Synergistic and Product Induction of the Enzymes of Tryptophan Metabolism in *Pseudomonas acidovorans*

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The process of induction of tryptophan oxygenase in *Pseudomonas acidovorans* is typical of many microbial enzyme induction systems, in that it (i) requires cell multiplication and de novo protein synthesis, (ii) is subject to catabolite repression, (iii) results in the formation of a stable enzyme, whose level, upon removal of inducer, is diluted out by cell proliferation, and (iv) exhibits product induction. L-Kynurenine was more effective than L-tryptophan as an inducer of both tryptophan oxygenase and the second enzyme of the pathway, kynurenine formamidase. The occurrence of product induction of these two enzymes by their common metabolite eliminated the possibility of sequential induction of these enzymes. DL-5-Fluorotryptophan, nonmetabolizable and devoid of any inducing activity, resulted in a concentration-dependent inhibition of the L-tryptophan-mediated induction of tryptophan oxygenase; kynurenine formamidase induction, however, was not influenced by the presence of DL-5-fluorotryptophan. DL-7-Azatryptophan, also nonmetabolizable and completely inactive as an inducer, acted synergistically with L-tryptophan and superinduced tryptophan oxygenase levels. When induction was conducted in a medium containing only L-tryptophan and 7-azatryptophan as inducing agents, then tryptophan oxygenase induction was enhanced, whereas the kynurenine formamidase level was essentially unchanged. These data indicate that various inducing conditions affect the relative proportions of tryptophan oxygenase and kynurenine formamidase, and thus indicate noncoordinate regulation of these enzyme activities.

The genus *Pseudomonas* is distinguished in its remarkable nutritional versatility, in that any one of hundreds of organic compounds can serve as a sole source of carbon and energy for growth. L-Tryptophan is one of the 18 amino acids which can serve as such a nutrient (5). It is thought that these bacteria adapt to the utilization of most, if not all, of such growth substances by the induction of specific catabolic enzymes. Induction of enzymes in response to tryptophan is of particular interest, since the first two enzymes, tryptophan oxygenase (which oxidatively converts L-tryptophan to L-formyl-kynurenine) and kynurenine formamidase (which hydrolytically removes the formyl group to yield kynurenine), are known to be under the control of various regulatory mechanisms in different microbial systems. Beyond the conversion of L-tryptophan to L-kynurenine, one of two pathways is encountered, depending on the bacterial strain used. L-Kynurenine may be converted to anthranilic acid and subsequently to succinic acid (the so-called "aromatic pathway"), or kynurenine may be converted by transamination to kynurenic acid and then transformed ultimately to glutamic acid the ("quinoline pathway"). Palleroni and Stanier (11) found that, in a strain of *P. fluorescens* utilizing the aromatic pathway, L-kynurenine was the true inducer of both tryptophan oxygenase and the subsequent enzyme, kynurenine formamidase; these enzymes appeared to be induced in a strictly coordinate manner. The process in which an enzyme(s) is induced by the product of its activity, rather than its substrate, is called product (or metabolite) induction (3). Tremblay et al. (15), investigating another strain of *P. fluorescens* which

1 Taken from the dissertation submitted to the faculty of Columbia University by H. Rosenfeld in partial fulfillment of the requirements for the Ph.D. degree.

2 Established Investigator, Health Research Council of the City of New York (J-104).
follows the quinoline pathway, provided evidence for a sequential mode of induction of these two enzymes. By using a strain of *P. acidovorans* which utilizes the quinoline pathway, we explored the biochemical mechanisms which regulate the catalytic efficiency and the physiological levels of tryptophan oxygenase and kynurenine formamidase. By use of tryptophan oxygenase purified from this organism, communications from this laboratory have described the role of tryptophan as an allosteric regulator which modulates catalytic efficiency of existing enzyme levels by virtue of tryptophan dependence on the affinity of the enzyme for its other substrate, oxygen (1, 9). This study, devoted to an exploration of the factors regulating enzyme levels during induction, indicates that (i) bacterial growth on either tryptophan or kynurenine results in the induction of high levels of tryptophan oxygenase and kynurenine formamidase, confirming the product inducibility of these enzymes, (ii) the ratio of in vivo induced activities of tryptophan oxygenase and kynurenine formamidase is alterable under various circumstances, indicating that these two enzymes are either noncoordinately induced at the transcriptional level or are each subject to separate translational regulation, and (iii) 7-azatryptophan, which lacks inducing ability per se, when added to media containing tryptophan, results in an augmented inducing efficiency of tryptophan upon tryptophan oxygenase synthesis (synergistic induction).

**MATERIALS AND METHODS**

*Pseudomonas* sp. ATCC 11299a was used throughout this study. We are grateful to R. Y. Stanier for classifying this organism as *P. acidovorans* on the basis of the following characteristics: absence of growth at 41 °C; failure to liquefy gelatin or given an egg yolk reaction; lack of pigmentation in King Medium; good growth on adipate, acetamide, glycollate, m- and p-hydroxybenzoate; absence of growth on testosterone, benzoate, salicylate, or any sugar tested except fructose. The tryptophan-adapted cells can be grown on kynurenic acid but not anthranilic acid, indicating this species utilizes the quinoline degradative pathway.

This strain of *P. acidovorans* was maintained at 5 °C on agar slants composed of (per liter of water): NaCl, 5 g; Difco Beef Extract, 5 g; Difco peptone, 10 g; Difco agar, 20 g. Cells were grown preautoclavably at 30 °C with vigorous aeration in a New Brunswick Cyramator incubator. The minimal medium consisted of (per liter of distilled water): NH4Cl, 5 g; K2HPO4, 1.5 g; KH2PO4, 0.5 g; MgSO4·7·H2O, 0.2 g. Prior to autoclaving, the pH was approximately 7. Where indicated, Difco yeast extract was added to the growth medium, and l-asparagine (Calbiochem, Los Angeles, Calif.), L- and D-tryptophan, and the tryptophan analogues, DL-5-fluorotryptophan and DL-7-azatryptophan (Nutritional Biochemicals Corp., Cleveland Ohio), and L-kynurenine (Calbiochem Co.) served as carbon sources or potential inducers, or both. Chloramphenicol (a gift of Parke, Davis & Co., Detroit, Mich.) was added to the medium, where indicated, to inhibit protein synthesis. Growth media containing the tryptophan analogues or kynurenine were sterilized by filtration (Millipore Corp., Bedford, Mass.) after adjusting the pH to 7.0.

After growth in the various media, the bacteria were isolated by centrifugation of the culture medium and were resuspended in 10 mm sodium phosphate buffer (pH 7.0) containing 1 mm L-tryptophan. Samples of the bacterial suspension were disintegrated by ultrasonic vibration at 0 °C for 1 min (Branson Sonifier, 20,000 cycles/sec). The presence of L-tryptophan in the suspending buffer served to stabilize the tryptophan oxygenase during disruption. Extracts were assayed for tryptophan oxygenase activity by continuous spectrophotometry as previously described (2). Kynurenine formamidase activity was measured as described by Knox (7). One enzyme unit (EU) is defined as that amount of catalytic activity which produces 1 μmole product per min at 25 °C. The optical density (OD) of the bacterial suspension at 600 nm was found to be linearly related to bacterial dry weight, such that an OD600 of 1.0 corresponds to 0.42 mg (dry weight) per ml. Specific activity was calculated by dividing the total EU in the extract by the OD600 of the bacterial suspension. All values depicted for specific activity in figures and tables are expressed as X 10–4 EU per OD600 equivalents.

**RESULTS**

When *P. acidovorans* was inoculated from a culture containing yeast extract into a medium containing L-tryptophan as well as yeast extract, a diauxic growth pattern was observed (Fig. 1). This growth response was also observed when the culture contained yeast extract and succinate, glutarate, or L-glutamate. Initially, the bacteria exhibited logarithmic growth for approximately 3 hr. It is thus presumed that the organisms utilized the yeast extract as a carbon source until its exhaustion; during this time no induction of tryptophan-catabolizing enzymes was detected. A growth lag then ensued, during which time rapid enzyme induction was initiated. As tryptophan oxygenase synthesis was induced, cell growth and multiplication were resumed, implying that the bacteria were now able to metabolize tryptophan to fulfill their energy and carbon requirements. The specific activity of tryptophan oxygenase augmented, reaching a final steady-state level of 27 times basal level which was maintained during continued bacterial growth.

To eliminate complicating factors resulting from modification of the energy supply of the cell, a gratuitous (i.e., nonmetabolizable) inducer idealy is used in studies of an enzyme-
inducing system. Since no such substance is known for tryptophan oxygenase, a carbon source, which would minimally influence the course of microbial enzyme induction, was sought. *P. acidovorans* was unable to utilize glucose for growth (14) and, therefore, manifested no “glucose effect,” or repression of enzyme induction (10; Table 1). Glutamate, succinate, glutarate, and asparagine all served as metabolizable carbon sources for growth, but the first two substrates were unsuitable for induction studies because they completely prevented tryptophan oxygenase induction by tryptophan. Although some degree of catabolite repression was evident with glutarate and L-asparagine, each permitted an approximate 10-fold tryptophan-induced elevation in the specific activity of tryptophan oxygenase. Since L-asparagine permitted better cell growth than glutarate, it was selected as the carbon source for subsequent experiments.

The kinetics of the induction of tryptophan oxygenase was investigated. Rapid growth with no lag phase and constant, low basal levels of tryptophan oxygenase was observed in cells growing in a medium containing exclusively L-asparagine (Fig. 2a). When unadapted cells were inoculated into a medium containing L-tryptophan as sole carbon source, no growth occurred for 4 hr; afterward elevated tryptophan oxygenase levels were induced, permitting enhanced tryptophan metabolism and cellular growth. With both L-tryptophan and L-asparagine in the medium, growth and tryptophan oxygenase induction ensued immediately; however, metabolite repression by the asparagine or its metabolites, or both, diminished the rate of induction and the steady-state specific activity of this enzyme. EU are plotted versus total growth or protein, giving a so-called “differential plot” of enzyme induction (Fig. 2b). These data also indicate that when *P. acidovorans* is grown in the presence of tryptophan alone, tryptophan oxygenase represents a greater proportion of the cellular components than in uninduced cells and that asparagine moderates the extent of enzyme induction. An analysis of the kinetics of tryptophan oxygenase induction as a function of inducer concentration indicates that the length of the latent period is an inverse function of the tryptophan concentration (Fig. 3a) and that 0.05 mM tryptophan resulted in no significant enzyme induction. The “differential plot” of these data (Fig. 3b) suggests that, since the slopes of the lines obtained for inducer concentrations of 5 and 0.5 mM were essentially parallel, in both instances the enzyme-synthesizing machinery is fully saturated and functions at equivalent rates.

A variety of tryptophan analogues and metabolites were tested as inducers of tryptophan oxygenase and kynurenine formamidase (Table 2). The bacteria neither grew upon nor manifested induced enzymatic elevation when supplied with anthranilic acid. L-Kynurenine proved to be as potent as tryptophan in inducing both enzymes. Nevertheless, the bacteria grew well

**Table 1. Catabolite repression of tryptophan oxygenase induction**

<table>
<thead>
<tr>
<th>Supplements to growth medium</th>
<th>Tryptophan</th>
<th>OD₆₅₀</th>
<th>Tryptophan oxygenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EU/100 ml</td>
</tr>
<tr>
<td>L-Tryptophan...</td>
<td>+</td>
<td>0.6</td>
<td>0.64</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>-</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>L-Glutamate...</td>
<td>+</td>
<td>0.6</td>
<td>0.64</td>
</tr>
<tr>
<td>Succinate</td>
<td>+</td>
<td>0.5</td>
<td>0.040</td>
</tr>
<tr>
<td>Glutarate</td>
<td>-</td>
<td>0.5</td>
<td>0.056</td>
</tr>
<tr>
<td>L-Asparagine...</td>
<td>+</td>
<td>0.8</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.3</td>
<td>0.78</td>
</tr>
</tbody>
</table>

a The concentration of L-tryptophan was 5 mM (0.1%); all other carbon sources were 0.2%.

b After 14-hr growth.
upon kynurenic acid, but neither this compound nor any generated metabolite served as an inducer. Thus, kynurenic acid (or a compound preceding it on the degradative pathway), but not any metabolite thereof, is the feedback inducer of these enzymes. The remote possibility that formylkynurenine is the true inducer cannot be eliminated at this time. D-Tryptophan also induced both enzymes but was slightly less effective than the L-isomer. The oxygenase formed in response to D-tryptophan showed absolute specificity for L-tryptophan and did not act upon D-tryptophan, thus suggesting the possible presence of a racemase that converts D-tryptophan to its L-isomer. The relative activities of kynurenine and tryptophan as inducing agents were compared at a variety of concentrations. At inducer concentrations below \(10^{-4}\) M, kynurenine and tryptophan were equally effective in inducing tryptophan oxygenase (Fig. 4); at 5 mM concentration and above, kynurenine was a considerably more potent inducer of this enzyme than tryptophan (Fig. 4; Table 2). This divergence

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**Figure 2.** (a) **Kinetics of tryptophan oxygenase induction.** Cells were precultured in medium containing 0.3% L-asparagine and 0.05% yeast extract; during late-log phase they were collected by centrifugation, and equal amounts of a heavy inoculum of bacteria were added to growth media containing 0.3% L-asparagine (○); 0.3% L-asparagine and 5 mM L-tryptophan (△); and 5 mM L-tryptophan (●); their specific activity was evaluated as described in the text. (b) **The differential rate of tryptophan oxygenase synthesis.** The experimental conditions are described above. Asparagine (●); asparagine and 5 mM L-tryptophan (△); 5 mM L-tryptophan (○).

**Figure 3.** (a) **Early kinetics of tryptophan oxygenase induction at various inducer concentrations.** Bacteria were grown in media containing 0.3% L-asparagine until the \(\text{OD}_{630}\) reached 0.4, and then additional media containing 0.3% L-asparagine and enough L-tryptophan to give the appropriate final tryptophan concentration were added: 5 mM L-tryptophan (●); 0.5 mM L-tryptophan (□); 0.05 mM L-tryptophan (○); no inducer (△). At the indicated intervals, portions of the incubation media were withdrawn, and the EU per 100 ml of culture were evaluated. (b) **The differential rates of tryptophan oxygenase induction at various L-tryptophan concentrations.** The experimental conditions are described above. L-Tryptophan at 5 mM (●); 0.5 mM (×); 0.05 mM (△); no inducer (○).
TABLE 2. Effect of tryptophan and its metabolites upon the induction of tryptophan oxygenase and kynurenine formamidase

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>O.D.₅₆₀ᵃ</th>
<th>Tryptophan oxygenase</th>
<th>Kynurenine formamidase</th>
<th>Tryptophan oxygenase: kynurenine formamidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Specific activity</td>
<td>Induction</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Asparagine (6.3%)</td>
<td>Supplement (5 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>0.92</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>L-Tryptophan</td>
<td>1.07</td>
<td>6.4</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td>Anthranilic acid</td>
<td>0.55</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Kynurenic acid</td>
<td>1.02</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>L-Kynurenine</td>
<td>0.92</td>
<td>7.6</td>
<td>660</td>
</tr>
<tr>
<td></td>
<td>D-Tryptophan</td>
<td>0.80</td>
<td>5.2</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>Kynurenic acid</td>
<td>0.87</td>
<td>0.8</td>
<td>0</td>
</tr>
</tbody>
</table>

ᵃ All cultures containing asparagine were allowed to grow for 7 hr; the kynurenic acid culture lacking asparagine was incubated for 20 hr to permit the cells to adapt to this carbon source.

To ascertain if tryptophan oxygenase induction requires protein synthesis, chloramphenicol, a powerful inhibitor of protein formation, was introduced into the medium. At a concentration of 50 μg/ml, chloramphenicol completely suppressed growth of P. acidovorans and, when added to an inducing medium containing L-asparagine and L-tryptophan, it completely prevented tryptophan oxygenase induction (Fig. 5). Thus, in common with other microbial enzyme induction systems, in P. acidovorans, substrate induction of tryptophan oxygenase is dependent on de novo protein synthesis or cellular growth, or both.

The reversibility of the induction and the stability of the induced tryptophan oxygenase were explored. Cells were precultured in media may reflect a difference in transport efficiencies.

The phenomenon of "product (or metabolite) induction," as typified by the L-kynurenine induction of tryptophan oxygenase, corroborates the findings of Palleroni and Stanier (11) with P. fluorescens and is in contrast to the findings of Tremblay et al. (15). The observed constancy of the ratios of per cent induction of the two enzymes evoked by the seemingly three different inducers would, per se, imply that the enzymes are coordinately regulated. However, the marked deviation between the induced and constitutive ratios of these enzymes would suggest their non-coordinate regulation.

![Figure 4. Induction of tryptophan oxygenase as a function of tryptophan and kynurenine levels. Unadapted (L-asparagine-grown) cells were inoculated into media containing L-asparagine with various concentrations of L-tryptophan (●) or L-kynurenine (○). After 7-hr growth, the specific activity of tryptophan oxygenase was determined.](http://jb.asm.org/)

![Figure 5. Effect of chloramphenicol on enzyme induction. Bacteria were precultured in L-asparagine, and then inoculated into minimal medium containing, in addition to L-asparagine: 5 mM L-tryptophan (▲); 5 mM L-tryptophan and 50 μg of chloramphenicol per ml (●); or no supplement (○).](http://jb.asm.org/)
containing the inducer tryptophan and then after all the inducer was metabolized, fresh minimal medium, to which L-tryptophan was either added or omitted, was introduced into the culture of preinduced cells (zero-time; Fig. 6). In the continued presence of inducer, the high specific activity of the induced enzyme was retained, and the total amount of enzyme was increased with bacterial growth. Cells, deprived of inducer, exhibited a continuous decline in specific activity; total EU, however, remained constant. These data thus indicate that the induced enzyme, in vivo, is stable, decreasing specific activity being due solely to dilution by cell growth.

Several tryptophan analogues were tested in an attempt to uncover gratuitous inducers of tryptophan oxygenase (Table 3). Each of the tryptophan analogues, tested alone in the minimal medium, acted as an amino acid antagonist, severely restricting growth on asparagine in the absence of tryptophan; tryptophan oxygenase specific activity, however, was unaffected. For many analogues the presence of L-tryptophan nullified this growth antagonism and allowed induction by tryptophan to proceed to near-normal enzyme levels. DL-5-Fluorotryptophan, however, without inhibiting cell growth, strongly inhibited the L-tryptophan-promoted enzyme induction. DL-7-Azatryptophan, on the other hand, although alone was incapable of enzyme induction, very significantly enhanced the induction by tryptophan. Figure 7 depicts the effects of graded concentrations of DL-5-fluorotryptophan and DL-7-azatryptophan on the induction of tryptophan oxygenase by L-tryptophan. Below a concentration of 1 mM, neither analogue alters tryptophan oxygenase levels; above this concentration, in the presence of L-tryptophan, DL-7-azatryptophan progressively augmented and DL-5-fluorotryptophan inhibited enzyme induction. No concentration of 7-azatryptophan caused enzyme induction in the absence of tryptophan nor did 7-azatryptophan alter the

**TABLE 3. Inhibitory and synergistic effects of tryptophan analogues upon tryptophan oxygenase induction by tryptophan**

<table>
<thead>
<tr>
<th>Growth medium supplement*</th>
<th>Tryptophan oxygenase specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Tryptophan</td>
</tr>
<tr>
<td>None</td>
<td>1.0</td>
</tr>
<tr>
<td>DL-5-Methyltryptophan</td>
<td>0.8</td>
</tr>
<tr>
<td>DL-a-Methyltryptophan</td>
<td>0.8</td>
</tr>
<tr>
<td>DL-5-Hydroxytryptophan</td>
<td>1.2</td>
</tr>
<tr>
<td>DL-5-Fluorotryptophan</td>
<td>1.4</td>
</tr>
<tr>
<td>DL-7-Azatryptophan</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* All media contained 0.3% asparagine; L-tryptophan and all tryptophan analogues were present at 5 mM. Cultures were incubated until early stationary phase.

![Fig. 6. The in vivo stability of induced tryptophan oxygenase during deadaptation. Induced cells were obtained by growth in one-half the usual tryptophan concentration (2.5 mM) and 0.05% yeast extract in 500 ml. When growth reached the stationary phase (OD<sub>600</sub> = 0.7) it was assumed that all the inducer had been metabolized. At this stage, 500 ml of fresh medium was added (zero-time) containing L-asparagine alone, (O—O) tryptophan oxygenase specific activity; (O——O) EU/10 ml. Addition of L-asparagine plus tryptophan, (●—●) specific activity; (●——●) EU/10 ml.](http://jb.asm.org/)

![Fig. 7. Effects of various levels of DL-5-fluorotryptophan and DL-7-azatryptophan upon tryptophan oxygenase induction by tryptophan. Unadapted cells were added to flasks containing L-asparagine and 5 mM L-tryptophan and the indicated concentrations of DL-5-fluorotryptophan (●) or DL-7-azatryptophan (○). After 7-hr growth, the specific activity of tryptophan oxygenase was determined.](http://jb.asm.org/)
cellular growth rate. Increasing concentrations of fluorotryptophan, however, progressively suppressed bacterial proliferation until minimal noninduced growth levels were approached, suggesting a competitive impairment of tryptophan utilization. Neither analogue served as sole carbon source for bacterial growth.

The possibility of the coordinate regulation of tryptophan oxygenase and kynurenine formamidase was tested by comparing, under various conditions, the relative effects of these tryptophan-analogue modifiers of induction by tryptophan upon the two enzyme activities (Table 4). Although DL-5-fluorotryptophan resulted in inhibition of tryptophan oxygenase induction, formamidase levels were unaffected or slightly elevated. In the presence of asparagine and tryptophan, DL-7-azatryptophan enhanced the induction of the two enzymes equally, but with L-tryptophan alone, this analogue markedly stimulated tryptophan oxygenase induction with no significant effect on the formamidase level. That the ratios of the activities of tryptophan oxygenase and kynurenine formamidase were clearly variable under the influence of various substrate analogues seems to preclude coordinate regulation of synthesis of these two enzymes.

**DISCUSSION**

In its basic attributes, the induction of tryptophan oxygenase is characteristic of most bacterial enzyme induction by the requirement for continued cell growth and de novo protein synthesis, formation of a stable enzyme which, upon removal of inducer, is diluted out by cell growth, and its sensitivity to catabolite repression. The phenomenon, analogous to the product induction of tryptophan oxygenase and kynurenine formamidase by kynurenine, likewise occurs in other microbial systems, e.g., the α-glycerophosphate pathway of *Escherichia coli* (3) and the histidine degradative pathway of *Aerobacter aerogenes* (12). Formally analogous to the inhibition of L-tryptophan-mediated induction of tryptophan oxygenase by 5-fluorotryptophan is the report by Hughes (4) that, in a strain of *P. fluorescens*, halogen-substituted benzoic acids compete with benzoic acid in the induction of benzoic acid-oxidizing enzymes.

7-Azatryptophan, itself inactive as an inducer, strikingly enhances the L-tryptophan-mediated induction of tryptophan oxygenase. 7-Azatryptophan is not a substrate, nor, in the concentrations tested, does it influence the activity, in vitro, of purified tryptophan oxygenase. It is, therefore, unlikely that 7-azatryptophan, or 5-fluorotryptophan (which is also not a substrate of purified tryptophan oxygenase) undergoes any chemical alteration while affecting the induction pattern. Leder and Perry (8) have recently reported that, in a galactokinase-deficient strain of *E. coli*, galactose can enhance, in an analogous manner, the induction of β-galactocidase by isopropylthiogalactoside. This effect was due to the induction of a galactoside permease by galactose. However, in *P. acidovorans*, 7-azatryptophan does not alter the activity or level of a tryptophan permease activity which is induced by tryptophan (unpublished data). Another possibility, that 7-azatryptophan acts by nullifying the repression elicited by asparagine (Table 1), was tested by cultivating bacteria in the presence of L-tryptophan and 7-azatryptophan, but in the absence of asparagine (Table 4). Although there was no metabolite repression to oppose, induction was again enhanced, thus indicating that this analogue must act at a site other than that of asparagine repression. Since an inducer

**Table 4. Comparative effects of tryptophan analogues upon the induction of tryptophan oxygenase and kynurenine formamidase**

<table>
<thead>
<tr>
<th>Growth medium supplementa</th>
<th>Tryptophan oxygenase specific activity</th>
<th>Kynurenine formamidase specific activity</th>
<th>Tryptophan oxygenase: kynurenine formamidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Asparagine (6.9%)</td>
<td>L-Tryptophan (5 mm)</td>
<td>Analogue (5 mm)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>5-Fluorotryptophan</td>
<td>1.0</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>4.0</td>
<td>1.74</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>7-Azatryptophan</td>
<td>9.8</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>10.2</td>
<td>3.2</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>5-Fluorotryptophan</td>
<td>16.0</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>7-Azatryptophan</td>
<td>9.2</td>
</tr>
</tbody>
</table>

a Those cultures containing asparagine were incubated for 7 hr; the remaining cultures were allowed to grow for 11 hr.
analogue may interact with, and influence the activity of, a repressor (6), the possibility exists that 7-azatryptophan may act as a coinducer by itself combining with the endogenous repressor and enhancing its affinity for the inducer(s) L-tryptophan or L-kyurenine, or both.

That tryptophan analogues cause differential effects upon the inductions of tryptophan oxygenase and kynurenine formamidase eliminates the possibility that these enzymes are coordinately regulated, unless one postulates additional regulatory processes which separately influence the levels of these two enzymes. In our strain of Pseudomonas, in contrast to a previous report with a different strain (15), the induction of these enzymes cannot be of the sequential type since a single compound, kynurenine, induces both enzymes, thereby violating the fundamental criterion of sequential induction, namely, that successive metabolites induce their respective enzymes (13).

Further studies will be required, by use of appropriate mutants, to determine if tryptophan and formylkynurenine are capable of direct action as inducing agents or if basal levels of tryptophan oxygenase and formamidase convert these precursors to kynurenine which exclusively acts as the inducer of these enzymes.

ACKNOWLEDGMENTS

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