wash medium and adjusted to an OD of 1.8 against distilled water at 540 \( \mu \)m. Such a suspension contains approximately 2.5 \( \times \) 10\(^8\) viable cells per ml.

\textit{Chemotaxis medium}. The medium used to demonstrate chemotaxis was that of Adler (1) with the addition of 1.6 \( \times \) 10\(^{-4}\) \( \text{M} \) \( \text{FeSO}_4\), 2.6 \( \times \) 10\(^{-4}\) \( \text{M} \) thiamine, 3.3 \( \times \) 10\(^{-5}\) \( \text{M} \) \( L \)-methionine, 3.7 \( \times \) 10\(^{-6}\) \( \text{M} \) \( L \)-leucine, 3.7 \( \times \) 10\(^{-3}\) \( \text{M} \) \( L \)-asparagine, 4.0 \( \times \) 10\(^{-5}\) \( \text{M} \) \( L \)-threonine, and 180 mg of agar per 100 ml to produce a semisolid medium. Galactose was added to a final concentration of 2.5 \( \times \) 10\(^{-4}\) \( \text{M} \) as the energy source. Approximately a 100-fold increase in the number of cells occurred during the response to be described below. A change in growth rate of the organism results in a change in the rate of movement of the ring of cells. Leucine, asparagine, and threonine were added to increase the growth rate of the organism. The same response is observed, but at a slower rate, when these amino acids...
are deleted. When the prototrophic strain was used, all the amino acids were deleted from the medium. During initial chemotaxis studies, it was observed that the inoculum frequently streaked across the agar surface rather than remaining as a distinct circle at the center of the plate. The addition of 0.05 ml of a 1:100 dilution of Tween 80 per 100 ml of the chemotaxis medium eliminated this problem. Each petri dish received 25 ml of this medium. After hardening, the plates were transferred to an incubator at 37°C and 50 µlitters of the washed-cell suspension, or about 6.0 × 10⁶ cells, were placed onto the center of the agar surface. The response normally observed was the appearance after 2 hr of two defined rings of cells around the periphery of the inoculum. These rings progressed outwards, and after 6 hr another ring appeared at the edge of the inoculum. This ring, with a highly reproducible 6-hr lag, was well defined, dense, and ribbonlike, and extended from the surface to the bottom of the agar. The 6-hr lag was reproducible even when all amino acids except methionine were deleted. The systematic deletion of the amino acids and galactose made it possible to determine that the dense ring was responding to galactose. Further work, including the data to be reported here, was done with the galactose band.

Effect of inhibitors. It was possible to observe the effects of an inhibitor on chemotaxis by adding a compound to the chemotaxis medium and directly measuring the radius of the galactose ring at various times. The effect on growth was determined by adding the compound to both the growth medium and the chemotaxis medium and making OD readings at 540 nm. The results presented are for the growth medium as the chemotaxis medium supports growth only to an OD of 0.30. The results were almost identical for both media, but the low levels of growth in the chemotaxis medium made it difficult to observe differences in the early growth rates. OD values greater than 0.8 were obtained by diluting the sample with additional growth medium and multiplying the resulting value by the dilution factor. Motility of the cells was observed in a Levy counting chamber with a preparation of cells from the growth medium supplemented with 0.18% agar and the test agent. The proportion of motile cells was evaluated by counting 50 to 100 cells in the chamber. At higher cell densities accuracy was improved by diluting the sample with additional growth medium supplemented with 0.18% agar. Cells exhibiting even weak motility were considered to be motile. The rate of motility was determined by averaging the time required for 20 to 40 random cells to pass between lines 50 µ apart in the counting chamber. The average rate of motility may give an indication of impaired motility in a culture which still exhibits a high proportion of motile cells. The Leifson staining procedure (Difco) was used to observe the presence or absence of flagella. Chloramphenicol and p-FPA were sterilized by filtration through membrane filters.

RESULTS

p-FPA inhibition. The effects of various levels of p-FPA on the migration of the galactose ring in semisolid medium are summarized in Fig. 1. The lowest levels tested had no apparent effect on chemotaxis, but 5 × 10⁻⁸ M and greater concentrations showed inhibition, as indicated by an increased lag period prior to the onset of movement of the galactose ring, and a slower rate of ring movement. Concentrations greater than 5 × 10⁻⁶ M completely eliminated any detectable
response. As shown in Fig. 2, p-FPA had only a slight effect on growth in broth at the same concentration. The primary result was an increased lag prior to the onset of log growth. Higher concentrations had a more pronounced effect. Figures 3 and 4 indicate the effects of various levels of p-FPA on the number of motile cells and the rate of motility of the cells, respectively. Figure 3 shows a sharp decrease in the number of motile cells after 12 hr with $5 \times 10^{-4}$ M p-FPA, and Fig. 4 indicates that this level not only reduced the proportion of motile cells but also caused a decrease in the rate of motility. Figure 4 shows a general periodicity in the average rate of motility. By comparing Fig. 3 and 4, it can be seen that the periodic decreases in rate did not correspond with any similar drop in the proportion of motile cells. The periodic drop in rate was a phenomenon found in all samples and reflected a general slowing in rate of movement in the population as a whole. Higher concentrations which completely eliminated motility were shown to result in cells which had no flagella, as compared to the control population which had one to five per cell.

Chloramphenical inhibition. The effects of various chloramphenicol concentrations on chemotaxis are summarized in Fig. 5. The higher levels, especially 1.5 and 2.0 $\mu$g/ml, inhibited chemotaxis, as shown by an increase in the length of the lag period on the chemotaxis medium from 6 hr to 34 and 39 hr, respectively. Figure 5 indicates that 0.5 and 1.0 $\mu$g of chloramphenicol per ml caused progressive decreases in the rate of band movement, as well as increasing the length of the lag. However, 1.5 and 2.0 $\mu$g/ml appeared to increase the lag with no apparent effect on the rate of band movement. Additional studies revealed that the return to normal rates of movement was the result of inactivation of the chloramphenicol.
There was an inhibited chemotaxis, and the plates a and plates being on in the medium, yielded essentially the same growth as the control growth flask. Even at 2.0 µg/ml, chloramphenicol did not significantly affect the rate of motility in the agar-supplemented growth medium. It was found that higher concentrations completely inhibited motility. A flagellum stain revealed that such populations have a normal complement of flagella. Figures 8 and 4 indicate an apparent periodicity in the average rate of motility of the cells. This, again, is a reflection of the general decrease in the rate of movement in the population as a whole, rather than an increase in the number of very slow-moving cells. Although the significance of this observation is questionable, it is interesting to note that the first sharp drop in the rate of motility coincides approximately with the onset of log growth. Inhibition studies with the prototrophic strain of the organism show no significant differences in the effect on chemotaxis, motility, or rate of motility.

**DISCUSSION**

The findings demonstrated that p-FPA inhibition of the chemotactic response to galactose...
The results showed a delay in onset of chemotaxis of approximately 28 hr when 1.5 \( \mu g \) of chloramphenicol per ml was added to the system. The effect of 1.5 \( \mu g \) of chloramphenicol per ml on chemotaxis would be even greater except for the inactivation of the chloramphenicol. This same concentration of chloramphenicol did not result in any decrease in growth rate, but delayed the onset of log growth in broth from 9 hr to 13.5 hr. As previously stated, 1.5 \( \mu g/ml \) has only a slight effect on the proportion of motile cells in the population or the average rate of motility.

The growth studies, and the growth observed on chemotaxis plates containing 1.5 and 2.0 \( \mu g \) of chloramphenicol per ml, show that the organism is still capable of utilizing galactose. This suggests that there is a galactose gradient produced and that the cells are incapable of sensing, or responding to, or both. The inhibitory effect of chloramphenicol on chemotaxis is too great to be accounted for in terms of its effects on the other characteristics described above. It is concluded that chloramphenicol, unlike \( p \)-FPA, does selectively inhibit chemotaxis at low concentrations.

It is postulated that the selective inhibition by chloramphenicol results from the interference with sensory or response mechanisms, as has been proposed for the inhibition of swarming by \( p \)-nitrophenylglycerol in \textit{Proteus} (7).

\section*{Literature Cited}