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Control of Actinomycin D Biosynthesis in *Streptomyces parvullus*: Regulation of Tryptophan Oxygenase Activity

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Tryptophan oxygenase (tryptophan 2,3-dioxygenase) activity increases immediately before the initiation of actinomycin D production by *Streptomyces parvullus*. We have attempted to discern whether this increase is due to a release from catabolite repression or to the synthesis of an inducer substance. The standard culture medium (glutamic acid-histidine-fructose medium) used in antibiotic production studies with *S. parvullus* contains L-glutamate as a major constituent. L-Glutamate is almost totally consumed before the onset of actinomycin D synthesis. The addition of 10 mM L-glutamate at this stage completely abolished actinomycin D production as well as tryptophan oxygenase synthesis. Fourteen amino acids were tested for a similar effect. Of these, L-glutamate and L-aspartate had the most dramatic effect on tryptophan oxygenase and β-galactosidase (β-D-galactosidase), another inducible enzyme. Standard glutamic acid-histidine-fructose medium, preincubated for 23 h to remove L-glutamate, allowed the synthesis of actinomycin D and tryptophan oxygenase by cells at a stage of growth normally considered too early for antibiotic production. A chemically defined medium lacking L-glutamate and adjusted to pH 8.0 was designed to simulate the preincubation medium. The transfer of cells to this artificial preincubation medium resulted in the appearance of tryptophan oxygenase as early as 19 h before normal synthesis occurred, eliminating the possibility that an inducer molecule is synthesized and excreted during the preincubation period. The results of these studies suggest that the increase in tryptophan oxygenase activity before the onset of actinomycin D synthesis, as well as the synthesis of actinomycin D itself, is due to a release from L-glutamate catabolite repression.

The antibiotic actinomycin D is a secondary metabolite produced by several *Streptomyces* species. Most of these species produce more than one form of actinomycin (5). *Streptomyces parvullus* is unusual in that it only synthesizes one class of actinomycin, class IV (12). Consequently, research into the biochemistry of antibiotic production in this organism is uncomplicated by the presence of multiple enzyme systems responsible for the production of more than one form of actinomycin.

Tryptophan has been implicated as a precursor of the actinocin ring structure of actinomycin D (6, 7). In addition, Lingens and Vollprecht (9) have presented evidence that, in at least one streptomycete, NAD is also synthesized from tryptophan. Figure 1, therefore, presents the pathways of tryptophan metabolism as they are believed to occur in *Streptomyces* spp. (19).

Tryptophan oxygenase (tryptophan 2,3-dioxygenase; L-tryptophan:oxygen 2,3-oxidoreductase; EC 1.13.11.11) is the first enzyme involved in both the primary metabolic pathway to NAD and the secondary metabolic pathway in actinomycin D. Its activity was first demonstrated in *S. parvullus* by M. J. M. Hitchcock and E. Katz (unpublished data), who demonstrated its apparent induction during the onset of actinomycin D biosynthesis. Their results also showed feedback inhibition of this enzyme by hydroxykynurinine. However, they were unable to experimentally induce tryptophan oxygenase with exogenously supplied tryptophan. This contrasts with the results obtained for other organisms (2, 16).

We report in this paper a more detailed analysis of tryptophan oxygenase induction and its relationship to actinomycin D production. Evidence is also presented which suggests that induction of this enzyme may actually be due to a release from glutamate-induced catabolite repression.

MATERIALS AND METHODS

Strains and culture conditions. *S. parvullus* strain C1 (19) was used throughout this study. Stand-

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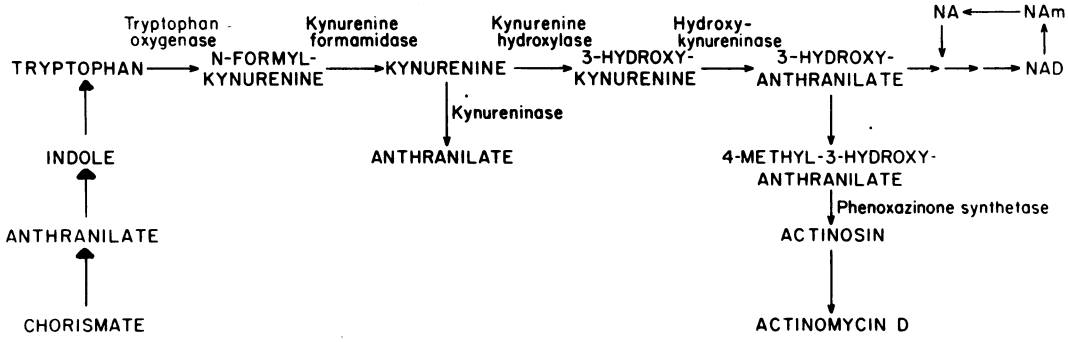

FIG. 1. *Pathways of tryptophan metabolism. NA, Nicotinic acid; NAm, nicotinamide.*

ard culture conditions were used and have been described in detail elsewhere (20). The major medium used was the glutamic acid-histidine-fructose medium (GHF) of Williams and Katz (20). Growth was measured by means of mycelial dry weight determinations (8).

Chemicals and reagents. All reagents were of analytical quality. [2-^{14}C]tryptophan was purchased from Schwarz/Mann, Orangeburg, N.Y. Puromycin was obtained from Sigma Chemical Co., St. Louis, Mo. Chloromycetin (chloramphenicol) was the generous gift of Parke, Davis & Co., Detroit, Mich.

Assays. Tryptophan oxygenase was measured with extracts prepared in 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA (1.0 g [wet weight] per 4 ml of buffer). Cells were passed twice through an Aminco French pressure cell at 1,200 psi and centrifuged at 10,000 × *g*, and the supernatant was desalted through a Sephadex G-25 column (1.0 by 10.0 cm). Coupled assays were performed in a solution (total volume, 400 μl) containing 20 μmol of potassium phosphate buffer (pH 7.0), 0.8 μmol of L-tryptophan containing 0.1 μCi of DL-[^{14}C]2-tryptophan, 20 μmol of L-ascorbic acid (pH 7.0), 9 U kynurinine formamidase (formamidase) (prepared as described by Peterkofsky [13]), and 100 to 200 μl of the enzyme preparation. Extracts were incubated for 30 min at 37°C with shaking in a Doubnoff shaking incubator. Reactions were stopped by the addition of 90 μl of 25% trichloroacetic acid. The tubes were centrifuged for 5 min at 1,000 × *g*, and the deproteinized supernatant was applied to a 0.5-ml AG 50W-X2 ion-exchange column (100 to 200 mesh, H+ form). The column was washed with 1.0 ml of deionized H_2O, and the eluant (containing [^{14}C]formate) was collected in a scintillation counting vial. Bray solution (10 ml) was added to the vial for scintillation counting.

β-Galactosidase (β-D-galactosidase) activity was measured by first preparing extracts of *S. parvullus* C1 in 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM dithiothreitol and 1 mM EDTA. The assay was performed by mixing 0.25 ml of extract with 0.5 ml of *o*-nitrophenyl-β-D-galactoside solution (2.5 mM in 50 mM potassium phosphate buffer [pH 7.0]). After incubation at 30°C for 5 min, 0.5 ml of 1 M Na_2CO_3 was added. The absorbance of *o*-nitrophenol was measured at 420 nm. The molar extinction coefficient for *o*-nitrophenol was 4700 $I \cdot mol^{-1} \cdot cm^{-1}$. All protein determinations were made by the method of Lowry et al. (10). Actinomycin D was extracted from the culture medium and assayed by the spectophotometric method of Katz and Weissbach (6).

RESULTS

Kinetics of tryptophan oxygenase induction. M. J. M. Hitchcock and E. Katz (unpublished data) discovered that tryptophan oxygenase activity began to increase 24 h after inoculation of *S. parvullus* C1 into chemically defined GHF medium. Maximum levels of this activity were achieved by 48 h. Figure 2 represents a more detailed examination of the induction phenomenon. Tryptophan oxygenase activity appeared approximately 21 h after inoculation. This occurred 3 h before the appearance of actinomycin D in the culture medium. Maximum tryptophan oxygenase levels were observed between 41 and 45 h after inoculation. The addition of various transcriptional and translational inhibitors 21 h after inoculation resulted in a complete lack of induction, indicat-

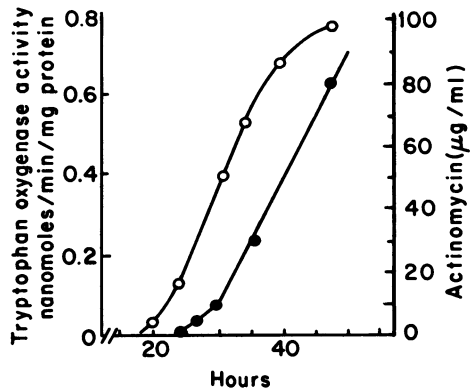

FIG. 2. *Induction of tryptophan oxygenase and actinomycin D production. S. parvullus was inoculated into GHF medium at time 0. Tryptophan oxygenase* (○) *and actinomycin D* (●) *were measured at the times indicated after inoculation.*

ing a requirement for new RNA and protein synthesis (Table 1).

Effect of L-glutamate on tryptophan oxygenase induction. Williams and Katz (20) reported that virtually all of the glutamate and histidine present in GHF medium was consumed by 20 h. This raised the question as to whether the proposed induction of tryptophan oxygenase before actinomycin D synthesis was actually a release from repression by glutamate or histidine or both. Experiments were conducted whereby these amino acids were added to GHF medium 23 h after inoculation. Tryptophan oxygenase activity was then measured 6 h later. The results indicated a dramatic effect on the synthesis of tryptophan oxygenase by glutamate (Table 2). Histidine failed to produce any significant effect. This evidence supports the theory proposed above. Further evidence (Fig. 3) reveals that cells exposed to 10 mM L-glutamate at 23 h recover from repression approximately 19 to 20 h later. This coincides with the results of Williams and Katz (20), who showed that the 13.6 mM glutamate present in GHF medium was totally consumed 20 h after inoculation. Furthermore, the increase in tryptophan oxygenase activity after glutamate recovery was accompanied by the onset of actinomycin D biosynthesis.

TABLE 1. *Effect of transcriptional and translational inhibitors on tryptophan oxygenase induction*

Addition[a]	Sp act[b] at:		
	0	3 h	6 h
Control	0.009	0.095	0.243
Puromycin (30)	0.009	0.000	0.000
Chloromycetin (30)	0.009	0.000	0.000
Actinomycin D (60)	0.012	0.007	0.079

[a] Numbers in parentheses represent the concentrations in micrograms per milliliter.

[b] Specific activity is expressed as nanomoles per minute per milligram of protein. Time 0 reflects the specific activity 21 h after inoculation.

TABLE 2. *Effect of L-glutamate and L-histidine on tryptophan oxygenase induction*

Addition[a]	Sp act[b] at:	
	23 h	29 h
None	0.09	0.36
10 mM Glutamate	0.11	0.08
10 mM Histidine	0.08	0.31
10 mM Glutamate + 10 mM histidine	0.09	0.07

[a] Additions were made 23 h after inoculation.

[b] Specific activity is expressed as nanomoles per minute per milligram of protein.

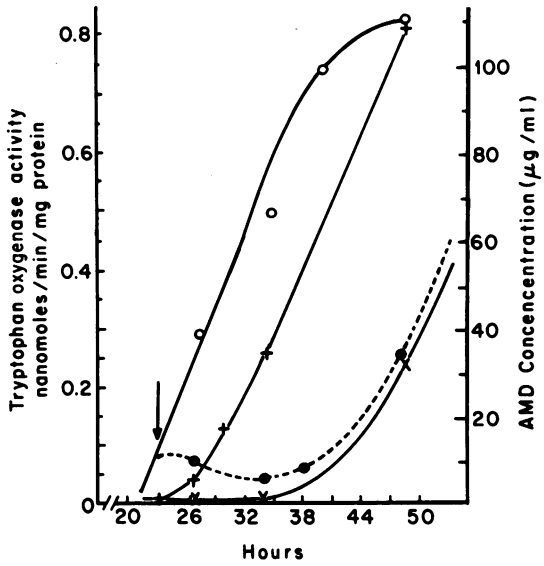

FIG. 3. *Effect of L-glutamate on the induction of tryptophan oxygenase and actinomycin D (AMD) production. Conditions were the same as those described in the legend to Fig. 2, with the exception that 23 h after inoculation (designated by the arrow), one set of flasks received 10 mM L-glutamic acid. Symbols: ○, tryptophan oxygenase induction in control flasks; +, actinomycin D production in control flasks; ●, tryptophan oxygenase induction in 10 mM glutamate flasks; actinomycin D production in 10 mM glutamate flasks.*

These results provide additional evidence that tryptophan oxygenase plays a role in the biosynthesis of actinomycin D.

Screening of various amino acids, carbohydrates, and organic acids for abilities to prevent tryptophan oxygenase synthesis. The results presented above suggest that the use of glutamate as a carbon source may result in catabolite repression. We examined whether other amino acids could exert a similar effect and, if so, whether this effect could be exerted on other inducible enzymes. Table 3 summarizes data obtained with a number of amino acids, carbohydrates, and organic acids. Their abilities to prevent the synthesis of tryptophan oxygenase and β-galactosidase are evident. β-Galactosidase activity was induced with the addition of 0.6% galactose at 23 h after inoculation. Specific activity for this enzyme increased linearly to 2.2 μmol/min per mg 31 h after inoculation.

The most pronounced inhibition of both tryptophan oxygenase and β-galactosidase was produced by L-aspartate and L-glutamate. The D isomers of these amino acids produced levels of repression close to those produced by the L

TABLE 3. *Effect of various amino acids, carbohydrates, and organic acids on tryptophan oxygenase and β-galactosidase activities*

Compound	Concn (mM)	% Repression[a] of: Tryptophan oxygenase	β-Galactosidase
Amino acid		0.0	0.0
L-Gluatamate	10	91.5	90.0
D-Glutamate	10	86.5	87.0
L-Aspartate	10	79.2	82.0
D-Aspartate	10	77.0	78.0
L-Proline	10	55.0	72.0
L-Alanine	10	72.0	47.0
L-Methionine	10	61.0	ND[b]
L-Valine	10	64.0	ND
D-Valine	10	52.0	ND
L-Threonine	10	30.0	44.0
Glutamine	10	40.0	37.0
L-Tyrosine	10	28.0	ND
L-Histidine	10	18.0	21.0
L-Arginine	10	11.0	16.0
L-Lysine	10	2.0	ND
L-Isoleucine	10	15.0	10.0
L-Ornithine	10	0.0	0.0
Carbohydrate			
D-Glucose	5.5	60.0	63.0
D-Glycerol	5.5	77.0	79.0
Organic acid			
α-Ketoglutarate	10	5.2	28.0
α-Ketoglutarate + NH_4[c]	10	11.0	ND
Citrate	10	22.0	ND
Fumarate	10	14.0	ND
Succinate	10	0.0	ND
Malate	10	24.0	ND

[a] For tryptophan oxygenase, additions to GHF medium were made 21 h after inoculation, and the cells were collected at 27 h. Induction of β-galactosidase was accomplished in GHF medium by the addition of 0.6% galactose at 23 h. Other additions were made 24.5 h after inoculation. Cells were collected at 30 h. The percent repression values were calculated as follows: (specific activity with addition/specific activity with no addition) × 100.

[b] ND, Not done.

[c] $Na_2(NH_4)HPO_4 \cdot 4H_2O$.

isomers. The other amino acids tested varied greatly in their ability to repress both enzymes. The values ranged from no repression to approximately 70%, depending upon the amino acid tested. Each individual amino acid, with the exception of glutamate and aspartate, also varied 10 to 15% between duplicate samples. Glutamate and aspartate, however, never varied by more than 4%. Thus, the repressive effect appears to be somewhat specific for the acidic amino acids.

The data presented in Table 4 suggest that glutamate and aspartate must be metabolized to exert their effects. Analogs of L-glutamate and L-aspartate failed to produce any significant repression of tryptophan oxygenase synthesis. However, one can see from Table 3 that α-ketoglutarate, an obvious metabolite of glutamate, did not significantly repress this enzyme. Thus, the precise nature of the metabolite responsible for repression remains obscure.

Glucose-6-phosphate dehydrogenase activity was also monitored in several of these extracts. The enzyme proved to be constitutive in that its activity did not fluctuate significantly with growth or carbohydrate source (24 μmol/min per mg). The addition of L-glutamate or D-glucose had no effect on the activity of this enzyme, in contrast to the effects observed for tryptophan oxygenase (data not shown).

Growth experiments. One possible explanation for the repressive effects of these amino acids is that their metabolism may affect the growth rate. Dry weight determinations showed that, once glutamate disappeared from GHF medium, the growth rate began to shift down (Fig. 4; 20). The addition of 10 mM glutamate at 24 h and again at 48 h prevented this shift down. Thus, the results agree with the hypothesis stated above. That is, an increase in the growth rate would cause the repression of inducible enzymes. To further test this theory, we added an amino acid (isoleucine) which exhibited almost no repression of either tryptophan oxygenase or β-galactosidase to cultures at 24 and 48 h. The result, also presented in Fig. 4, was an even greater increase in the growth rate. Repression, therefore, does not appear to be directly related to the rate of growth.

Effect of cyclic nucleotides on the repression of tryptophan oxygenase. As catabolite

TABLE 4. *Effect of glutamate, aspartate, and glucose analogs on tryptophan oxygenase activity*[a]

Compound	Concn (mM)	Sp act[b]	% Repression
Control		0.222	0.0
L-Glutamate	10	0.022	90.0
L-Methionine-DL-sulfoxide	10	0.178	20.0
L-Aspartate	10	0.047	79.0
α-Methyl aspartate	10	0.186	16.0
β-Methyl aspartate	10	0.204	8.0
D-Glucose	5.5	0.089	60.0
α-Methyl glucoside	5.5	0.193	13.0

[a] All additions were made 21 h after inoculation. Cells were collected and assayed 6 h later.

[b] Nanomoles per minute per milligram of protein.

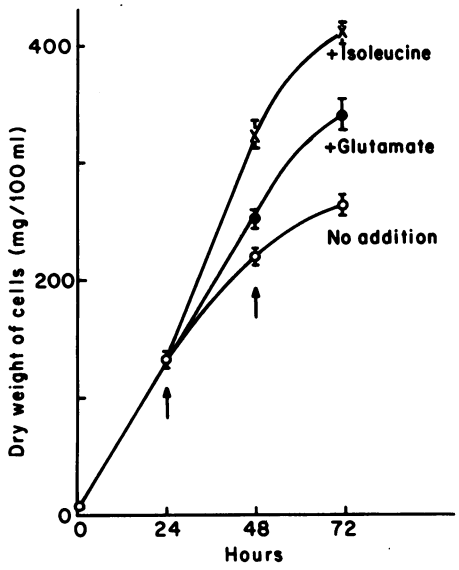

FIG. 4. *Effect of L-glutamate and L-isoleucine on the growth of S. parvullus. Replicate flasks containing GHF medium were inoculated at time 0. One-third of the cultures received 10 mM L-glutamate at the times indicated by arrows. Another one-third of the cultures received 10 mM L-isoleucine at the same time points. The remainder of the flasks received no additions. Dry weight determinations were made in triplicate 0, 24, 48, and 72 h after inoculation. The error bars represent the standard errors of the means.*

repression is known to involve cyclic AMP (cAMP) in several microorganisms and other investigators have demonstrated the uptake of cAMP and cyclic GMP (cGMP) by streptomycetes (14, 15), we examined the effect of dibutyryl-cAMP and cGMP on glutamate repression. The basic experiment involved adding 10 mM glutamate and either dibutyryl-cAMP or cGMP 23 h after inoculation (2 h after the onset of tryptophan oxygenase synthesis). After 6 h of incubation, the cells were collected and assayed for tryptophan oxygenase activity. Neither cAMP nor cGMP at 5 mM had any effect on relieving glutamate repression. Similar experiments were performed with β-galactosidase as the test enzyme, with identical results.

Early synthesis of tryptophan oxygenase. All of the evidence presented thus far suggests that the synthesis of tryptophan oxygenase is actually due to a release from glutamate repression rather than to an induction phenomenon. One could predict, then, that removing cells from media with glutamate would result in the synthesis of tryptophan oxygenase and the subsequent appearance of actinomycin D. To test this hypothesis, we preincubated GHF medium with *S. parvullus* C1 for 23 h and then filtered and reinoculated the medium with cells from two 15-h GHF cultures (100 ml each). This 23-h preincubated medium (PIM) should have been lacking in glutamate but should have contained any other component necessary for tryptophan oxygenase synthesis. With this procedure, tryptophan oxygenase synthesis began at 16.5 h (Fig. 5A), 4.5 h before it did in the control cultures. Treatment of 23-h PIM with 10 μg of trypsin, pronase, DNase, RNase, and lipase per ml had no effect on tryptophan oxygenase synthesis (data not shown).

Additional proof that the increase in tryptophan oxygenase activity was not associated with any extracellular factor is presented in Fig. 5B. Artificial PIM was prepared by leaving glutamate and histidine out of the standard GHF medium and adjusting the pH to 8.0. This medium was inoculated with 15-h cells as described above. Synthesis of tryptophan oxygenase as well as actinomycin D accumulation again occurred at 16.5 h, 90 min after resuspension. Finally, removing cells from artificial PIM after 90 min by filtration and resuspending them in fresh artificial PIM caused almost no lag in tryptophan oxygenase synthesis (Fig. 5C). The lag observed was due mainly to the time required to filter, wash, and resuspend the cells. Thus, extracellular products or components do not appear to be involved in the process.

Effect of chloramphenicol on preinduction. One question which remained concerned what occurred during the 90-min lag period. Two possibilities immediately present themselves: (i) Intracellular pool levels of glutamate are consumed or (ii) an intracellular inducer protein is synthesized. The second possibility was examined in the following manner. Cells from 15-h GHF cultures were suspended in artificial PIM containing 30 μg of chloramphenicol per ml for 90 min. After the 90-min preinduction period, the culture was filtered, washed twice with 20 ml of saline, and resuspended in fresh artificial PIM. Tryptophan oxygenase activity was measured at various intervals thereafter. A control culture was filtered and assayed in a similar manner for comparison. The results of this experiment (Fig. 5D) reveal that, although the overall rate of induction by the chloramphenicol culture was less than that of the control culture, induction occurred at the same time. Thus, one can conclude that an intracellular inducer protein is probably not required for tryptophan oxygenase induction.

Inducibility of variously aged cells. The synthesis of tryptophan oxygenase and, therefore, actinomycin D biosynthesis might require

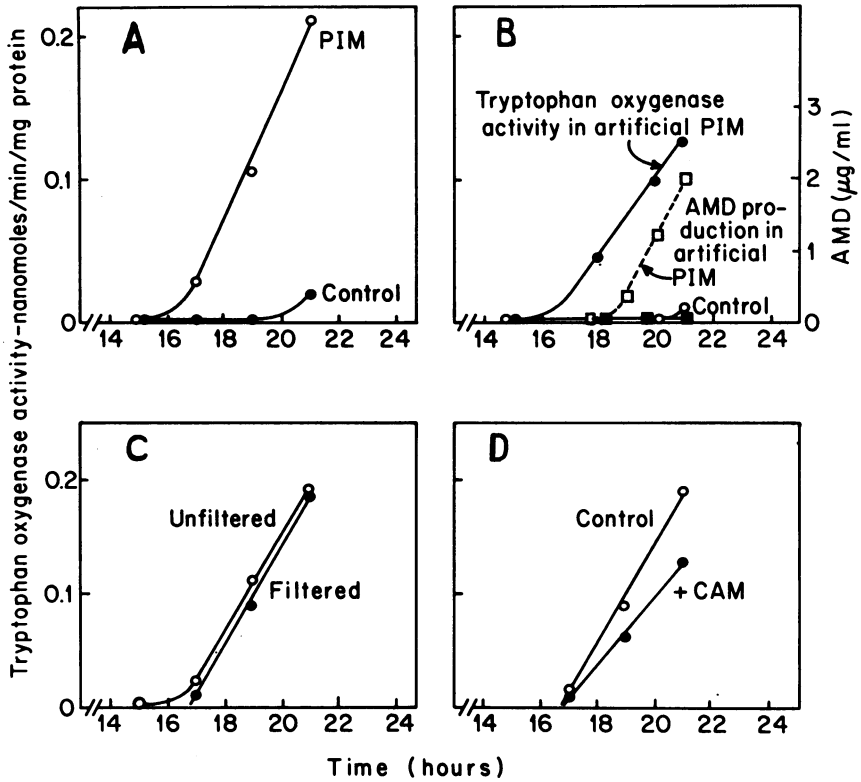

FIG. 5. *(A) Effect of 23-h PIM on tryptophan oxygenase production. S. parvullus was grown in GHF medium for 23 h, at which time the cells were removed by filtration. This PIM was then inoculated with cells from two 15-h GHF cultures (100 ml each) for each 100 ml of PIM. Tryptophan oxygenase activity was then monitored at 2-h intervals. Symbols: ○, tryptophan oxygenase activity measured in cells switched to PIM; ●, tryptophan oxygenase activity measured in control cultures allowed to remain in the original GHF medium. (B) Tryptophan oxygenase activity and actinomycin D (AMD) production after incubation in artificial PIM. Artificial PIM was prepared by leaving glutamate and histidine out of the standard GHF medium and adjusting the pH to 8.0. This medium was inoculated with 15-h cells, and tryptophan oxygenase (●) and actinomycin D (□) were measured. Control cultures were run to show that neither tryptophan oxygenase (○) nor actinomycin D (■) was induced before 21 h. (C) Effect of filtration on the induction of tryptophan oxygenase. Symbols: ●, tryptophan oxygenase activity induced in 15-h cells transferred to artificial PIM; ●, tryptophan oxygenase activity in 15-h cells transferred to artificial PIM, incubated for 90 min, refiltered, and added to fresh artificial PIM. (D) Effect of Chloromycetin (CAM) on the induction of tryptophan oxygenase. Symbols: ○, 15-h cells transferred to artificial PIM; ●, addition of 30 μg of Chloromycetin per ml to a parallel culture for 90 min.*

a certain amount of cell growth. This theory was examined by suspending 5-, 12-, and 15-h cells in artificial PIM. Because of the relative amount of growth present at each of these time points, cells from six 5-h GHF medium flasks were combined and resuspended in 100 ml of artificial PIM. Two flasks per PIM were used for both the 12- and 15-h cells. The results revealed that even 5-h cells were capable of synthesizing tryptophan oxygenase as well as actinomycin D (Fig. 6). Therefore, extensive growth is not required to prepare cells for antibiotic synthesis. However, growth in glutamate for the normal period of 24 h did result in a much higher antibiotic titer than that achieved when cells were prematurely suspended in PIM (ca. 500 versus 80 μg/ml). Growth of young cells in PIM was rather poor. Thus, the difference in titers could be the result of cell numbers as well as other undetermined physiological parameters.

DISCUSSION

Tryptophan oxygenase was studied with regard to its synthesis before actinomycin D biosynthesis. The results showed that this enzyme is synthesized 21 h after inoculation, 3 h before any significant actinomycin D accumulation in

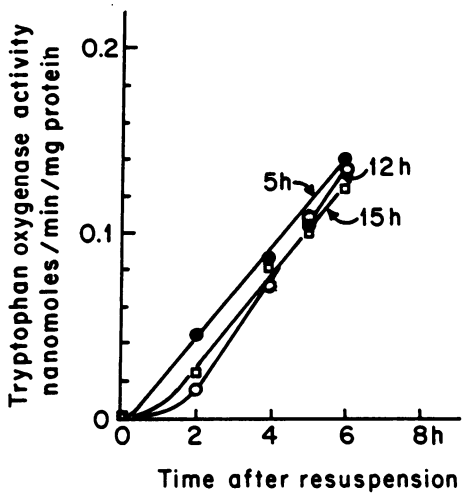

FIG. 6. *Tryptophan oxygenase induction in variously aged cells. Cells (5, 10, and 15 h) were transferred to artificial PIM, and tryptophan oxygenase activity was measured at subsequent 2-h intervals.*

the culture medium. The specific activity of this enzyme increased for approximately 20 h. These data differ somewhat from those obtained with kynurenine formamidase in that induction of kynurenine formamidase occurs at the same time as actinomycin D accumulation (3). Treatment of mycelium with translational and transcriptional inhibitors during synthesis of tryptophan oxygenase suggests that new RNA and protein synthesis are required.

Several questions were posed when this study began. First, what causes the increase of tryptophan oxygenase activity in *S. parvullus*? Second, could the synthesis of tryptophan oxygenase as well as actinomycin D production be experimentally triggered earlier than the normal 21 h after inoculation? The data presented suggest that the transfer of washed mycelium from preinoculation N-Z amine medium (20) to chemically defined GHF medium immediately imposes some form of catabolite repression on the cells by L-glutamate. Once the glutamate has been consumed, tryptophan oxygenase activity begins to increase, as does actinomycin D production. Removing glutamate from cells as young as 5 h releases them from repression, with a subsequent "induction" of tryptophan oxygenase and actinomycin D biosynthesis. We consistently found a lag period between the removal of glutamate and the initial increase in tryptophan oxygenase activity. The results suggest that this lag is caused by the consumption of internal levels of glutamate and not by the synthesis of an inducer protein (Fig. 5A–D).

This report of catabolite repression by glutamate is not without precedent. Bouknight and Safoff (2) suggested a similar effect of glutamate on the induction of tryptophan oxygenase in *Bacillus cereus*. Our results, however, showed that L-aspartate has an effect as dramatic as that of glutamate. In addition, the D isomers of glutamate and aspartate also caused pronounced inhibition of tryptophan oxygenase induction. Subsequent examination of another, unrelated inducible enzyme, β-galactosidase, revealed that glutamate and aspartate also prevented its induction. No effect was observed on the constitutive enzyme glucose-6-phosphate dehydrogenase, however. Thus, the effect appears to be general in nature, acting upon various inducible enzymes of *S. parvullus*. The nature of the compound directly responsible for this repression remains unknown. Analogs of glutamate and aspartate had little effect on tryptophan oxygenase induction, suggesting that both glutamate and aspartate must be metabolized to exert their effects. However, neither α-ketoglutarate, NH_4^+, nor a combination of the two produced significant repression.

On the assumption that catabolite repression usually involves some cyclic nucleotide, we tested both cAMP and cGMP for their ability to reverse glutamate repression of tryptophan oxygenase. Neither compound had any effect. In addition, cAMP failed to reverse either the glucose or glutamate repression of β-galactosidase. There are two possible explanations for these results. The first is that possibly neither nucleotide plays a role in catabolite repression in *S. parvullus*. There are reports of other organisms in which cAMP and cGMP are either not found (1, 4, 11, 17, 21) or are found, but their extracellular and intracellular levels fail to vary significantly during catabolite repression (18). The report from Yeung et al. (21) on *Bacillus megaterium* included data which revealed that, even though cAMP could not be detected intracellularly or extracellularly, an unknown nucleotide, the levels of which did vary during catabolite repression, was detected. The situation in *S. parvullus* could be similar to that just described. Alternatively, cAMP or cGMP or both could be present but not involved in catabolite repression. A case for this type of phenomenon has been presented for *Pseudomonas aeruginosa* (18). The measurement of cAMP in *S. parvullus* would lend support to the latter theory. A second explanation for the failure of either cyclic nucleotide to reverse catabolite repression is that *S. parvullus* may be impermeable to these compounds. Further experimentation will be required to resolve this point.

Thus far, all attempts to increase the level or rate of tryptophan oxygenase induction through

the addition of L-tryptophan have been unsuccessful. Either some other tryptophan metabolite is involved or there is not a true induction phenomenon. Instead of induction, the increase in tryptophan oxygenase may be related to a release from repression imposed by the metabolism of various amino acids and carbohydrates. The data presented in this paper provide substantial support for this proposal.

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