Induction by L-Tryptophan and an Analogue, α-Methyl-DL-Tryptophan, of the Enzymes Catabolizing L-Tryptophan in Pseudomonas

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Received for publication 19 August 1966

ABSTRACT

An investigation was made of the pattern of induction of the enzymes that metabolize L-tryptophan through kynurenic acid (the quinoline pathway) in Pseudomonas fluorescens. The first four enzymes in the pathway were not induced in the same proportions or in the same time courses. This lack of coordinate induction excludes a mechanism of regulation of these enzymes at a single site as proposed in the operon model. The enzymes were induced in a sequential pattern in the order of their position on the pathway, when they became the limiting reactions. The nonmetabolizable analogue, α-methyl-DL-tryptophan, caused a measurable elevation in the levels of the first three enzymes of the same pathway. Evidence is presented that growth of the cells in the presence of α-methyl-DL-tryptophan caused the accumulation of endogenous tryptophan, and that induction by the nonmetabolizable analogue is induction by the endogenous tryptophan.

Adaptation of Pseudomonas cells to any intermediate of the tryptophan pathway resulted in the acquisition of the ability to catabolize the subsequent metabolites, but not the precursors, of that intermediate (14, 17, 18). These and other findings gave rise to the view that the induction of the enzymes of a metabolic pathway proceeds in a sequential fashion, each enzyme being induced as a result of the accumulation of its substrate (7, 14, 15, 17, 18). The operon model suggested another mechanism by which the levels of enzymes may be regulated (5). This model requires, by definition, that the ratio of the amount of any enzyme to the amount of any other enzyme of the same operon be constant regardless of the extent of induction (coordinate induction). Palleroni and Stanier (11) examined a strain of Pseudomonas which oxidizes tryptophan via the aromatic pathway, through anthranilic acid, and found that, whereas the later enzymes in the pathway (kynureninase, pyrocatechase, and the lactonizing enzyme) were induced sequentially, the earlier enzymes (tryptophan pyrrolase and formamidase) appeared to be induced coordinately. The present communication reports the pattern of induction of the enzymes involved in the catabolism of tryptophan via the quinoline pathway, through kynurenic acid, in a strain of P. fluorescens. The enzymes were induced during growth in a medium containing either L-tryptophan or a nonmetabolizable analogue, α-methyl-DL-tryptophan.

Evidence is presented indicating that the first four enzymes in the pathway were not induced coordinately by tryptophan. Although the first three of these enzymes were induced by α-methyltryptophan, they did not appear to be induced directly by the nonmetabolizable analogue. Induction with α-methyltryptophan is thought to result from the accumulation of endogenous tryptophan, brought about by the action of the analogue. The findings are incompatible with regulation of these enzymes according to the operon model, but are consistent with the concept of sequential induction.

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MATERIALS AND METHODS

Materials. Formamidase was prepared from rat liver according to the method of Knox (8), and N-formyl-DL-kynurenine was synthesized according to the method of Auerbach and Knox (1). All other chemicals were obtained commercially. α-Methyl-DLtryptophan, purchased from the Regis Chemical Co., Chicago, Ill., showed no impurities when subjected to paper chromatography by use of the two solvent systems of Jepson (6).

Growth and harvest of cells. Cultures of Pseudomonas strain RYS-1 (16) were maintained on agar slants (Difco Nutrient Broth, 8.0 g; Difco Agar, 30.0 g; 0.04 M sodium potassium phosphate buffer, pH 7.2; 1 liter) at 5°C. The cells were grown for experimental use by transfer from an agar slant to a liquid culture medium of the following composition: ammonium nitrate, 1.0 g; magnesium sulfate, 0.2 g; yeast extract (Difco), 1.0 g; ferrous sulfate heptahydrate, 0.001 g; sodium succinate hexahydrate, 1.35 g (final concentration, 5 × 10−4 M); 0.04 M sodium potassium phosphate buffer, pH 7.2, in a sufficient quantity to make 1 liter of solution. A suspension, in sterile buffer, of freshly harvested and washed cells grown in the absence of inducer was used to inoculate media containing inducer. Inducers were added, when indicated, at a final concentration of 5 × 10−4 M. The suspension of cells was placed on a mechanical shaker at room temperature for the duration of the logarithmic-growth period. The cells were harvested by centrifugation in an International PR-2 refrigerated centrifuge. The harvested cells were resuspended in 0.04 M sodium potassium phosphate buffer (pH 7.2), the centrifugation was repeated, and the washed cells were dispersed in buffer for respirometric studies, or were extracted for the analysis of enzymatic activities.

Respirometric studies. The ability of whole cells to consume oxygen at the expense of L-tryptophan and its metabolites was measured in air at 30°C by use of a Warburg apparatus. The main chamber of the Warburg flasks contained 2.0 ml of a suspension in buffer of 2.6 mg of wet cells per ml. The center well contained paper wicks and 0.20 ml of a 10% solution of potassium hydroxide, and the side arm contained a solution of 2 μmoles of test substrate.

Preparation of cell-free extracts. The washed cells were transferred to a chilled mortar and were ground in the cold with twice their wet weight of levigated alumina (Norton Co., Worcester, Mass.) until a tacky mass was obtained (4, 10). The broken cells were extracted with 0.04 M sodium potassium phosphate buffer, pH 7.2 (5 ml of buffer per gram of wet cells), and the resulting suspension was fractionated at 20,000 × g for 20 min in a Spinco model L refrigerated centrifuge. The supernatant fluid from the low-speed centrifugation was decanted from the sediment of alumina, whole cells, and cell fragments, and was centrifuged at 78,000 × g for 60 min in the centrifuge. The high-speed supernatant fluid was used as the source of the enzymes which catabolize tryptophan.

Enzymatic assays. Each of the enzymes was assayed spectrophotometrically by use of a Beckman model-DK Ratio Recording spectrophotometer. The reference cell contained all the ingredients of the incubation mixture except the substrate. In each of the assays, there was a linear relationship between the concentration of cell-free extract in the incubation mixture and the rate of reaction. The enzymatic activities are expressed as micromoles of product formed (or substrate lost) per minute per milligram of protein at 25°C. The concentrations were calculated from the molecular extinction coefficients of 4.500 at 360 μM for kynurenine and 10,000 at 333 μM for kynurenic acid. Protein determinations were made according to the biuret method (9). The solvent used for all of the components of the incubation mixtures described below was 0.04 M sodium potassium phosphate buffer, pH 7.2, with the exception of ferrous ammonium sulfate which was dissolved in water. All reagents used in each of the enzymatic assays were freshly prepared.

Tryptophan pyrrolase. The activity of tryptophan pyrrolase (EC 1.13.1.12) was determined according to the method of Tanaka and Knox (19). Since the determination of tryptophan pyrrolase by this method is dependent upon the accumulation of kynurenine, the formamidase was added to the assay mixture. Each incubation mixture contained: 0.02 M ascorbic acid, 0.10 ml; bacterial extract, 0.10 to 0.40 ml; formamidase (8), 0.47 μ mole/min under the conditions for the assay of formamidase described below; 0.02 M l-tryptophan, 0.40 ml; 0.04 M phosphate buffer, pH 7.2, in the quantity needed to bring the final volume to 3.50 ml. The rate of increase in optical density at 360 μM was used as a measurement of the activity of tryptophan pyrrolase.

Formamidase. The activity of formamidase (EC 3.5.1.19) was determined according to the method of Knox (8), the determination being dependent upon the accumulation of kynurenine. Each complete incubation mixture contained: bacterial extract, 0.05 to 0.30 ml; 0.01 M N-formyl-DL-kynurenine, 0.20 ml; 0.04 M phosphate buffer, pH 7.2, to give a final volume of 3.50 ml. The rate of increase in optical density at 360 μM was used as a measurement of the activity of formamidase.

Kynurenine transaminase. The activity of kynurenine transaminase (EC 2.6.1.7) was determined by measuring the initial rate of disappearance of kynurenine in an incubation mixture of the following composition: 0.001 M pyridoxal phosphate, 0.30 μM; 0.005 μM α-ketoglutarate, 0.30 μM; bacterial extract, 0.05 to 0.30 ml; 0.005 M L-kynurenine sulfamate, 0.15 μM; 0.04 M phosphate buffer, pH 7.2, in sufficient quantity to give a final volume of 3.25 ml. The rate of decrease in optical density at 360 μM was used as a measurement of the activity of kynurenine transaminase.

Interference by kynurenine transaminase with the accumulation of kynurenine in the assays of the first two enzymes was evaluated by measuring the rate of increase in optical density at 333 μM (maximum of kynurenic acid) as well as at 360 μM (maximum of kynurenine), the value at 333 μM being corrected for absorption by kynurenine. The rate of formation of kynurenic acid from kynurenine during the assays of the tryptophan pyrrolase and the formamidase was about 20% of the rate of formation of kynurenine except in extracts of cells not exposed to tryptophan,
where no interference was detected. Correction of the activities of tryptophan pyrrolase and formamidase for this interference by kynurenine transaminase did not alter the pattern of induction of these enzymes.

Kynurenic acid hydroxylase. The assay devised for kynurenic acid hydroxylase (EC 1.14.1.2) depended upon the discovery that the oxidation of kynurenic acid by this enzyme required iron and reduced nicotinamide adenine dinucleotide \((\text{NADH}_2)\), and was inhibited by the particulate components of the cell (2, 20). Quantitative analysis of the activity of this enzyme in the high-speed supernatant fluids of bacterial extracts was achieved by use of the following incubation mixture: alcohol dehydrogenase from yeast, 30 units (stock no. 340-26, Sigma Chemical Co., St. Louis, Mo.); 1.1 \times 10^{-4} \text{M NADH}_2, 0.15 ml; 2.2 \times 10^{-4} \text{M ferrous ammonium sulfate}, 0.15 ml; 1.6 \text{mM} \text{DL-tryptophan}, 0.15 ml; bacterial extract, 0.50 to 2.00 ml; 3.76 \times 10^{-4} \text{M kynurenic acid}, 0.10 ml; 0.04 \text{M phosphate buffer, pH 7.2}, in a sufficient quantity to give a final volume of 3.30 ml. The activity of kynurenic acid hydroxylase was linearly proportional to the concentration of protein above 3 mg per incubation mixture. Accordingly, the activity of the enzyme was determined by use of at least two concentrations of protein above 3 mg per incubation mixture. The activity of the enzyme was determined routinely by measuring the rate of disappearance of kynurenic acid spectrophotometrically at 333 nm.

Kynurenic acid hydroxylase catalyzes the formation of 7,8-dihydroxykynurenic acid-7,8-diol from equimolar amounts of kynurenic acid and molecular oxygen (20). The rate of oxygen uptake, measured in a Warburg apparatus, was found to be 70% higher than the stoichiometric amount required for the rate of disappearance of kynurenic acid by this reaction, measured spectrophotometrically. The consumption of oxygen in excess of the amount required for the disappearance of kynurenic acid could be attributed to the further oxidation of 7,8-dihydroxykynurenic acid-7,8-diol (20), which resulted in the formation of a yellow compound.

**Results**

\(\alpha\)-Methyl-DL-tryptophan is a nonmetabolized inducer of tryptophan pyrrolase in rats (3, 12). \(\alpha\)-Methyltryptophan added to the culture medium delayed the growth of *Pseudomonas* cells. After the period of delayed growth, these cells gained the ability to metabolize added L-tryptophan. The nature of the adaptation to L-tryptophan induced by \(\alpha\)-methyltryptophan was compared in whole cells and in cell-free extracts with that induced by L-tryptophan, to establish whether \(\alpha\)-methyltryptophan was a nonmetabolized inducer of tryptophan pyrrolase in *Pseudomonas* as well as in rats.

**Adaptive patterns of intact cells.** The rates of growth of the cells at the expense of succinate, in the absence of inducer or in the presence of tryptophan or \(\alpha\)-methyltryptophan, are illustrated in Fig. 1. The retardation of growth by \(\alpha\)-methyltryptophan and the prevention of the retardation by tryptophan will be discussed in a later section. The cells were harvested at the end of their particular growth phase, washed, resuspended, and tested in a Warburg apparatus for their ability to consume oxygen at the expense of tryptophan and its metabolites. Typical respirometric patterns are illustrated in Fig. 2 and 3. Cells adapted to tryptophan consumed a total of 5.60 \text{umoles} of oxygen per \text{umole} of tryptophan and 4.26 \text{umoles} of oxygen per \text{umole} of kynurenic acid (Fig. 2). These values represent equally complete metabolism, since the difference of 1.34 \text{umoles} between the consumption of oxygen with tryptophan and with kynurenic acid reflects the oxygen uptake in the reactions catalyzed by tryptophan pyrrolase and formic acid oxidase, the theoretical value for which is 1.50 \text{umoles} of \(\text{O}_2\) per \text{umole} of substrate. The consumption of oxygen at the expense of anthranilic acid was only slightly higher than the endogenous respiration (0.60 \text{umole} of oxygen consumed in 90 min per \text{umole} of anthranilic acid), illustrating that tryptophan is catabolized in this organism mainly through the quinoline pathway.

Cells induced by \(\alpha\)-methyltryptophan consumed oxygen at the expense of tryptophan, but not kynurenic acid or anthranilic acid (Fig. 3).
These results suggested that the enzymes induced by α-methyltryptophan did not include kynurenic acid hydroxylase. Cells grown in the absence of either inducer did not consume oxygen at the expense of any of the test substrates for the first 60 min, but after this time oxygen uptake usually commenced as a result of adaptation of the cells to the test substrate. Noninduced cells, cells induced by tryptophan, and cells induced by α-methyltryptophan did not consume oxygen at the expense of α-methyltryptophan even after several hours.

Cells induced with α-methyltryptophan and exposed to tryptophan accumulated metabolites, which indicated the extent of the enzymatic pathway induced by α-methyltryptophan. Spectra were taken of neutralized metaphosphoric acid filtrates prepared from the reaction mixtures of these cells incubated with tryptophan for 60 min in the Warburg apparatus. These spectra showed that kynurenic acid accumulated, the observation being confirmed by chromatographic analysis (6). A major portion (73%) of the tryptophan metabolized could be accounted for as accumulated kynurenic acid. Analogous experiments with tryptophan-induced and non-

induced cells showed no accumulation of kynurenic acid or other absorbing intermediates. In addition, the noninduced cells showed virtually no disappearance of tryptophan during 60 min of incubation. These data supported the view that α-methyltryptophan induced some early enzymes of tryptophan catabolism, but not kynurenic acid hydroxylase or the later enzymes in the pathway that were induced by tryptophan.

Enzymatic activities of cell-free extracts. The number of enzymes that were induced by α-methyltryptophan, as indicated by the respirometric and chromatographic studies in whole cells, was confirmed by analysis of the enzymatic activities in cell-free extracts. The abilities of α-methyltryptophan and tryptophan to induce the first four enzymes involved in the dissimilation of tryptophan are compared in Table 1. A fifth enzyme, formic acid oxidase, was present constitutively in excess in the cells. Spectral analysis during the course of the reaction with kynurenine in extracts of cells induced with either tryptophan or α-methyltryptophan showed only kynurenic acid (formed by kynurenine transaminase) and no evidence for the formation of anthranilic acid (formed by kynureninase). Although less effective as an inducer than tryptophan, α-methyltryptophan induced tryptophan pyrrolase and kynurenine transaminase to levels which were approximately 300% higher than the levels found in noninduced cells. The specific activity of formamidase was the highest of the enzymes assayed in noninduced cells, and it was elevated the least by α-methyltryptophan. The activity of kynurenic acid hydroxylase was found only in cells induced by tryptophan for several hours, and was not detectable in either non-

FIG. 2. Ability of cells induced by L-tryptophan to consume oxygen at the expense of L-tryptophan, kynurenic acid, and anthranilic acid; 2 μmoles of substrate were used in each case. The endogenous oxygen uptake has been subtracted from the values.

FIG. 3. Ability of cells induced by α-methyl-DL-tryptophan to consume oxygen at the expense of L-tryptophan, kynurenic acid, and anthranilic acid; 2 μmoles of substrate were used in each case. The endogenous oxygen uptake has been subtracted.
induction of tryptophan pyrrolase, formamidase, and kynurenine transaminase. An inoculum of 6.0 g of noninduced cells was suspended in 3 liters of culture medium made 5 × 10⁻³ M in L-tryptophan. The culture was divided into three portions of 1 liter each, and was allowed to grow at room temperature on a mechanical shaker. The portions were harvested, and the cells were washed after 30, 90, and 190 min of growth. Cell-free extracts were prepared immediately, and measurements of the enzymatic activities were begun as soon as the high-speed supernatant fluids were available. The specific activities of these enzymes, in three separate experiments of this type, after exposure to tryptophan for periods up to 190 min are listed in Table 2. With the exception of formamidase, which was initially present in highest amount, the enzymes were induced in the order of their place in the metabolic pathway. Induction of tryptophan pyrrolase preceded that of kynurenine transaminase, whereas kynurenic acid hydroxylase was not induced during the same period but at some time after 190 min (Table 1).

Coordination of induction of the enzymes would require that they be increased by the same proportions throughout the course of induction. The specific activity of formamidase was not increased over a period of 90 min of exposure to inducer, whereas, during the same period, the specific activities of tryptophan pyrrolase and kynurenine transaminase were elevated to levels 4 and 1.7 times their initial values, respectively. Between 90 and 190 min of exposure to inducer, the specific activities of formamidase and tryptophan pyrrolase increased by 3 times their values

### Table 1. Enzymatic activities in cell-free extracts

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Non-induced cells</th>
<th>Cells induced by α-methyl-DL-tryptophan</th>
<th>Cells induced by L-tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan pyrrolase</td>
<td>1.9²</td>
<td>5.4</td>
<td>61.2</td>
</tr>
<tr>
<td>Formamidase</td>
<td>10.5</td>
<td>15.8</td>
<td>78.7</td>
</tr>
<tr>
<td>Kynurenine transaminase</td>
<td>7.4</td>
<td>21.3</td>
<td>144.4</td>
</tr>
<tr>
<td>Kynurenic acid hydroxylase</td>
<td>&lt;0.50</td>
<td>&lt;0.50</td>
<td>4.53</td>
</tr>
</tbody>
</table>

² Pseudomonas cells were harvested at the end of their growth phases, without inducer, with α-methyl-DL-tryptophan or with L-tryptophan. Soluble extracts were prepared for enzyme assays, as described in the text.

The finding that growth of the cells in the presence of a nonmetabolizable inducer resulted in the induction of the first three enzymes in the pathway would be compatible with the notion that these enzymes were regulated by a single operon, if they were induced together (coordinate). Evidence on the conditional point was obtained by measuring the rates of the inductions of these enzymes.

Cells were subcultured in the succinate medium lacking inducer, harvested, and washed at the end of the growth phase; they were then used as the inoculum for the studies of the kinetics of

### Table 2. Activities of the first four enzymes involved in the catabolism of L-tryptophan during 190 min of exposure to L-tryptophan

<table>
<thead>
<tr>
<th>Time of exposure to L-tryptophan</th>
<th>Expt no.</th>
<th>Tryptophan pyrrolase</th>
<th>Formamidase</th>
<th>Kynurenine transaminase</th>
<th>Kynurenic acid hydroxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>1</td>
<td>1.9 (100)</td>
<td>10.5 (100)</td>
<td>7.4 (100)</td>
<td>&lt;0.50</td>
</tr>
<tr>
<td>2</td>
<td>2.2 (100)</td>
<td>13.1 (100)</td>
<td>7.1 (100)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.7 (100)</td>
<td>9.6 (100)</td>
<td>5.5 (100)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>1</td>
<td>2.2 (116)</td>
<td>9.3 (88.5)</td>
<td>7.9 (107)</td>
<td>&lt;0.50</td>
</tr>
<tr>
<td>2</td>
<td>2.4 (109)</td>
<td>11.4 (87.0)</td>
<td>6.8 (96)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.1 (374)</td>
<td>9.2 (86.5)</td>
<td>13.7 (185)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.8 (309)</td>
<td>11.0 (84.0)</td>
<td>9.6 (135)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.0 (530)</td>
<td>8.5 (88.5)</td>
<td>10.2 (185)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>190 min</td>
<td>1</td>
<td>18.3 (965)</td>
<td>26.8 (255)</td>
<td>58.2 (788)</td>
<td>&lt;0.50</td>
</tr>
<tr>
<td>2</td>
<td>20.2 (920)</td>
<td>34.2 (261)</td>
<td>43.8 (618)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>29.2 (1715)</td>
<td>22.5 (235)</td>
<td>49.0 (890)</td>
<td>&lt;0.50</td>
<td></td>
</tr>
</tbody>
</table>

² Enzyme activities (expressed as millimicromoles per minute per milligram of protein) in soluble extracts of cells, subcultured without inducer and then grown for different times in 5 × 10⁻⁴ M L-tryptophan, are given for three separate experiments. The activities as per cent of those at zero-time are given in parentheses.
at 90 min, whereas that of kynurenine transaminase increased by 4.5 times. Since the specific activities of these enzymes in the noninduced cells were not the same, these relative kinetics of their inductions were graphically illustrated by setting all of the noninduced levels at 100% and plotting the per cent of the initial activity found after intervals of exposure to the inducer (Fig. 4).

The possibility exists that the apparent levels of tryptophan pyrrolase, formamidase, and kynurenine transaminase in noninduced cells represent, in part, nonspecific activities, only fractions of which are inducible by tryptophan. Therefore, a second analysis of the kinetics of induction was made in which the initial level of enzymatic activity was subtracted from that found after intervals of exposure to inducer. The difference represents solely the amount of activity induced, and this treatment of the data again illustrates the marked lack of coordination in the induction of these enzymes (Fig. 4). It is clear from Fig. 4 and 5 that the kinetics of induction of these enzymes are not coordinate, and could not be controlled by a single operon.

Mechanism of induction by a-methyltryptophan. With the elimination of a single operon on which an inducer could act, another explanation was needed for the induction of the first three enzymes by the nonmetabolizable analogue, a-methyltryptophan. The structural similarity between a-methyltryptophan and the three natural substrates of these enzymes suggested that a-methyltryptophan might act at the same sites as the natural inducers of each of the enzymes. The ability of a-methyltryptophan to interact with sites specific for tryptophan and kynurenine was tested by measuring the effect of a-methyltryptophan on the activities of tryptophan pyrrolase and kynurenine transaminase. The activity of tryptophan pyrrolase was significantly inhibited by a-methyltryptophan present in a concentration equal to or greater than that of L-tryptophan, but kynurenine transaminase was very slightly inhibited by concentrations of a-methyltryptophan four times greater than the concentration of L-kynurenine sulfate (Fig. 6).

The affinities of both enzymes for a-methyltryptophan ($K_i$) were nevertheless similar ($2 \times 10^{-8}$ and $4 \times 10^{-8}$ M) (Fig. 6), and both were induced to comparable extents by a-methyltryptophan (Table 1). These findings do not eliminate, but also do not compel, belief in a hypothesis that a-methyltryptophan induces tryptophan pyrrolase and kynurenine transaminase by virtue of its affinities for the separate sites specific for the substrates of each enzyme.

The pattern of growth of the cells exposed to a-methyltryptophan suggested another mechanism by which a-methyltryptophan could induce the first three enzymes of the pathway. The cells grew promptly and equally well on tryptophan, kynurenine, or kynurenic acid, alone or in combination with succinate. The onset of growth of cells in the medium containing a-methyltryptophan was delayed about 4 hr (Fig. 1). The period
of retardation was followed by growth at a rate and to an extent which approached that of cells grown in succinate or in succinate plus tryptophan. The induction of the enzymes by \( \alpha \)-methyltryptophan appeared after the delayed growth and during the period of rapid growth. It is probable that the retardation of growth caused by \( \alpha \)-methyltryptophan was eventually overcome by the intracellular accumulation of endogenous tryptophan, which also induced the tryptophan-catabolizing enzymes. The delay before the commencement of growth in the presence of \( \alpha \)-methyltryptophan was significantly shortened by the addition to the medium of one-tenth as much tryptophan as \( \alpha \)-methyltryptophan, and the delay was completely avoided by the addition of an equimolar concentration of tryptophan (Fig. 1). The addition of an equimolar concentration of kynurenic acid did not alter the delay of growth, suggesting that the effect of tryptophan was specific, and not the effect of an added nutrient. Addition of kynurenine to the medium containing \( \alpha \)-methyltryptophan caused complete failure of growth for 15 hr or more, during which time the growth could be started by the addition of sufficient tryptophan to the culture. Kynurenine is a very effective inducer of tryptophan pyrrolase (17) and does not itself alter the rate of growth. Apparently, the induction of tryptophan pyrrolase by kynurenine prevented the necessary accumulation of endogenous tryptophan in the presence of \( \alpha \)-methyltryptophan.

**Discussion**

The long series of enzymes induced by L-tryptophan and its metabolites in *P. fluorescens* RYS-1 (16) provided this opportunity to test the general applicability of schemes for the regulation of such a group of enzymes at a single site, such as that proposed in the operon model (5).

A study of the kinetics of induction of the first four enzymes in the pathway showed that there was no coordination of their induction, and indicated that the mechanism of regulation of these enzymes was incompatible with that described in the operon model. Moreover, the kinetics of induction of these enzymes followed a pattern which was generally sequential, each enzyme being induced in the order of its appearance in the pathway. During the first 90 min of exposure to tryptophan, a rapid rate of induction of tryptophan pyrrolase occurred, without induction of formamidase and concomitantly with a small degree of induction of kynurenine transaminase (Fig. 4). The initial lack of induction of formamidase, the specific activity for which was about five times greater than that of tryptophan pyrrolase at zero-time, was an exception to the sequential pattern by which the first four enzymes were elevated. Formamidase was induced only after 90 min when the specific activity of tryptophan pyrrolase had risen to a value approaching the initial level of formamidase. It is inferred that the activity of formamidase became inadequate at this point to metabolize the formylkynurenine accumulated by the action of the elevated levels of tryptophan pyrrolase. The relative rate of induction of the tryptophan pyrrolase was, at first, faster than that of kynurenine transaminase, but later the rate of induction of kynurenine transaminase significantly increased (Fig. 5). Thus, kynurenine transaminase was also induced in the wake of the induction of tryptophan pyrrolase. It can again be inferred that the induction of kynurenine transaminase increased after the conversion of tryptophan to kynurenine increased with the action of the induced tryptophan pyrrolase and formamidase. These ordered events, plus the induction of kynurenic acid hydroxylase even later than 190 min after exposure to trypto-
The sequential induction of the first four enzymes of tryptophan catabolism reported here is essentially a descriptive characterization, for which no hypothetical mechanism is proposed that is comparable in sophistication to the inapplicable repressor-operon model.

Each of the enzymes may be regulated by the level of its substrate. Similar evidence of sequential appearance was obtained by Palleroni and Stanier (11) for the enzymes of the alternative (aromatic) pathway of tryptophan metabolism in *Pseudomonas*, except that their measurements did not distinguish a difference between the rates of induction of tryptophan pyrrolase and formamidase.

The induction of a series of enzymes by a non-metabolizable analogue has in the past been accepted as provisional evidence that the series is regulated at a single site. However, such analogues generally induce fewer enzymes of the pathway than does the natural inducer (11), thus limiting the possibility of regulation at a single site to the earliest enzymes in the pathway, as in the present instance. Yet, the experimental elimination of a single site for the control of the first four enzymes by tryptophan also eliminated this obvious explanation for the induction of the first three enzymes by the nonmetabolizable analogue, α-methyltryptophan. Closer scrutiny indicated two other possible ways that α-methyltryptophan might act. It showed definite affinities as an inhibitor for both tryptophan pyrrolase and kynurenine transaminase, and it temporarily inhibited growth. Since α-methyltryptophan can compete with tryptophan for the catalytic site of tryptophan pyrrolase, it is reasonable to suspect that α-methyltryptophan may also compete for the catalytic sites involved in the incorporation of tryptophan into protein. Such a competition was perhaps reflected in the second effect, the initial retardation of the rate of growth. The cells eventually recovered their ability to grow in the absence of added tryptophan, although added tryptophan completely avoided the delay in growth. This suggests that in the presence of α-methyltryptophan the cells can, in time, accumulate additional tryptophan to support growth.

Thus, the most likely explanation for the induction of tryptophan pyrrolase by α-methyltryptophan is that α-methyltryptophan causes the cells to accumulate the minimal inducing levels of extra endogenous tryptophan. After this induction of tryptophan pyrrolase, the induction of formamidase and kynurenine transaminase occurs more or less sequentially, as each becomes limiting. Either the insufficient amount of this endogenous tryptophan or the short time it is available to the cells during growth might account for the failure of the fourth enzyme, kynurenic acid hydroxylase, to be induced by α-methyltryptophan. A similar action is that of the non-metabolizable α-methylhistidine, which has been reported to exert its inductive effect on the enzymes catalyzing the degradation of histidine by causing an accelerated rate of synthesis of histidine (13).

**Acknowledgments**

We wish to acknowledge the capable technical assistance of Peter Smith.

Dr. Tremblay was supported by American Cancer Society Fellowship PF-274. This investigation was also supported by Public Health Service grant AM 00567 and Research Career Award AM-K6-2018, both from the National Institute of Arthritis and Metabolic Diseases, and by U.S. Atomic Energy Commission Contract AT(30-1)-901 with the New England Deaconess Hospital (NYO-901-58).

**Literature Cited**


