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Formation and Interrelationships of Tryptophanase and Tryptophan Synthetases in *Escherichia coli*

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ABSTRACT

NEWTON, W. AUSTIN (University of California, Berkeley), AND ESMOND E. SNELL. Formation and interrelationships of tryptophanase and tryptophan synthetases in *Escherichia coli*. J. Bacteriol. **89**:355–364. 1965.—In addition to the classical tryptophan-repressible tryptophan synthetase (TSase-*tr*), tryptophan auxotrophs of *Escherichia coli* contain another distinct tryptophan synthetase (TSase-*ti*) which is induced by tryptophan and is identical with tryptophanase (TPase). *Escherichia coli* B (wild type) forms only TSase-*tr* when the growth medium lacks tryptophan. When tryptophan is supplied, parallel induction of TPase and TSase-*ti* occurs while TSase-*tr* is repressed. Antiserum prepared against purified TPase neutralized TPase and TSase-*ti* equally, but not TSase-*tr*. TPase-negative strains of *E. coli* do not form TSase-*ti*. Unlike TSase-*tr*, TSase-*ti* is not readily detected by whole-cell assays. In the tryptophan auxotroph, *E. coli* B/1t7, a direct correlation exists between the effectiveness of 4-, 5-, and 6-methyltryptophan in inducing TPase and in promoting growth in the presence of indole. In a mutant of this organism, *E. coli* B/1t7-A, which is constitutive for TPase, 5-methyltryptophan and other substrates of TPase increased the rate of growth on limiting indole, a result ascribed to their ability to inhibit degradation of tryptophan and to supply the 3-carbon side chain for synthesis of tryptophan by TPase. This organism produced maximal amounts of TPase when inocula from log-phase cells grown in tryptophan-supplemented minimal medium were allowed to undergo two cell generations in an enriched broth medium.

When certain tryptophan-requiring strains of *Escherichia coli* are grown in the presence of high levels of tryptophan, an inducible enzyme is formed, which catalyzes the synthesis of tryptophan according to reaction 1 (Newton and Snell, 1962, 1964).

L-serine + indole → L-tryptophan + water (1)

This tryptophan-inducible synthetase is distinct from the tryptophan-repressible tryptophan synthetase of *E. coli*, which also catalyzes reaction 1 and has been studied by Yanofsky (1960). For clarity in the discussion that follows, we shall refer to the inducible synthetase as TSase-*ti* and to the repressible synthetase as TSase-*tr*. TSase-*tr* is composed of an A and a B protein, and this enzyme, but not TSase-*ti*, also catalyzes reactions 2 and 3.

indole-3-glycerolphosphate → indole + glyceraldehyde-3-phosphate (2)

L-serine + indole-3-glycerolphosphate → L-tryptophan + glyceraldehyde-3-phosphate (3)

TSase-*ti* has been obtained in apparently pure form and has been shown to be identical with TPase, which catalyzes reaction 4:

L-tryptophan + water → indole + pyruvate + NH_3 (4)

This same enzyme also catalyzes several other α,β-elimination and replacement reactions, including the formation of pyruvate and NH_3 from serine; pyruvate, NH_3, and H_2S from cysteine; and tryptophan from cysteine or α-methylcysteine and indole (Newton and Snell, 1964). Although TPase (TSase-*ti*) is not normally involved in the synthesis of tryptophan in wild-type *E. coli*, it can supply the tryptophan required for growth under certain conditions. For example, strains of *E. coli* which lack TSase-*tr* and are constitutive for TPase grow on minimal medium supplemented with indole (Newton and Snell, 1962; Gartner and Riley, 1965).

In several published studies of the induction of TPase, the simultaneous induction of tryptophan synthetase activity (TSase-*ti*) has not been observed (Freundlich and Lichstein, 1960; Lester and Yanofsky, 1961; Scott and Happold, 1962). Since such studies were in apparent con-

flict with our conclusion that TPase and TSase-*ti* are identical, further studies were undertaken. We present herein observations which clarify these apparent discrepancies and reinforce the conclusion that TPase and TSase-*ti* are identical.

MATERIALS AND METHODS

Bacterial cultures. The strains of *E. coli* used were the following: strains B and K-12 are wild-type; strain B/1t7 is a deletion mutant for TSase-*tr* (Yanofsky and Lennox, 1959) obtained from C. Yanofsky; strain B/1t7-A is a mutant of B/1t7 which is constitutive for TPase (Newton and Snell, 1962); strain B/1t, a deletion mutant for TSase-*tr* (Yanofsky and Lennox, 1959), was obtained as a lyophilized culture from A. Pardee and was found to be also TPase-negative; strains TP-1 and TP-2 are TPase-negative mutants which resulted from transduction of two different TPase-negative mutations into *E. coli* AB 1450 (Gartner and Riley, 1965).

Growth media and conditions. The minimal medium contained the salts mixture of Rickenberg, Yanofsky, and Bonner (1953) with the supplements indicated in individual experiments. The broth medium contained 1% of yeast extract, 1% of Tryptone (Difco), and 0.5% of dibasic potassium phosphate. Unless indicated otherwise, the cells were cultured from 1% inocula by shaking 100 ml of medium in 250-ml Erlenmeyer flasks on a rotary shaker at 30 or 37 C. The cells were generally harvested in the stationary phase after 12 to 18 hr of growth.

Enzyme assays. The TSase activity of whole and of toluene-treated cells was determined by the procedure of Lester and Yanofsky (1961). The preparation of cell-free extracts by ultrasonic oscillation and the assay of TPase and TSase in the extracts have been described (Newton and Snell, 1962). A unit of TPase activity is that amount of protein which forms 0.1 μmole of indole from L-tryptophan in 10 min. In most cases, a unit of TSase activity is that amount of protein which catalyzes disappearance of 0.1 μmole of indole in 10 min in the presence of excess L-serine; in specified instances it represents the formation of 0.1 μmole of L-tryptophan in 10 min in the presence of L-serine and either indole or indole-3-glycerolphosphate. [This TSase unit is larger than that used in a previous study from this laboratory (Newton and Snell, 1962), in which the unit was based on disappearance of 0.1 μmole of indole in 40 min. The larger unit allows more direct comparison of the degradative and synthetic activities of TPase (TSase-*ti*).] The actual assay periods for TSase and TPase were 40 and 10 min, respectively; L-tryptophan was determined microbiologically (Newton and Snell, 1962, 1964). Specific activities in each case represent the number of units per milligram of protein.

Preparation and assay of anti-TPase serum. A 2-ml amount of a purified TPase preparation (specific activity 800; 40 mg of protein) was combined with 1.6 ml of paraffin oil (Bayol F) containing 4.8 mg of heat-killed dried mycobacteria and 3 ml of mannide monooleate (Arlacel A). After making control bleedings from the rabbit used in the experiment, three 1.0-ml injections of the above emulsion were made intramuscularly into the animal at 1-week intervals. The rabbit was bled by heart puncture 1 week after the last injection. Since the control serum at high levels inhibited the TSase reaction (apparently due to the presence of sodium salts), all sera were heated for 30 min at 56 C to destroy complement, and ammonium sulfate was added at 4 C to 50% of saturation. The precipitated material was dissolved in an amount of 0.02 M potassium phosphate (*p*H 7.0) equal to about one third of the original volume of serum, and dialyzed overnight against the same buffer at 4 C.

The activity of the antisera against TPase and TSase was assayed by a slight modification of the standard assays for these enzymes. Buffer, antiserum, enzyme, and, for the TSase reaction only, serine, were combined in the reaction vessel at 0 C, diluted to 0.15 ml with distilled water, and allowed to stand 15 min. The remaining components of the reaction mixtures, except substrate, were added, and the vessels were transferred to the 37 C bath for 20 min. The reactions were then initiated by addition of the appropriate substrate: tryptophan for determination of TPase activity, or indole for determination of TSase activity. Other details of each procedure were as described previously (Newton and Snell, 1962).

Extracts of the tryptophan-induced, TPase-negative mutants of *E. coli* were tested for cross-reacting material corresponding to TPase by determining whether they protected the active enzyme against neutralization by the antiserum. The cell extracts were incubated with the anti-TPase serum for 30 min at either 4 or 37 C before addition of active TPase and completion of the above assay.

Chemicals. Yeast extract, acid-hydrolyzed casein, Tryptone, Bayol F, and Aracel A were Difco brand; 4-, 5-, and 6-methyltryptophan were purchased from Mann Research Laboratories, Inc., New York, N.Y. Indole-3-glycerol phosphate and the purified A and B proteins of TSase-*tr* were kindly supplied by C. Yanofsky.

RESULTS

Effect of methyltryptophans on growth of E. coli B/1t7 and induction of TPase. E. coli B/1t7, a deletion mutant for the A and B genes of TSase-*tr*, cannot grow on a minimal medium supplemented with either indole or 5-methyltryptophan, but does grow on a minimal medium containing both of these supplements (Newton and Snell, 1962). Although they are much less effective than 5-methyltryptophan, 4- and 6-methyltryptophan also support growth of these cells in the presence of indole (Table 1). A direct correlation exists

between the effectiveness of 4-, 5-, and 6-methyltryptophan in promoting growth and their effectiveness in inducing formation of TPase (TSase-*ti*) by resting-cell suspensions of this organism (Table 2). The order of activities of these tryptophan analogues for growth and TPase induction differs appreciably from the order of their activities as substrates for TPase, as determined by Hall, Leeson, and Tweddle (1960) for both the cell-free enzyme and washed cell suspensions.

TABLE 1. *Comparative growth response of Escherichia coli B/1t7 to tryptophan or indole in the presence of methyl-substituted tryptophans*

Supplement*	Cell density (μg/ml, dry wt)
Indole	0
L-Tryptophan	340
L-Tryptophan + 5-methyl-DL-tryptophan	700
4-Methyl-DL-tryptophan	0
4-Methyl-DL-tryptophan + indole	12
5-Methyl-DL-tryptophan	0
5-Methyl-DL-tryptophan + indole	450
6-Methyl-DL-tryptophan	0
6-Methyl-DL-tryptophan + indole	80

* The minimal medium was supplemented with 0.2% of glucose and with 10 μg/ml of indole or tryptophan and 100 μg/ml of the methyltryptophans, as indicated. The cells were cultured at 37 C in 1-in. test tubes containing 10 ml of medium. Absorbance readings were taken after 96 hr of incubation.

TABLE 2. *Induction of TPase and TSase-ti in resting cells of Escherichia coli B/1t7 by methyl-substituted tryptophans*

Supplement*	Specific activity	
	TPase	TSase-*ti*
None	1.3	0.22
4-Methyl-DL-tryptophan	2.3	0.49
5-Methyl-DL-tryptophan	16.1	3.8
6-Methyl-DL-tryptophan	4.2	1.8

* *E. coli* B/1t7 was grown for 12 hr in minimal medium containing 0.16% of glycerol, 0.05% of acid-hydrolyzed casein, and 10 μg/ml of L-tryptophan. Cells from this culture were resuspended at a concentration of 2.0 mg (dry weight) per ml in this same medium, to which 100 μg/ml of the indicated supplements were added, and incubated at 37 C with shaking for 3 hr. There was no significant increase in cell density during this last incubation. The cells were washed three times in 0.9% sodium chloride, then disrupted, and assayed for TPase and TSase.

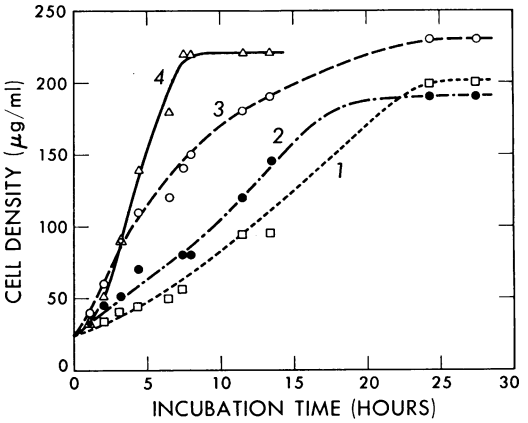

FIG. 1. *Growth response of Escherichia coli B/1t7-A to limited indole in the presence of: curve 1, no supplement; curve 2, serine; curve 3, 5-methyltryptophan; and curve 4, S-methylcysteine. Minimal medium containing 0.2% glucose, 0.01 mM indole, and other supplements at 0.5 mM was inoculated with 22 μg/ml of washed cells and incubated with shaking at 37 C.*

Therefore, it appears that the structural features required of an effective inducer of TPase in this series are not the same as those for a substrate of TPase, and that the primary effect of the methyltryptophans in permitting growth of mutants lacking TSase-*tr* lies in their action as inducers of TPase (TSase-*ti*), which then forms tryptophan from indole.

Effect of amino acid substrates of TPase on growth of E. coli B/1t7-A with limiting indole. 5-Methyltryptophan substantially increases growth of *E. coli* B/1t7 with low levels of tryptophan (Table 1). Does this effect result solely from induction of TPase, or is it related in part to the action of 5-methyltryptophan as a substrate for TPase? Figure 1 shows that 5-methyltryptophan, *S*-methylcysteine, and, to a lesser extent, serine, all stimulate the rate (but not the extent) of growth of *E. coli* B/1t7-A with limited indole. We assume that this effect results from an enhanced rate of conversion of indole to tryptophan. However, formation of TPase in this organism is constitutive, and is relatively little affected by addition of substances such as 5-methyltryptophan, which act as inducers in the parent strain. We conclude, therefore, that these substrates of TPase enhance the growth rate of this organism on limiting indole by some mechanism other than the induction of TPase. Operation of this additional mechanism also may contribute to the enhanced growth with low levels of tryptophan observed when organisms such as

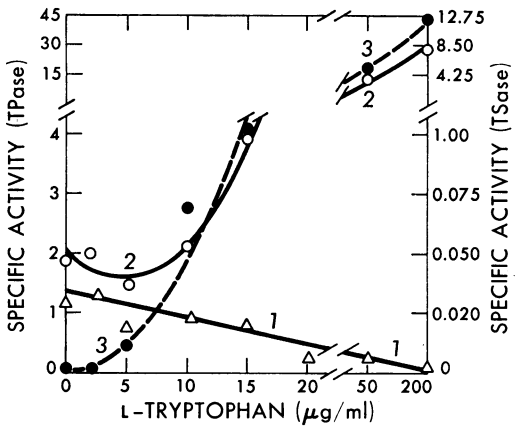

FIG. 2. *Effect of concentration of* L*-tryptophan in the growth medium on the activity of extracts of Escherichia coli B in catalyzing tryptophan synthesis from indole-3-glycerol phosphate (curve 1), synthesis of tryptophan from indole (curve 2), and the formation of indole from tryptophan (curve 3). Cultures were grown from 5% inocula at 37 C for 12 hr in minimal medium containing 0.2% glucose, 0.1% acid-hydrolyzed casein, and* L*-tryptophan as indicated.*

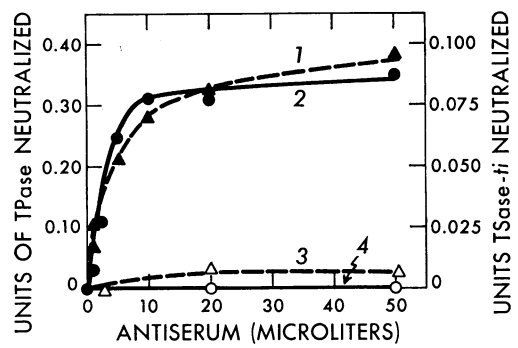

FIG. 3. *Neutralization of TPase (curve 1) and TSase-ti (curve 2) in crude extract from Escherichia coli B/1t7-A by anti-TPase serum. The effects of equal additions of nonimmune serum are shown in curves 3 and 4.*

E. coli B/1t7 are grown in the presence of 5-methyltryptophan (Table 1).

Relationship of TPase to TSase in prototrophic strains of E. coli. If the TPase and TSase-*ti* of *E. coli* B/1t7 are identical and not related to TSase-*tr*, then both the inducible and repressible TSase activities should be present in extracts of the tryptophan-induced parent strain, *E. coli* B; Fig. 2 shows that this is the case. Curve 1 of this figure shows that TSase-*tr*, assayed specifically in cell extracts as the formation of tryptophan from indoleglycerol-3-phosphate by reaction 3, decreases as the tryptophan content of the growth medium is increased. The synthesis of tryptophan from indole through the combined activities of TSase-*tr* and TSase-*ti* (curve 2) decreases moderately at low levels of tryptophan, but increases to very high levels as the tryptophan of the medium is increased to levels that largely repress formation of TSase-*tr* and induce the formation of high levels of TPase (curve 3) and TSase-*ti*. At high levels of tryptophan, the latter two activities increase in parallel (curves 2 and 3), as would be expected if they represent separate activities of a single enzyme.

Neutralization of TPase and TSase activity by anti-TPase serum. Increasing levels of antiserum prepared against purified TPase (see Materials and Methods) neutralized TPase and TSase-*ti* in parallel (Fig. 3), and the amount of enzyme neutralized is nearly directly proportional to the antiserum added, until about 75% of the activity initially present has been neutralized. The amount of both enzymatic activities neutralized varies in the same way with the amount of enzyme present (Fig. 4).

Anti-TPase serum inhibits both the TSase-*ti* present in extracts of *E. coli* B/1t7-A (constitutive for TPase) and that in extracts of the parent *E. coli* B induced on high levels of tryptophan (Table 3). There is, however, no cross-reaction of this antiserum with TSase-*tr* of *E. coli* B grown on minimal medium, or with the purified A and B proteins of TSase-*tr*. These immunological assays thus support the conclusion that cells of *E. coli* B may form two distinct tryptophan

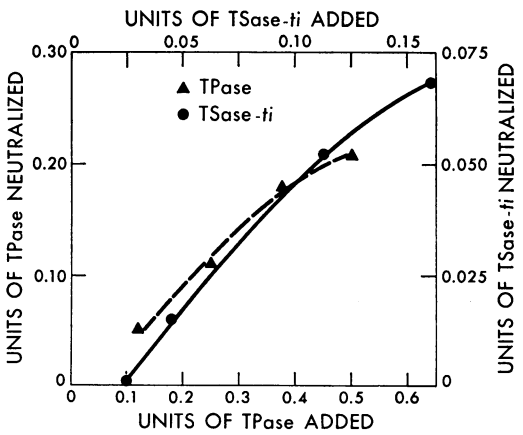

FIG. 4. *Effect of enzyme concentration on the neutralization of TPase and TSase-ti in crude extracts of Escherichia coli B/1t7-A by anti-TPase serum. Various amounts of the same crude extract employed in Fig. 3 were assayed in the presence of a constant amount (5 μliters) of anti-TPase serum.*

synthetases: TSase-*tr*, formed in minimal medium and repressed by tryptophan, and TSase-*ti*, which is identical with TPase (Fig. 2). (The converse experiment has also been carried out with antisera to the A and B components of TSase-*tr* kindly supplied by C. Yanofsky. Under conditions where TSase-*tr* activity of purified A and B proteins was almost completely neutralized, no reduction in tryptophan synthesis by TPase in crude extracts of *E. coli* B/1t7-A was observed.)

Repression of TSase in TPase-negative strains of E. coli. Three TPase-negative mutants of *E. coli* were examined to gain genetic evidence for the identity of TPase and TSase-*ti*. If both reactions are catalyzed by a single enzyme, no TSase-*ti* should be observable in these mutants. This result was observed with all of the TPase-negative strains tested, even though the mutants were grown under conditions which induced high levels of TPase and TSase-*ti* in the parent K-12 and B strains (Table 4). TSase was present in extracts of *E. coli* TP-1 and TP-2 prepared from cells grown on the minimal medium, but this enzyme was strongly repressed when the cells were grown with high levels of tryptophan, and thus corresponded to the biosynthetic enzyme, TSase-*tr;* no TSase-*ti* appeared under these conditions. These findings are consistent with the failure to find TSase activity under any conditions in extracts of *E. coli* B/1t, a mutant of *E. coli* which lacks TPase and is also a deletion mutant for the A and B genes of TSase-*tr* (Yanofsky and Lennox, 1959). Under the conditions employed, no cross-reacting material to TPase antiserum was detected in either of the TPase-negative strains, TP-1 and TP-2, even though the cells were grown with high levels of tryptophan which induce TPase in the parent strain, *E. coli* K-12.

TABLE 3. *Effect of anti-TPase serum on TSase activity from different sources*

Source of enzyme	Enzyme prepn	Anti-TPase serum	TSase activity*	
			Units found	Units neutralized
	μliters	*μliters*		
Crude extract from *Escherichia coli* B (minimal medium)†	10	0	.14	—
		5	.12	0.02
		10	.14	0
		20	.13	0.01
E. coli B (minimal medium + tryptophan)	2	0	.38	—
		5	.23	0.15
		10	.16	0.22
E. coli B/1t7-A (minimal medium)	1	0	.37	—
		10	.12	0.25
Purified A and B proteins of TSase-*tr*	‡	0	.18	—
		5	.18	0
		10	.18	0
		20	.18	0

* The units used throughout this table are those defined by Crawford and Yanofsky (1958) and are about one fourth as large as the units used in the remainder of this paper.

† The minimal medium contained 0.2% of glycerol, 0.1% of acid-hydrolyzed casein, and 10 μg/ml of L-tryptophan, where indicated. Cells were grown from 5% inocula, harvested after 18 hr, disrupted, and assayed as described in the text.

‡ Assay tubes contained approximately 0.4 units of B protein and 1.2 units of A protein.

TABLE 4. *Tryptophan synthetase activity of Escherichia coli K-12 and of TPase-negative mutants of E. coli K-12 and E. coli B*

Strain of *E. coli*	L-Tryptophan in culture medium*	Specific activity of crude extract	
		TPase	TSase
	mg/ml		
K-12	0	0.9	2.3
	1	155	49
TP-1	0	0	1.2
	1	0	0.48
TP-2	0	0	1.5
	1	0	0.45
B/1t	0.01	0	0
	1	0	0

* All strains were cultured overnight on minimal medium containing 0.2% of glycerol, 0.1% of acid-hydrolyzed casein, and L-tryptophan at the concentration indicated; 5% inocula were used in each case.

Comparative activities of whole cells and cell extracts in catalyzing the TSase reaction. Although previous investigators demonstrated with whole cells the repression of TSase-*tr* and the induction of TPase by growth in the presence of tryptophan, they did not observe the induction of TSase-*ti*. As shown earlier, the latter two activities appear in parallel under our assay conditions when cell-free extracts are used (Fig. 2). Direct trial showed (Table 5) that TSase-*ti* activity is not observed in whole cell assays, although the repressible activity of TSase-*tr* is readily observed. The former activity, although not striking, is observable by these same assay procedures when toluene-treated cells are used. The contrast between either untreated or toluene-treated cells assayed by the older procedure (which employs a comparatively low concentration of serine) and the activity of cell-free extracts assayed in the

TABLE 5. *Comparative synthetase (TSase-tr plus TSase-ti) activity of Escherichia coli B as determined in whole cells, toluene-treated cells, or cell-free extracts*

L-Tryptophan in growth medium[a]	Total TSase activity[b] of		
	Whole cells[c]	Toluene-treated cells[c]	Cell-free extracts[d]
μg/ml			
0	20	35	14
5	15	20	
10			56
15	0	20	
200	0	50	1350

[a] Cells were grown for 18 hr in minimal medium containing 0.16% of glucose and tryptophan as indicated.

[b] Expressed as millimicromoles of indole disappearing in 30 min per milligram (dry weight) of cells or per milligram of protein in the cell extract.

[c] These assays for TSase were performed by the procedure of Lester and Yanofsky (1961). Toluene treatment consisted in shaking the cells plus assay mixture vigorously with 2 drops of toluene before incubation at 37 C.

[d] Prepared and assayed as described by Newton and Snell (1962). This assay method employs a fourfold higher serine concentration than that used for the two cell preparations.

TABLE 6. *Apparent effect of size of inoculum on TPase formation by Escherichia coli B/1t7-A in supplemented minimal medium*

Inoculum used* (percentage of final volume of culture)	TPase activity of final culture† (units/mg of protein)
1	64
2	78
5	95
10	113

* The inoculum was grown in minimal medium containing 0.16% of glucose and 4 μg/ml of indole, and contained approximately 0.8 mg (dry weight) of cells per ml.

† The minimal medium was supplemented with 10 μg/ml of indole and 1% of acid-hydrolyzed casein. Cells were harvested after 18 hr at 37 C.

presence of higher serine concentrations is striking. The TSase activity of the toluene-treated cells is enhanced only moderately, and that of untreated cells almost not at all, by increasing the concentration of serine to that (0.16 M) used in the cell-free assay. The factors that interfere with detection of TSase-*ti* activity in whole cells have not been investigated further.

Effect of cultural conditions on formation of TPase by E. coli B/1t7-A. Although *E. coli* B/1t7-A forms TPase constitutively, the amount formed is greatly influenced by cultural conditions. These effects were investigated more carefully since a critical aspect of the purification procedure for TPase (Newton and Snell, 1964) is the use of cell extracts of very high specific activity.

When inocula grown on a minimal medium were transferred to minimal medium supplemented with acid-hydrolyzed casein, the specific activity of extracts of the resulting cells was markedly dependent upon the size of the inoculum used (Table 6). Further investigation showed that the initial activity of the cell extracts was increased as much as two to fourfold when cells grown in minimal medium were transferred to enriched media for short growth periods. Maximal specific activities were obtained in cultures that had undergone about two cell divisions in broth medium (Fig. 5). Data of Fig. 6, in which the specific activities of extracts from cultures which had undergone approximately two cell generations in broth medium (curve 2) are plotted as a function of age and growth phase of the inoculum used (curve 1), show that maximal activities are obtained when inoculum cells from the minimal medium are in the exponential phase of growth. It should be emphasized that these high specific activities are not obtained if the inocula used are grown in the enriched medium; the increase in specific activity is produced in some unexplained

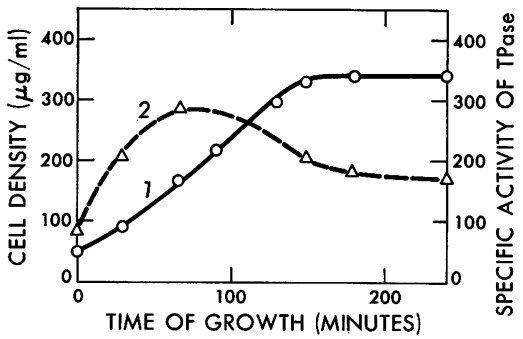

FIG. 5. *Effect of time of growth in broth medium on cell yield (curve 1) of Escherichia coli B/1t7-A and the specific activity for TPase (curve 2) of cell extracts. Inocula were grown in minimal medium containing 0.1% glucose and 20 μg/ml of L-tryptophan. When a cell density of 0.3 mg/ml was reached, 40 ml of this culture were used as inoculum for each of a series of 500-ml Erlenmeyer flasks containing 200 ml of broth medium. At the times indicated, a single flask was chilled, the cells were harvested, and the TPase activity was determined on extracts of the cells. All incubations were at 37 C.*

fashion by the "shift-up" from minimal to enriched medium.

DISCUSSION

The observations that 4-, 5-, and 6-methyltryptophan support growth of tryptophan auxotrophs of *E. coli* only in the presence of indole (Newton and Snell, 1962; and Table 1), and that the growth-promoting activities of these tryptophan analogues fall in the same order as their activities as inducers of TPase (Table 2), support the concept that these analogues promote growth by inducing TPase, which then catalyzes synthesis of tryptophan from exogenous indole and an endogenous amino acid supplying the 3-carbon side chain such as serine (reaction 1) or cysteine (Newton and Snell, 1964). That this pathway can supply the tryptophan required for growth is shown by the existence of mutants such as B/1t7-A, which lack TSase-*tr* completely, are constitutive for TPase, and grow when indole alone is added to minimal media (Newton and Snell, 1962; Gartner and Riley, 1965).

Although TPase is usually considered to act as a degradative enzyme, these results show that it also can act as a biosynthetic enzyme under certain conditions. The intracellular factors which determine whether the enzyme acts in a degradative or synthetic capacity are largely unknown. That the degradative function takes precedence under conditions of tryptophan excess is unquestioned. Some evidence indicates that this degradative activity can assume significance even in cultures of tryptophan auxotrophs grown with limiting amounts of tryptophan, where the amount of induced TPase present would be minimal. For example, tryptophan auxotrophs, blocked in the conversion of indole to tryptophan through lack of TSase-*tr*, reach a lower final level of growth on limiting amounts of tryptophan than do other tryptophan auxotrophs not so blocked (Yanofsky, 1960). It has been assumed that both classes of mutants degrade a portion of the tryptophan to indole via the TPase reaction, and that the blocked mutants achieve lower growth levels because they are unable to reconvert the indole thus formed to tryptophan. Since TPase can also catalyze synthesis of tryptophan from indole, it is not entirely clear why the degradative function should take precedence over the synthetic function in this instance, and lead to a net loss in tryptophan in the TSase-*tr*-negative mutants. However, the affinity of TPase for tryptophan is far greater than that for serine, and it seems likely that degradation of the small amounts of tryptophan involved does not lead to accumulation of sufficient indole to inhibit the tryptophanase reaction (reaction 4) and initiate the synthetase reaction (reaction 1).

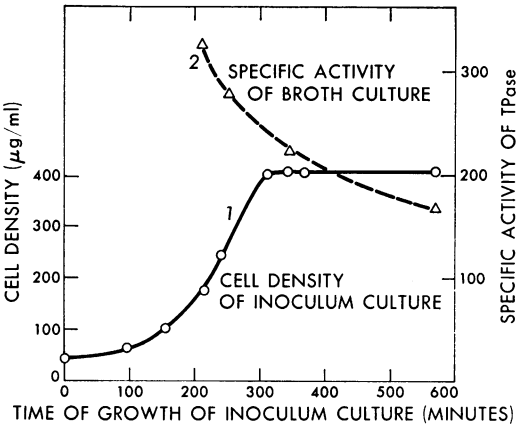

FIG. 6. *Relation of age and growth phase of the inoculum to the TPase activity of cell extracts of Escherichia coli B/1t7-A grown for two generations in broth medium. Inocula were grown in minimal medium as described in Fig. 5, and the cell density (curve 1) was followed with time. At the times indicated by the points in curve 2, 40 ml of this inoculum culture were used to inoculate 200 ml of broth medium contained in a 500-ml Erlenmeyer flask. The cells were grown with shaking at 37 C for two cell generations, then harvested, disrupted, and assayed for TPase.*

According to the foregoing hypothesis, however, factors which increase the TPase content of the cell [e.g., 5-methyltryptophan (Table 2)] should reduce growth of such tryptophan auxotrophs on limiting tryptophan still further. Instead, a pronounced enhancement of growth with limiting tryptophan is observed on addition of 5-methyltryptophan (Table 1). Yanofsky (1960) suggests that this effect, which was observed previously by Lester (*unpublished data*, cited by Yanofsky, 1960), results from inhibition of TPase by the analogue. This suggestion is consistent with the observation (Hall et al., 1960) that 4-, 5-, and 6-methyltryptophan are degraded by TPase to the corresponding methylindoles, and thus would serve as competitive substrates for this enzyme and correspondingly reduce formation of indole from tryptophan. This explanation, however, is insufficient to explain the similar favorable effect (Fig. 1) of 5-methyltryptophan on utilization of limiting amounts of indole as a source of tryptophan by the mutant, *E. coli* B/1t-7A, which lacks TSase-*tr* but is constitutive for TPase (TSase-*ti*). This effect is also observed in the presence of other substrates of TPase, such as *S*-methylcysteine and serine, and its magnitude seems to correlate with the effective-

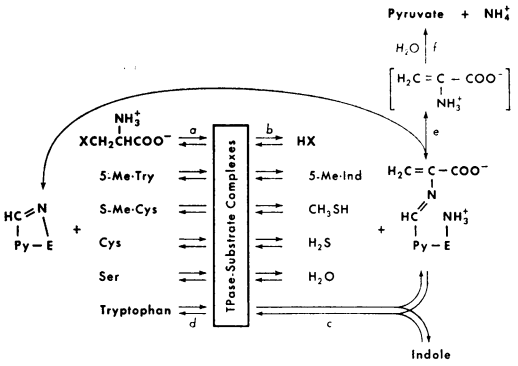

FIG. 7. *Proposed mechanism for TPase action illustrating how substrates other than tryptophan can be either degraded to pyruvate and ammonia or used to supply the 3-carbon side chain in the synthesis of tryptophan from indole. Abbreviations are as follows: 5-Me·try, 5-methyl-L-tryptophan; S-Me·cys, S-methyl-L-cysteine; cys, L-cysteine; ser, L-serine. HoloTPase is abbreviated by the structure at the left where Py represents the pyridine nucleus of pyridoxal phosphate.*

ness of these compounds as substrates for TPase (Newton, Morino, and Snell, J. Biol. Chem., *in press;* Newton and Snell, 1964). A more complete explanation for these effects is suggested by Fig. 7, which presents in modified form a previously suggested mechanism (Newton and Snell, 1964) proposed to explain the varied degradative and synthetic activities of TPase. According to this scheme, β-substituted amino acids of appropriate structure, such as tryptophan, 5-methyltryptophan, serine, etc., all react with TPase (reaction *a*, Fig. 7) to form an enzyme substrate complex from which the β-substituent plus a proton (HX = indole, 5-methylindole, etc.) is eliminated to form reversibly in each instance an enzyme-bound α-aminoacrylic acid (reaction *b*). In the presence of sufficient indole, operation of these steps in the reverse direction (reactions *c*, *d*) leads to tryptophan; in its absence, α-aminoacrylate is liberated (reaction *e*) and hydrolyzes irreversibly to pyruvate and ammonia (reaction *f*). Thus, it is highly probable that added 5-methyltryptophan, *S*-methylcysteine, etc., in addition to minimizing breakdown of any tryptophan formed through their action as competitive substrates, stimulate growth of *E. coli* B/1t7-A with limited indole by supplying the three-carbon side chain required for tryptophan synthesis more efficiently than this can be supplied endogenously. In further support of this interpretation is the observation (Newton et al., *in press*) that, in the presence of indole, crystalline TPase also catalyzes formation of tryptophan from 5-methyltryptophan. [Beerstecher and Edmonds (1956) claimed without details that indole was an essential catalyst for the formation of 5-methylindole from 5-methyltryptophan by the TPase of *E. coli*, and that tryptophan was formed as an essential intermediate in this conversion. Formation of tryptophan from indole and 5-methyltryptophan is in accord with the concept developed here, and this reaction is catalyzed by crystalline TPase. However, under our conditions, formation of 5-methylindole from 5-methyltryptophan occurs in the absence of added indole, and is not catalyzed by the addition of indole (Newton et al., *in press*).]

The repression by tryptophan of the biosynthetic TSase in *Aerobacter aerogenes* (Monod and Cohen-Bazire, 1953) can be observed without interference from any inducible TSase activity, since this bacterium cannot form TPase. An analogous case is provided by the two TPase-negative mutants of *E. coli* K-12, TP-1, and TP-2, in which partial repression of TSase-*tr* by the presence of excess tryptophan in the growth medium is also readily observed in cell-free extracts (Table 4). In the more complex case of wild-type *E. coli* B, only a moderate repression of tryptophan synthesis from indole and serine is observed in extracts, and only at levels of tryptophan (5 μg/ml) where the induction of TPase is not pronounced (Fig. 2). At higher concentrations of tryptophan, induction of TSase-*ti* activity occurs in parallel with TPase (Fig. 2). The ability to catalyze reaction 1 is thus shared by the TPase of wild-type *E. coli*, and does not result from a modification of this enzyme coincident with loss of the genes for TSase-*tr* production. The repression of TSase-*tr* activity observed in extracts of cells grown at higher levels of tryptophan may not be as complete as it appears, since it may result in part from degradation by the induced TPase of the tryptophan formed by TSase-*tr* from indole-3-glycerol phosphate. Several investigations (Cohen and Jacob, 1959; Scott and Happold, 1962; Lester and Yanofsky, 1961) have dealt with repression of TSase and the induction of TPase in *E. coli* by tryptophan, and it is surprising that the TSase activity of TPase was not observed earlier. Several factors appear to have prevented its recognition. (i) In those investigations limited to repression of TSase-*tr*, the levels of tryptophan employed were frequently not sufficient to induce appreciable levels of TSase-*ti*. (ii) TSase-*ti* has an unusually low affinity for serine (K_m = 0.16 M), and assay mixtures used by previous investigators, although they contained adequate serine for assay of TSase-*tr*, contained suboptimal amounts for assay of TSase-*ti*. For example, the report (Burns and DeMoss, 1962) that ultracentrifugally homogene-

ous TPase possessed no TSase activity appears to have resulted primarily from the low serine concentrations used. When higher levels of serine were employed, the expected TSase activity was found (DeMoss, *personal communication*). (iii) Finally, for reasons not yet apparent but possibly connected with the uptake of serine, the whole-cell assays used by most investigators for determination of TSase-*tr* do not reflect the TSase-*ti* activity of the cell extracts (Table 5).

The neutralization of TSase-*ti* and TPase by anti-TPase serum is dependent upon the concentration of both enzyme and antiserum. The close similarity of the neutralization curves (Fig. 3 and 4) supports the conclusion that both reactions are catalyzed by the same enzyme. The behavior of the anti-TPase serum in neutralization of the enzyme activity is qualitatively similar to that observed by Lerner and Yanofsky (1957) in a study of the interaction of anti-TSase serum with TSase-*tr* of *E. coli*. There is, however, no cross-reaction of the anti-TPase serum with the A or B proteins of TSase-*tr*; thus, it is possible to distinguish this enzyme from TSase-*ti* (TPase) in crude cell extracts by an immunological assay (Table 3).

Since formation of cross-reacting material corresponding to TPase was not detected in extracts of the TPase-negative mutants of *E. coli*, TP-1 or TP-2, the possibility cannot be eliminated that the loss of TPase (and hence of TSase-*ti*) is due to an "operator-type" mutation (Jacob and Monod, 1961) rather than to mutation in the structural gene for TPase.

The pronounced effect of growth conditions on the formation of TPase by the constitutive mutant, *E. coli* B/1t7-A (Fig. 5 and 6), remains unexplained. The fact that extracts of higher specific activity are obtained from cells in the exponential phase of growth has been observed previously for inducible strains of *E. coli* by Freundlich and Lichstein (1960) and perhaps can be attributed to accumulation of catabolites that repress enzyme formation during the later stages of growth (Fig. 5). Cells growing exponentially in a minimal medium should contain relatively small amounts of these catabolites, and transfer of such cells to a rich medium, which represses synthesis of many biosynthetic enzymes, might favor formation of certain other constitutive enzymes, such as TPase. This effect may not be a general one, however, since, in preliminary experiments, conditions optimal for maximum TPase production by *E. coli* B/1t7-A did not prove optimal for production of the induced enzyme in its parent, *E. coli* B/1t7.

From the results presented herein, and from catalytic activities of the purified enzyme protein (Newton and Snell, 1964; Newton et al., *in press*), it is clear that the inducible tryptophan synthetase (TSase-*ti*) is identical with tryptophanase. Henceforth, the synthesis of tryptophan from indole and an appropriate amino acid, as well as its degradation, should be considered normal catalytic activities of the latter enzyme.

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