Derepression of β-Galactosidase Synthesis in Escherichia coli K-12 by p-Fluorophenylalanine

SOOSANG KANG, PAUL ROCKEY, AND ALVIN MARKOVITZ

Department of Microbiology and LaRabida-University of Chicago Institute, University of Chicago, Chicago, Illinois 60637

Received for publication 13 May 1968

p-Fluorophenylalanine (FPA) derepresses β-galactosidase synthesis at 35 C but not at 25 C in Escherichia coli K-12, strain MC132 (lac II, 2), a strain with a temperature-sensitive lac repressor. In contrast, strain MC130 (lac I+) is not derepressed by FPA at 35 C. Temperature-shift experiments with strain MC132 in the presence of FPA and other reagents (isopropyl-1-thio-β-D-galactoside or chloramphenicol) are consistent with the following mechanism. FPA is incorporated into the genetically altered lac repressor at all temperatures. This further alteration due to incorporation of analogue makes the lac repressor protein inactive at 35 C but active at 25 C. Once an active tertiary structure is formed at 25 C, it is stable at 35 C. However, the inactive tertiary structure of the FPA-containing repressor can assume an active tertiary structure when the temperature is shifted from 35 to 25 C. In the discussion of the results, "inactive tertiary structure" is equated with "monomers" and "active tertiary structure" with oligomers.

Previous studies showed that low concentrations of DL-p-fluorophenylalanine (FPA) caused a limited derepression of several enzymes involved in capsular polysaccharide synthesis and consequent excess synthesis of capsular polysaccharide in a wild-type strain of Escherichia coli K-12 (9). Genetic studies indicate that the regulator gene product that controls capsular polysaccharide synthesis and radiation sensitivity [capR (14) or Lon (6)] is a protein (14, 15) composed of subunits (15).

Although FPA did not cause derepression of alkaline phosphatase in wild-type E. coli strains, it did cause complete derepression of alkaline phosphatase synthesis (100-fold) in a strain with a temperature-sensitive regulator gene mutation [phoS, previously designated R2 pho (3)]. These results may be interpreted as follows: FPA was incorporated into protein products of regulator genes (and other proteins). In the strain with the temperature-sensitive phoS mutation, the mutationally altered protein product of the phoS gene was further altered by incorporation of FPA and was inactive. No preferential inactivation of the protein product of the wild type (phoS+) gene occurred.

The present study was undertaken to determine whether or not the β-galactosidase control system could also be altered by incorporation of FPA. The product of the lacI gene, the repressor, has been isolated and shown to be a protein (4). Furthermore, Gilbert and Müller-Hill recently demonstrated that the lac repressor binds specifically to deoxyribonucleic acid (DNA) from the lac region of the chromosome (5), as predicted by Jacob and Monod (8). A preliminary report of the present work has been published (S. Kang, P. Rockey, and A. Markovitz, Federation Proc. 36:678, 1967).

Materials and Methods

Bacteria and transduction. All experiments were done with strains of E. coli K-12. Transductions were carried out with bacteriophage P1kc (12). The following donors were used for the construction of isogenic strains containing the indicated lacI allele: strain AB259 [lacZ, lacI, proC]; strain 3.300 [lacZ, lacI, proC+ (13)]; strain E303 [lacZ+, proC+ (19)] kindly provided by A. Novick (a strain that contains two mutations in the lacI gene; one, designated previously as i, prevents induction of β-galactosidase by known inducers; from this, a secondary mutant was obtained that synthesizes β-galactosidase at 42 C, but not at 30 C, in the presence or absence of inducers; the double mutant was previously designated i,786 (20) and is hereafter designated lacI1,2). The recipient used was strain MC129, a galactose-positive transductant of strain MC100 (14) that is sensitive to P1kc. Strain MC129 has the following genotype leu-I, proC1, purE1, trp-I, str-I, lacZ2. The lac genes and proC are cotransducible (13), the order being lacZ, lacI, proC. Therefore, lacZ+ and proC+ could
be selected simultaneously from the donors on media that contained lactose without added proline. The temperature-sensitive double mutant at lac I (lac I 1, 2) was selected at 42°C to insure growth of transductants on the lactose-containing agar. Out of 97 proC+ lacZ+ transductants obtained at 42°C, only one showed significant growth on the selection medium at 30°C. This indicated that lacI1, 2 was transduced from 97 times when lacZ+ and proC+ were cotransduced.

Media. Penassay Broth (Difco) and L broth (12) were used as complex liquid media. M9 minimal medium (1) was supplemented with 10 μg of thiamine HCl per ml and 50 μg of L-leucine, adenine, and L-tryptophan per ml. The carbon and energy source was 0.6% glucose, 0.3% glycerol, or 1% lactose (filter-sterilized) as indicated. Agar (1.5%) was added to solidify liquid media for plating experiments. The concentration of FPA used in all experiments was 3.2 × 10⁻⁴ M unless stated otherwise.

Chemicals. FPA was purchased from Calbiochem, Los Angeles, Calif. Isopropyl-1-thio-β-D-galactoside (IPTG) and o-nitrophenyl-β-D-galactoside (ONPG) were purchased from Mann Research Laboratories, New York, N.Y. Chloramphenicol succinate was purchased from Parke, Davis and Co., Detroit, Mich.

Assay of β-galactosidase. Enzyme activity was assayed by a modification of the method of Pardee, Jacob, and Monod (18). Cultures were centrifuged, and resuspended in 0.1 M Na₃HPO₄-NaH₂PO₄, pH 7.0, to a given optical density (OD) at 540 μm in the Bausch & Lomb Spectronic-20 colorimeter using a cylindrical tube 1.16 cm in diameter. The cells were disrupted by treatment with toluene (0.05 ml of toluene per ml of cell suspension) at 30°C for 40 min. A 1-ml portion of appropriately diluted extract was incubated with 1 ml of 3 × 10⁻⁵ M ONPG in 0.1 M Na₃HPO₄-NaH₂PO₄, pH 7.0, at 25°C. The reaction was stopped by the addition of 1 ml of 1 M Na₂CO₃. Parallel samples were incubated without ONPG. At the end of the incubation period, 1 ml of ONPG and 1 ml of 1 M Na₂CO₃ were added to these tubes which were used as blanks. The increase in OD at 420 μm (absorption maximum of o-nitrophenol) was followed by use of a Bausch & Lomb Spectronic-20 colorimeter. A unit of β-galactosidase activity (enzymatic activity) is the amount which will hydrolyze 0.212 μmoles of o-nitrophenyl galactoside in 1 min at pH 7.0 at 25°C. Specific enzymatic activity was defined as units of enzymatic activity per unit of cell turbidity at 540 μm. A unit of cell turbidity at 540 μm contains 0.42 mg of cell (dry weight) per ml.

**RESULTS**

Characterization of isogenic transductants with different lacI alleles. Table 1 presents the results of β-galactosidase assays of isogenic strains MC130 (lacI⁺), MC131 (lacI⁻), and MC132 (lacI1, 2) grown at high or low temperature in the presence or absence of the inducer IPTG. All three strains responded to temperature and IPTG as expected. In particular, strain MC132 synthesized β-galactosidase at 42°C but not at 30°C and IPTG did not induce β-galactosidase synthesis. These results agree with those of Sadler and Novick on strain E303 (20), the donor used to construct strain MC132. The results with strain MC132 demonstrate for the first time that both the super-repressed mutation and the temperature-sensitive mutation are cotransducible with the lacZ and proC region of the chromosome. Therefore, the genetic designation lacI1, 2 (previously designated as iB3, T88; 20) is supported.

**Derepression of β-galactosidase synthesis by FPA.** Various concentrations of FPA were tested on cells growing in glycerol media. β-Galactosidase synthesis by strain MC132 (lacI, 2) was derepressed a maximum of 25-fold by growth in FPA (1.6 × 10⁻⁴ to 3.2 × 10⁻⁴ M) at 35°C after four generations. In contrast, β-galactosidase synthesis by strain MC130 (lacI⁺) was not affected by FPA (8 × 10⁻⁵ to 8 × 10⁻⁴ M) either in the presence or absence of 10⁻³ M IPTG at 37°C. β-Galactosidase synthesis by the constitutive strain (MC131, lacI3) was increased twofold by growth in FPA (1.6 × 10⁻⁴ to 8.0 × 10⁻⁴ M) at 37°C, in either the presence or the absence of IPTG.

Although we pursued the present study with strain MC132, our preliminary studies showed that other nonisogenic strains with mutations in the lacI gene were also derepressed by growth in FPA; strain E103, a strain with a temperature-labile lacI gene mutation that produces β-galactosidase immediately after heat shock in buffer

---

**Table 1. Characteristics of isogenic strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>lacI allele</th>
<th>Conditions</th>
<th>IPTG concn</th>
<th>Temp</th>
<th>Specific enzymatic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC130</td>
<td>+</td>
<td>0</td>
<td>23</td>
<td>4 × 10⁻³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>42</td>
<td>4 × 10⁻³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻³</td>
<td>30</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻²</td>
<td>42</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>MC131</td>
<td>3</td>
<td>0</td>
<td>30</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>42</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻³</td>
<td>30</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻²</td>
<td>42</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>MC132</td>
<td>1, 2</td>
<td>0</td>
<td>30</td>
<td>5 × 10⁻³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>42</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻³</td>
<td>30</td>
<td>5 × 10⁻³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻²</td>
<td>42</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

*Cells were grown in glycerol media for 20 hr in the presence or absence of IPTG and at high or low temperatures. Specific enzymatic activities were determined as described in Materials and Methods.*
Fig. 1. Derepression of $\beta$-galactosidase synthesis by FPA at $35^\circ C$. Strain MC132 (lac $11,2$) was grown in glycerol medium at $35^\circ C$. FPA ($3.2 \times 10^{-4} \text{M}$) was added to exponentially growing cells (OD at 540 $\mu\text{m} = 0.06$) at zero-time. At 210 min, $6.4 \times 10^{-4} \text{M}$ L-phenylalanine was added to half of the culture.

Fig. 2. Effect of temperature shift ($35^\circ C \rightarrow 25^\circ C$) on derepression of $\beta$-galactosidase synthesis by FPA. FPA ($3.2 \times 10^{-4} \text{M}$) was added to strain MC132 growing exponentially at $35^\circ C$. At times indicated by the arrows, samples were transferred to $25^\circ C$. IPTG ($5 \times 10^{-4} \text{M}$) was added to another sample at 225 min, and growth continued at $35^\circ C$. 

141
(7, 20) was derepressed only 2.8-fold by growth in FPA at 30°C; strain E321, a strain that is inducible by either growth at 43°C or addition of IPTG (20), was derepressed 10-fold by growth in FPA at 35°C.

The kinetics of FPA-induced β-galactosidase synthesis in strain MC132 are presented in Fig. 1. In the presence of FPA at 35°C, strain MC132 started to synthesize β-galactosidase after a lag of 0.6 generation. However, there was only a minimal increase of specific enzymatic activity without FPA under the same conditions. As mentioned above, it took four generations before the specific enzymatic activity reached a maximum. The addition of a two-fold excess of L-phenylalanine (with respect to FPA) at 210 min resulted in repression only after a lag of 0.4 generation (60 min). The growth rate in FPA was 50 to 60% of the normal growth rate in minimal medium, and the addition of L-phenylalanine partially reversed the growth inhibition.

The fact that derepression of β-galactosidase by FPA could be observed in strain MC132 (lacI1,2) but not in the strain MC130 (lacI+) indicated that the effect of FPA is related to the mutationally altered product of the lacI gene. These results parallel those obtained with the temperature-sensitive regulator gene mutation in the alkaline phosphatase system (10). It is noteworthy that derepression by FPA and re-repression by phenylalanine require at least 10 times as long as changes effected by an inducer such as IPTG (2).

Effect of temperature shift on FPA-induced derepression of β-galactosidase synthesis. Preliminary studies demonstrated that FPA-induced β-galactosidase synthesis occurred at 35°C but not at 25°C with strain MC132. Therefore, we used temperature-shift experiments in an attempt to determine the cause of the lags before derepression and re-repression of β-galactosidase synthesis by FPA and phenylalanine, respectively. When the temperature was shifted from 35 to 25°C, after derepression of enzyme synthesis had occurred, a rapid reduction in the differential rate of enzyme synthesis was observed (Fig. 2). The addition of 5 × 10⁻³ m IPTG at 35°C also resulted in a rapid reduction in the differential rate of enzyme synthesis. These changes occurred with little, if any, lag, and were apparently different from the re-repression effected by phenylalanine addition (see Fig. 1), which required 60 min at 35°C. Since cells grown in FPA are subject to catabolite repression (see below), it was important to demonstrate that the cessation of β-galactosidase synthesis on shifting from 35 to 25°C was not due to a rapid increase of catabolite repression when the temperature was lowered. Strains MC130 (lacI+) and MC131 (lacI3) were tested in experiments similar to those of Fig. 2, except that strain MC130 was exposed to 2.5 × 10⁻⁴ m IPTG for 45 min. Both were then shifted from 35 to 25°C. The differential rate of β-galactosidase synthesis did not decrease in either strain. Thus, catabolite repression cannot explain the decrease in the differential rate of β-galactosidase synthesis when strain MC132 was shifted from 35 to 25°C.

Previous growth in FPA at 25°C for 2, 4 (results not shown), or even 14 hr did not reduce the lag in derepression of β-galactosidase synthesis when cells were shifted to 35°C (Fig. 3). Heat-shock treatment for 1 hr in buffer at 43°C, between the shift from 25 to 35°C, shortened the lag for derepression to the same extent in cells grown at 25°C with or without FPA (Fig. 3). The lag was not reduced further by prolonging the heat-shock treatment. These results suggested that FPA incorporation into the repressor did not enhance its inactivation or degradation during the heat shock.

Effects of glucose and chloramphenicol on FPA-induced derepression of β-galactosidase synthesis.
Attempts were made to investigate the inactivation of repressor at a reduced rate of β-galactosidase synthesis caused by catabolite repression (Fig. 4). Strain MC132 was grown overnight at 25°C in glycerol medium that contained FPA. As shown previously, these cells produce little β-galactosidase. At time-zero (Fig. 4), these cells were transferred to glucose medium with FPA at 35°C. After 210 min, a sample was shifted to glycerol medium with FPA. The lag in initiation of derepression after the shift to glycerol with FPA was shortened to 40 min (data from experiment of Fig. 4), compared with a lag of 100 min in glycerol medium with FPA at 35°C (data from experiment of Fig. 1). The lag was further reduced to less than 0.1 generation (20 min; data from experiment of Fig. 4) when cells were grown at 43°C for 1 hr just prior to transfer to glycerol medium with FPA (Fig. 4). In some experiments, the lag was completely eliminated by this latter treatment. The effect of chloramphenicol on FPA-induced derepression is presented in Fig. 5. Cells grown in glycerol-FPA medium at 25°C were incubated with 50 μg of chloramphenicol succinate per ml in glycerol medium at 35 or 43°C for 1 hr. Control cells were incubated in buffer for 1 hr at 35 or 43°C. All groups were then resuspended in glycerol FPA medium at 35°C, and β-galactosidase synthesis and growth (OD at 540 μM) were measured at 30-min intervals. No lag in growth was noted in the cultures treated with either chloramphenicol or buffer. Treatment with chloramphenicol at 35 and 43°C reduced the lag significantly. In fact, the cells at 43°C with chloramphenicol exhibited no detectable lag in initiation of β-galactosidase synthesis (Fig. 5).

**DISCUSSION**

The brilliant studies of Gilbert and Müller-Hill showed that the repressor controlled by the lacI locus is a protein of 150,000 to 200,000 molecular weight (4), that the purified repressor binds to lac region DNA, and that the inducer IPTG releases the repressor from lac region DNA (5).

We will discuss the data in terms of the subunit model presented in Fig. 6, which is modified from the model of Sadler and Novick (20). The assumption that the active repressor is an oligomer

![Figure 4](http://jb.asm.org/) Effect of catabolite repression on FPA-induced derepression of β-galactosidase synthesis. Strain MC132 was grown overnight in glycerol medium that contained FPA (3.2 × 10^{-4} μM) at 25°C and was transferred to glucose medium with FPA at 35°C when the OD at 540 μM was 0.024 (0 min). One sample was centrifuged at 35°C and resuspended in prewarmed FPA-containing glycerol medium at 210 min (O). Another sample was shifted to 43°C for 1 hr just prior to transfer to glycerol medium with FPA at 210 min (X).
composed of four monomers is based on the estimated molecular weight of the isolated repressor protein (4) and on kinetic experiments (20). FPA is incorporated into protein (9, 10, 16, 19), and thus cells grown in the presence of FPA will contain lac repressor that contains FPA. Incorporation of amino acid analogues into specific proteins leads to alterations in stability (11, 17). Kepes showed that β-galactosidase produced in the presence of FPA is more heat-labile than β-galactosidase produced in its absence (11). The fact that FPA caused derepression of β-galactosidase synthesis in the temperature-sensitive mutant (strain MC132, lacI1,2) but not in the wild type (lacI1) demonstrates that the effect of FPA is related to the mutationally altered repressor. Even with strain MC132, FPA caused derepression at 35 C but not at 25 C. Thus, at 25 C the mutationally altered lac repressor was not inactivated by incorporation of FPA. These results are easily understood if strain MC132 (lacI1,2) is a mutant in which assembly of inactive monomers into FPA containing active repressor takes place at 25 C but not at 35 C. The same explanation was suggested to explain similar results with FPA in a temperature-sensitive regulator gene mutation for alkaline phosphatase synthesis (10). Kinetic studies utilizing FPA at 35 C demonstrated that approximately 0.6 generation was required before derepression began, and subsequent addition of L-phenylalanine initiated re-repression only after 0.4 generation. These lags may be a measure of the time required for: (i) de novo synthesis of repressor containing FPA (derepression) or phenylalanine (re-repression); or (ii) dilution of existing repressor by growth. In contrast, a shift of an FPA-induced culture from 35 to 25 C results in a rapid reduction in the differential rate of β-galactosidase synthesis (Fig. 2). This latter result is consistent with rapid assembly of FPA monomers into active repressor at the low temperature. Sadler and Novick showed that, with a lacI1,2 gene mutant, IPTG caused stabilization of repressor at a high temperature which was manifest by the unusual result that addition of IPTG at 43 C caused immediate repression (20). We have observed a similar phenomenon in strain MC132 (lacI1,2) derepressed by growth in FPA at 35 C (Fig. 2). Such results indicate that stable

![Fig. 5. Effect of chloramphenicol on FPA-induced derepression of β-galactosidase synthesis. Cells were previously grown in glycerol medium that contained FPA (3.2 × 10^-4 M). Samples were incubated in buffer at 35 or 43 C and in glycerol medium with chloramphenicol (CM, 50 μg/ml) at 35 or 43 C. After 1 hr, cells were centrifuged at 35 C and resuspended in prewarmed FPA-containing glycerol medium at 35 C.

![Fig. 6. Hypothetical model of mutational effects of FPA and chloramphenicol (CM).]
inactive monomers are present at high temperature. In this respect, our model differs from that of Sadler and Novick (20).

Attempts to reduce the 0.5-generation lag in FPA-induced β-galactosidase synthesis at 35°C by previous growth in FPA at 25°C were not successful. Thus, it seems likely that FPA-containing lacI2 repressor, once formed at 25°C, does not readily dissociate into inactive monomers at 35°C. This may be contrasted with the result observed with the temperature-sensitive regulator gene mutant in the alkaline phosphatase system. In that system, growth in FPA at 22°C resulted in inactivation of the lacI2 repressor in FPA-induced alkaline phosphatase synthesis when the culture was shifted to 30°C (10). In the present study, we were able to eliminate the lag in the initiation of β-galactosidase synthesis either by employing catabolite repression or by growth at 25°C in FPA followed by exposure to chloramphenicol at 43°C (Fig. 5). However, exposure to 43°C in buffer did not substitute for chloramphenicol treatment (Fig. 3 and 5). Such results indicate that inactivation of FPA-containing repressor does not require extensive protein synthesis. Further work is necessary to understand the effect of chloramphenicol.

Perhaps the value of these studies with FPA in the well-defined lacI2 genetic system will be to provide a tool to search for regulator genes in mammalian cells. One would predict that FPA would cause increases in certain specific enzyme syntheses where a hypothetical repressor would be more readily inactivated than the enzyme protein being measured.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Grant AI-06966 from the National Institute of Allergy and Infectious Diseases. One of us (S. K.) is a Postdoctoral Fellow of the Arthritis Foundation. The authors thank M. Stodolsky and W. Epstein for suggestions concerning certain experiments.

LITERATURE CITED