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Formation of Tryptophansynthase and its Regulation during the Active Growth Phases of *Escherichia coli*

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1. The formation of tryptophansynthase by *E. coli* has been investigated in cells that were transferred from a medium containing amino acids and vitamins to a simple glucose-mineral salt medium. Also the effect of tryptophan on the formation of the enzyme was studied.

2. A maximal repression of the formation of tryptophansynthase was effected by tryptophan in concentrations of 75–100 μM; concentrations of 35–45 μM repressed the formation to 50 %.

3. The amount of tryptophansynthase formed increased 10–15-fold, but in cells which had a high initial activity at the beginning of the lag phase, the increase was only about 1.5-fold.

4. The repression took effect immediately when L-tryptophan in 0.5 mM concentration was added to the medium containing the *E. coli* cells in the lag phase.

5. A high tryptophanase activity in the cells caused an increase in the formation of tryptophansynthase.

6. Indole inhibited the formation of tryptophansynthase.

Previous papers from this laboratory have dealt with the formation of B_6 vitamins during the active growth phases of *Escherichia coli* [1,2] and the formation of certain B_6 enzymes during the lag phase of growth.[2,3] It was observed that the different enzymes were formed in widely differing amounts during the growth cycle.

The present paper deals with the formation of tryptophansynthase (C.E. code number 4.2.1.20,[5] also known as tryptophansynthetase and tryptophandesmolase) during the active growth phases of *E. coli*, and the way in which this process is regulated. Tryptophansynthase catalyzes three different reactions in the biosynthesis of tryptophan;[6] in the present work the formation of the catalyst of one of these, the reaction

$$\text{L-serine} + \text{indole} \rightarrow \text{L-tryptophan} + H_2O$$

was followed during the growth of *E. coli*. Previously tryptophan was found to repress the formation of tryptophansynthase during the growth of the bacte-

rial cell.[7,8] To our knowledge, however, no data relating to the formation of tryptophansynthase during the active growth phases has been reported. In the present work the formation of tryptophansynthase was followed in cells transferred from an inoculum medium containing amino acids and vitamins to a simple glucose-mineral salt medium. At the same time, the significance of the tryptophan concentration for the formation of this enzyme was examined under the same conditions.

EXPERIMENTAL

Growth organism and media. The organism *Escherichia coli* was isolated as reported earlier.[9] A GSHT medium containing amino acids and growth factors was employed as inoculum medium, and a simple glucose-mineral salt medium as growth medium in the tests. The compositions of these media are described in an earlier paper.[3]

Determination of enzyme activity. The activity of tryptophansynthase was determined by the method of Yanofsky [10] in which the disappearance of indole from the reaction mixture is followed. The enzyme preparation consisted of a cell extract dissolved in a 0.04 M phosphate buffer of pH 7.8 which had been twice frozen to −40° and warmed to + 37°. In each determination the reaction mixture contained 0.15−0.20 mg (dry weight) of cells. When a medium lacking DL-serine was employed in control experiments no loss of indole was observed under the experimental conditions. In all the activity determinations pyridoxal phosphate was added to the reaction mixture, and before each determination a curve plotting the reaction rate was drawn. On the basis of this curve the reaction time, usually 10−30 min, was chosen. Also a correlation curve was established between the different enzyme concentrations and the reaction rates employed in the experiments. The tryptophanase activity was determined by the method of Gunsalus *et al.*[11] The cell extract was prepared as above.

Procedure. E. coli was transferred from an agar slant, first into some 5 ml of GSHT medium which was incubated 3−5 h and then, before the inoculation, into 1 litre of GSHT medium of + 2° which was incubated without shaking in a water bath at 37° for 15−16 h. The cells were then centrifuged 10 min in a refrigerating Servall RC-2 centrifuge at 12 000 *g* and washed three times with ice-cold 0.9 % NaCl solution.

The growth medium was inoculated with a previously homogenized mass of cells. Before the inoculation, the medium was warmed to 37° and during the growth it was mixed with an electric stirrer. Samples 5−10 ml in volume were withdrawn with a pipette and their turbidities were measured with a Klett-Summerson colorimeter employing

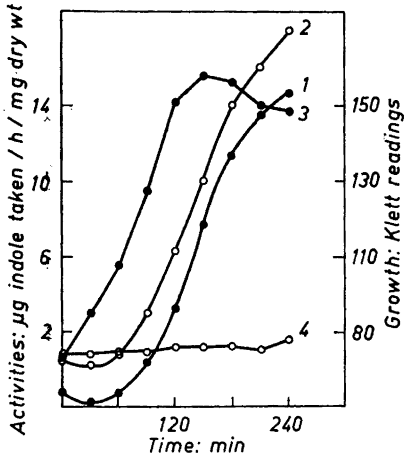

Fig. 1. Formation of tryptophansynthase during the active growth of *E. coli*. Effect of L-tryptophan on the formation of the enzyme.

1. Growth curve for *E. coli* in medium MM (right-hand scale).
2. Growth curve for *E. coli* in medium MM when L-tryptophan had been added at the beginning of growth to give a 2 mM solution.
3. Formation of tryptophansynthase in medium 1. The activity is expressed in µg of indole consumed/h/mg dry wt.
4. Formation of tryptophansynthase in *E. coli* cells growing in medium 2. Activity expressed as under 3.

Table 1. Effect of L-tryptophan on the formation of tryptophansynthase during the active growth of *E. coli*.

L-tryptophan μg/ml	Activity μg indole/h/mg
0	32.0
3	27.9
6	18.5
13	10.2
26	7.7
51	7.8
102	7.5
204	7.7
0	7.7 *

E. coli was grown in medium GSHT at 37° for 15 h, after which the cells were inoculated in medium MM, to which the amounts of tryptophan indicated in the table had been added at the beginning of growth. Growth stopped after 210 min when most of the cells were in the same, the exponential growth phase. The Klett readings varied between 168 and 172.

* Activity (per mg) at the beginning of growth.

red filter 62. The samples were then cooled and centrifuged as above and the cells were washed once with ice-cold saline. The enzyme preparation was made from these cells by freezing and thawing them as described above.

RESULTS

It was mentioned above that tryptophan represses the formation of tryptophansynthase during cell growth.[7,8] As shown in Fig. 1, this was the case also when 2 mmoles of L-tryptophan per litre was added to the glucose-mineral salt medium for *E. coli*. According to curve No. 3 in Fig. 1 the activity of the tryptophansynthase increased from the original value of 1 μg of indole used/h/mg dry wt. to a maximum of 15 μg of indole used/h/mg dry wt., but according to curve No. 4 in the same figure no tryptophansynthase was formed when

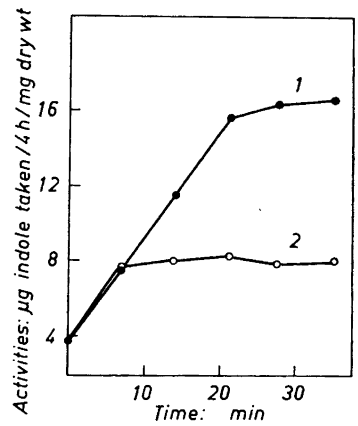

Fig. 2. Formation of tryptophansynthase during the lag phase of *E. coli*, and the effect on this formation of L-tryptophan added to the medium during the lag phase.
1. Formation of tryptophansynthase in medium MM during the lag phase. Activity expressed in μg of indole consumed/h/mg dry wt.
2. Formation of tryptophansynthase during the lag phase in medium MM to which 100 μg of tryptophan had been added per ml 7 min after the inoculation.

In both cases the lag phase lasted about 40 min.

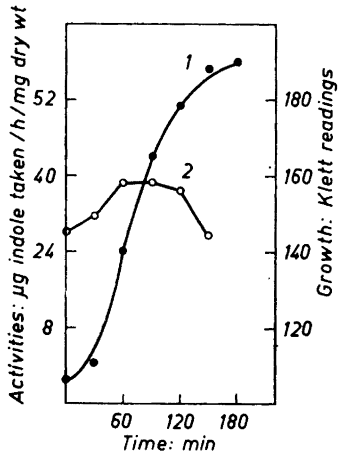

Fig. 3. Formation of tryptophansynthase during the active growth of *E. coli* cells in which the enzyme activity was higher than normal at the beginning of growth.
1. Growth curve for *E. coli* in medium MM after precultivation in the same medium up to the end of the exponential phase (when the tryptophansynthase activity is high).
2. Formation of tryptophansynthase during the active growth of *E. coli*. Activity: µg of indole consumed/h/mg dry wt.

the medium was 2 mM in L-tryptophan. Usually the activity increased 10—15 times under the experimental conditions, and the maximum was reached at the end of the exponential phase of growth (for the classification of the growth phases, see Monod [12]).

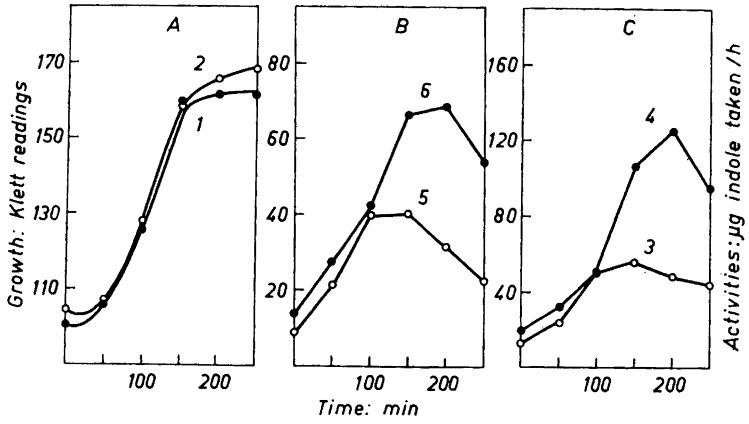

Fig. 4. Formation of tryptophansynthase in cells with a high tryptophansynthase activity during the active growth of *E. coli*.
1. Growth curve for *E. coli* when the inoculum medium was a normal GSHT medium.
2. Growth curve for *E. coli* when the GSHT inoculum medium lacked glucose. Because of the absence of glucose repression, the tryptophanase activity per cell was about 7 times the activity in cells which were precultivated in medium 1. The growth medium in both instances was medium MM.
3. Total tryptophansynthase activity, expressed in µg of indole consumed/h, corresponding to growth curve 1.
4. Total tryptophansynthase activity corresponding to growth curve 2.
5. Specific enzyme activity, expressed in µg of indole consumed/h/mg dry wt., corresponding to growth curve 1.
6. Specific activity corresponding to growth curve 2.

Table 1 shows the degrees of repression effected by different concentrations of tryptophan. Maximal repression of the formation of tryptophansynthase occurred when the concentration of tryptophan was 15—20 μg/ml (75—100 μM) and a 50 % repression when the concentration was 7—9 μg/ml (35—45 μM).

As appears from Fig. 2, a repression occurred already in the lag phase. When L-tryptophan, in a concentration of 100 μg/ml (0.5 mM), was added to the medium containing *E. coli* 7 min after the inoculation, the formation of tryptophansynthase stopped (Fig. 2, curve 2).

Fig. 3 shows the formation of tryptophansynthase in the glucose-mineral salt medium when the concentration of the enzyme was exceptionally high at the beginning of the lag phase: the value obtained was 28 μg of indole used/h/mg dry wt. In this case the formation curve (Fig. 3, curve 2) differed clearly from curve No. 3 in Fig. 1 in that far less tryptophansynthase was formed. An about 1.4-fold increase in the formation was observed, and maximum was also in this case reached at the end of the exponential phase.

Fig. 4 illustrates the formation of tryptophansynthase in cells with a high tryptophanase activity. The high activity was obtained by cultivating the cells in a glucose-free medium before the inoculation; glucose inhibits the formation of tryptophanase.[13,14] More tryptophansynthase is formed in cells with a high tryptophanase activity than in normal cells, as can be seen from Fig. 4 (curves 3—6).

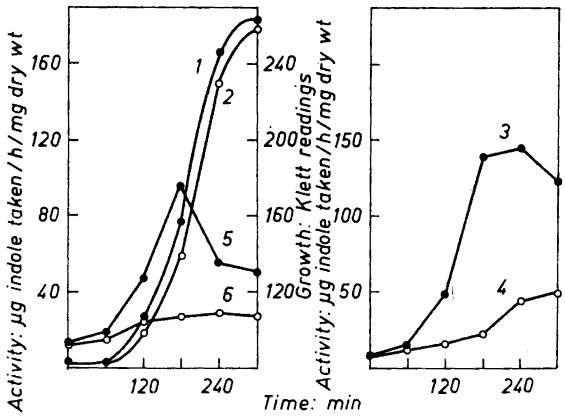

Fig. 5. Effect of indole on the formation of tryptophansynthase during the active growth of *E. coli* in medium MM.

1. Growth curve for *E. coli* in medium MM (right-hand scale).
2. Growth curve for *E. coli* in medium MM when indole was added to the medium at the beginning of growth (0.5 mM).
3. Total tryptophansynthase activity, expressed in μg of indole consumed/h, corresponding to growth curve 1.
4. Total tryptophansynthase activity corresponding to growth curve 2.
5. Specific activity, expressed as μg of indole consumed/h/mg dry wt, corresponding to growth curve 1.
6. Specific activity corresponding to growth curve 2.

Fig. 5 shows the effect of indole on the formation of tryptophansynthase in a glucose-mineral salt medium. It can be seen from the figure that, even though it slightly inhibited the bacterial growth (curve 2), indole also caused a marked decrease in the formation of tryptophansynthase (curves 4 and 6).

DISCUSSION

The results obtained show that tryptophansynthase is formed in large amounts in the glucose-mineral salt medium when the cells are transferred from an amino acid-vitamin medium to a tryptophan-free medium (Fig. 1, curve 3; Fig. 2, curve 1; Fig. 4 and Fig. 5, curve 3). Very likely no tryptophansynthase is formed in the inoculum medium because the medium contained tryptophan derived from Bacto-tryptone and yeast extract. The content was always maximal at the end of the exponential phase, and the high initial activity did not in any case precede this maximum on the growth curve. In addition, our previous observation [4] that the formation of tryptophansynthase starts immediately after the inoculation is confirmed by the results shown in curve 1 of Fig. 2.

It should also be noted that the total tryptophansynthase activity decreases after the maximum (Fig. 4, curve 4 and Fig. 5, curve 3). The decrease could not very well be caused by changes in pH during the growth, because the enzyme was similarly inactivated when the pH was kept constant (at 6.7—6.8) during the growth by additions of sodium hydroxide.[15] The maximum amount of enzyme present was normally 10—15 times higher than when its formation began, but the increase was considerably smaller when the initial activity was comparatively high (Fig. 3, curve 2). Probably in this case the activities of all enzymes connected with the biosynthesis of tryptophan were equally high and tryptophan was formed at a higher rate in these cells than in the control cells and caused a marked repression.

The results shown in Fig. 4 suggest also that the tryptophan concentration in the cells functions as a regulator of the formation of tryptophansynthase. An attempt was made to diminish the amount of tryptophan within the cells by increasing the amount of tryptophanase, and thus obtain a further increase in tryptophansynthase, with the results shown by curves 4 and 6 in Fig. 4. Results of another attempt to change the tryptophan content in the cell are shown in Fig. 5. Indole, while inhibiting the tryptophanase reaction,[16] is supposed to increase the amount of tryptophan, the repressor, in the cells and thereby to repress the formation of tryptophansynthase (Fig. 5, curves 4 and 6).

The results obtained thus confirm our suggestion that the concentration of tryptophan in the cells greatly influences the formation of tryptophansynthase during the active growth of *E. coli* in a glucose-mineral salt medium.

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