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Variations in the Transaminase Activity of *Escherichia coli* Cells during the Lag Phase of Growth

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Variations in transaminase activity during the lag growth period of *Escherichia coli* have been investigated using α-ketoglutaric acid and eight amino acids as transaminase reactants. A rise was observed in the activities of the following transaminases: valine-glutamic, leucine-glutamic, *nor*valine-glutamic, *nor*leucine-glutamic and *iso*-leucine-glutamic. The transaminase activities rose to a maximum after approximately 40 min of lag growth and remained constant or, in a few cases, decreased slightly during the remaining part of the lag phase. Under the experimental conditions the lag phase lasted 50 min. During this time no variations were observed in the activities of the aspartic-, phenylalanine-, and tryptophan-glutamic transaminases.

A recent study carried out in this laboratory revealed that the rate of synthesis of vitamin B_6 in *Escherichia coli* cells rises to a maximum during the acceleration growth phase and then decreases slowly to the initial level towards the end of the exponential phase of growth [1,2]. It is a well-known fact that pyridoxal phosphate and pyridoxamine phosphate act as coenzymes of vitamin B_6 and participate in, for instance, transaminase reactions. With these facts in mind, it seems probable that variations should occur also in the transaminase activity during the growth phases in question. However, we find no published information on variations in the transaminase activity of bacterial cells during the early growth phases; in fact, there seems to be very little information about variations in enzyme activity in general during the lag and acceleration phases of growth. There is, however, a paper by Mc Carty [3] on the variations in dehydrogenase and deaminase activity during bacterial growth, and hence also during the lag phase. The present study was undertaken to determine whether any variations take place in the transaminase activity during the lag phase of growth of *E. coli*. It constitutes a part of a more extensive study on variations in enzyme activity and in rates of biosynthesis of the vitamin B complex and vitamin B coenzymes during the early growth phases of *E. coli* cells.

EXPERIMENTAL

Growth organism. The *Escherichia coli* strain U5-41 isolated as reported earlier [2] was used in all the experiments.

Culture methods. The *E. coli* strain was transferred monthly and incubated for inoculation of the media described in the previous report [2]. The growth medium contained 2.0 g of glucose, 1.4 g of dihydrogenpotassium phosphate, 100 mg of trisodium citrate, 200 mg of ammonium sulphate, 200 mg of ammonium chloride and 20 mg of magnesium sulphate heptahydrate in 100 ml of distilled water. The medium was neutralized with sodium hydroxide to pH 6.7—6.8 and briefly heated before the inoculation.

Procedure. E. coli was transferred from the agar slant into approximately 5 ml of the inoculum medium and incubated for 6—9 h. The cell suspension was then poured aseptically into one litre of the same inoculum medium and again incubated 12—14 h at 37°. The cells were centrifuged 15 min in a Wifug centrifuge at 2 500 rpm and washed three times with 0.9 % saline. Before transfer to the growth medium, the cells were stored in a refrigerator for not more that 30 min. All the cells were added as inoculum and one millilitre of the growth medium contained approximately 0.2 mg of *E. coli* cells, calculated as dry weight. The growth medium was heated to 37° on a water bath and mixed with an electric stirrer during the whole experiment. The first sample was taken 5 min after the inoculation in order to ensure a homogeneous suspension. After this, samples were taken at intervals of 3—5 min into tubes which were immediately immersed in an ice bath. After cooling, the turbidities of the samples were measured with a Klett-Summerson colorimeter using filter 62. After this the samples were centrifuged 15 min at 3 500 rpm.

Preparation of extracts. The centrifuged cells were treated with cold acetone (−20°) for 10—20 min, the precipitate was centrifuged down and the acetone poured out. The remaining acetone was removed in a vacuum and the cell powder transferred to a refrigerator. Before the enzyme assays the cell powder was suspended in a 0.01 M tris-buffer of pH 8.0, and stored overnight in the refrigerator. This suspension was used in all enzyme assays.

Transaminase assay. For the determination of enzyme activity, 0.050 ml volumes of a substrate solution containing 5 μmoles of L-amino acid (or 10 μmoles of DL-amino acid) and 10 μmoles α-ketoglutaric acid in a 0.05 M tris-buffer of pH 8.0 were pipetted into 60 × 7 mm test tubes and neutralized with potassium hydroxide. When pyridoxal-5-phosphate was added as activator, the amount was 2.5 μg per 0.050 ml of the substrate solution. The substrate solution was prepared some time before the assay and the tubes were stored in ice water. A 0.050 ml volume of the cell extract containing 0.25—0.30 mg (dry wt.) of the cells was pipetted into the tubes and they were shaken. After an incubation period of 60 min at 37°, the reaction was stopped by pipetting 0.020 ml volumes of 70 % trichloroacetic acid solution into the test tubes. The precipitated protein was removed by centrifuging 5-10 min at 3500 rpm.

Glutamic acid formed in the reaction was determined chromatographically. For this purpose samples (0.020 ml) of the clear supernatant were pipetted onto Whatman No. 1 paper. The glutamic acid was separated from the substrate amino acids by the ascending technique in a 17 × 68 × 62 cm tank. For the separation of threonine, alanine, serine, and glycine, an ethanol-water-ammonia (18:1:1) mixture was used as solvent system. For aspartic acid, a pyridine-acetic acid-water (10:7:3) mixture was used. In the first case the development time was 40—50 h and in the latter 20—30 h. In other cases an *n*-butanol-glacial acetic acid-water (120:30:50) mixture was used as solvent system. After the runs, the chromatograms were dried in a hood and sprayed with 0.5 % ninhydrin in acetone containing 5 % pyridine as color intensifier. After the chromatograms had been sprayed, the color was allowed to develop at room temperature for several hours and finally at 60° for 30 min in a moist oven. Pieces of paper, all of the same size, containing the glutamic acid spots were cut out and the color extracted with 4.0 ml of methanol for 2 h. The density was measured with a Beckman DU spectrophotometer at wavelength 570 mμ. The assays were carried out in duplicate on the same paper. The average density was calculated from the two values. In each assay one of the following substances was omitted from one specimen: cell extract, α-ketoglutaric acid, and amino acid, or one of the two substrates. A standard curve for glutamic acid (shown in Fig. 1) was used to determine the amount of glutamic acid formed.

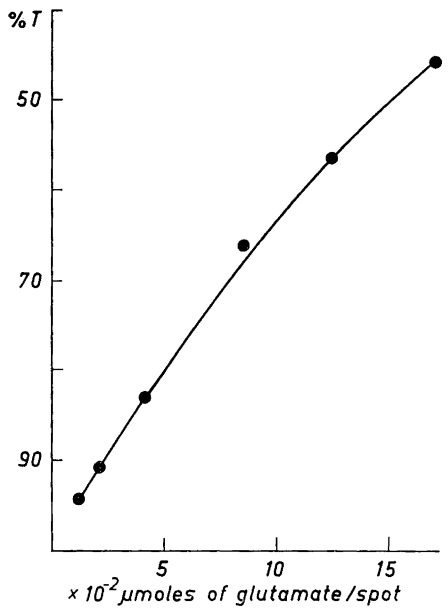

Fig. 1. Standard curve for glutamic acid determination.

The transaminase activity is expressed as the number of μmoles of glutamic acid formed per mg (dry weight) of the cells in one hour. Fig. 2 shows the progress curves obtained under the assay conditions with leucine, *iso*leucine, aspartic acid, tryptophan, phenylalanine, valine, *nor*leucine, and *nor*valine, as amino group donors to α-ketoglutaric acid.

As can be seen from Fig. 2, the reaction time employed, 60 min, was not too long a time for revealing variations in the activity under the experimental conditions.

Chemicals. DL-Ornithine and α-ketoglutaric acid were obtained from L. Light & Co Ltd, DL-tryptophan, glycine, DL-methionine, L-proline and DL-*iso*leucine from British Drug Houses Ltd, DL-lysine, DL-valine, DL-phenylalanine, DL-serine, L-tyrosine, L-leucine,

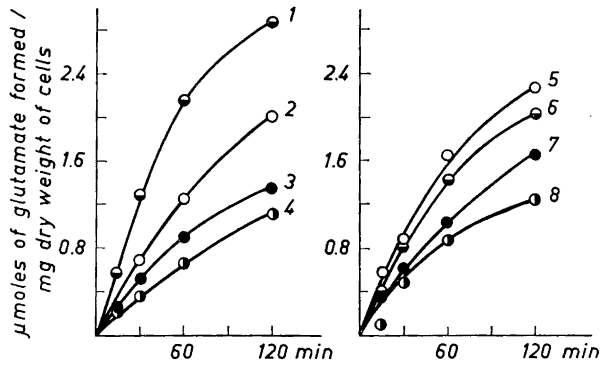

Fig. 2. Progress curves for transaminase reactions between α-ketoglutaric acid and certain amino acids. 1,aspartic acid; 2,tryptophan; 3,*nor*valine; 4,*nor*leucine; 5,*iso*leucine; 6,leucine; 7,phenylalanine; 8,valine.

Table 1. Transamination between α-ketoglutaric acid and amino acids. Crude *E. coli* cell extracts have been used in all experiments.

Amino acid	Feldman and Gunsalus[4], μmoles glutamate formed in one hour	Rudman and Meister[5], μmoles glutamate formed in two hours	Our experiments, glutamate formed in one hour: percentage from the aspartic acid activity (= 100)
D-Alanine			0
L-Alanine	0	w *	6
Arginine		w	5
Asparagine			94
Aspartic acid	25	41.0	100
Cysteine			0
Cystine		w	12
Glutamine			16
Glycine	10	w	0
Histidine	10	w	0
Hydroxyproline			0
*Iso*leucine	10	16.4	61
Leucine	20	16.9	47
Lysine	10		6
Methionine	15	12.5	42
*Nor*leucine	20	15.9	42
*Nor*valine		17.3	48
Ornithine		w	12
Phenylalanine	15	15.3	56
Proline	0	w	0
Serine		w	0
Threonine	8	w	0
Tryptophan	15	29.8	48
Tyrosine	12	10.3	22
Valine	25	12.3	52

* w = weak or not detectable.

L-cystine, L-cysteine, L-arginine, L-asparagine, and L-glutamic acid from E. Merck AG, DL-threonine, L-glutamine, L-histidine, DL-homocystine, L-hydroxyproline, DL-*nor*valine, L-aspartic acid, L-alanine, and D-alanine from Hoffman La Roche & Co Ltd, and DL-*nor*-leucine from General Biochemicals, Inc. Pyridoxal-5-phosphate was obtained from Hoffman La Roche & Co Ltd. In enzyme assays the buffer used was prepared from Sigma 7 — 9 reagent and obtained from Sigma Chemical Co. All other chemicals used in the experiments were AnalaR reagents obtained from E. Merck AG and British Drug Houses Ltd.

RESULTS

The first aim of the study was to find out what transaminase reactions involving the transaminase systems formed by α-ketoglutaric acid and the 25 different amino acids take place in *E. coli* cells. Pyridoxal phosphate was added as coenzyme to all these transaminase systems. The *E. coli* cells were harvested in the middle of the exponential growth phase. The results obtained are shown in Table 1. Under the experimental conditions used, the following amino acids transaminated weakly or not at all: DL-threonine, L-alanine, D-alanine, DL-serine, L-cysteine, L-histidine, L-hydroxyproline, L-proline, L-cystine, DL-orni-

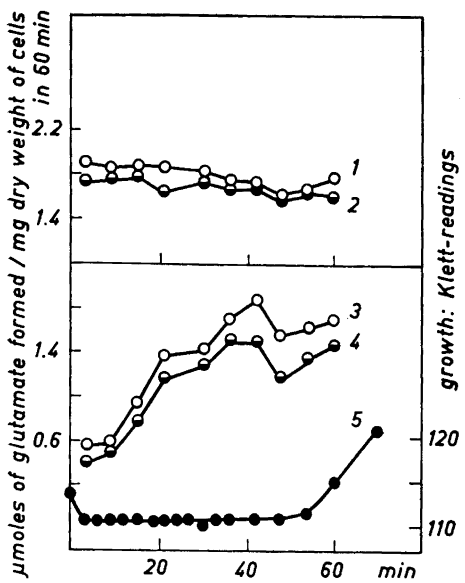

Fig. 3. Transaminase activity during the lag phase of growth of *E. coli*. 1,glutamic-aspartic transaminase (pyridoxal phosphate was added); 2,glutamic-aspartic transaminase; 3,glutamic-leucine transaminase (pyridoxal phosphate was added); 4,glutamic-leucine transaminase, 5,growth curve (right-hand scale).

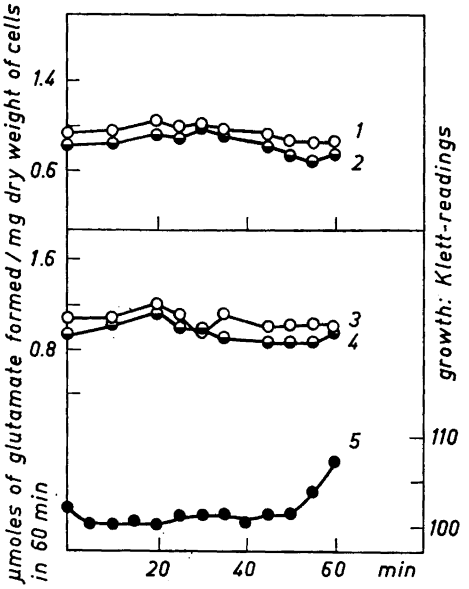

Fig. 4. Transaminase activity during the lag phase of growth of *E. coli*. 1,glutamic-phenylalanine transaminase (pyridoxal phosphate was added); 2,glutamic-phenylalanine transaminase; 3,glutamic-tryptophan transaminase (pyridoxal phosphate was added); 4,glutamic-tryptophan transaminase; 5, growth curve (right-hand scale).

thine, L-arginine, L-glutamine, and DL-lysine. Amino acids which definitely exhibited transamination activity were DL-*nor*leucine, DL-phenylalanine, DL-tryptophan, DL-*nor*valine, DL-valine, L-tyrosine, DL-*iso*leucine, DL-methionine, L-leucine, and L-aspartic acid. All the amino acids of this latter group except tyrosine and methionine were included in the experiments in which the variations in transaminase activity were studied during the lag phase of growth. In the transamination reaction of asparagine and α-ketoglutaric acid, glutamic acid was synthesized in large amounts, but also aspartic acid was produced in quite considerable amounts. The results of the determinations of transaminase activity are shown in Figs. 3—6.

The length of the lag phase varied between 30 and 50 min in these transamination experiments. The initial culture contained 0.20—0.22 mg (dry wt.) of cells per ml of growth medium, and had an optical density of 100—195 with filter 62. The values of the optical density at the beginning of the lag phase were higher than those measured later because the culture suspensions could not be made homogeneous at once with the experimental procedure used. The high values thus do not indicate any increase in bacterial count as compared to the count in the later part of the lag phase. In order to ensure an accurate determination of the length of the lag phase, the growth was followed into the acceleration phase; as seen from the figures, the transaminase activities were

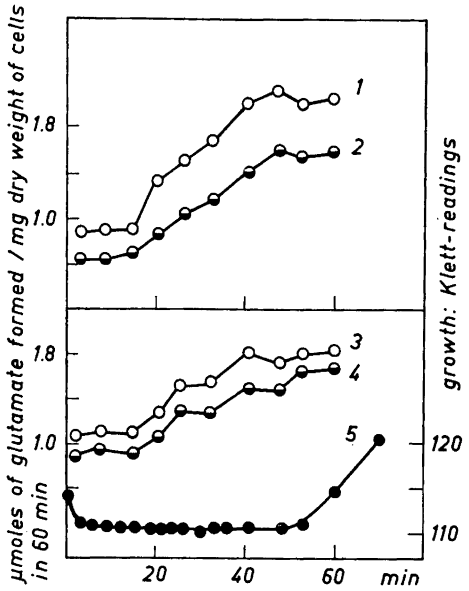

Fig. 5. Transaminase activity during the lag phase of growth of *E. coli*. 1,glutamic-*iso*-leucine transaminase (pyridoxal phosphate was added); 2,glutamic-*iso*leucine transaminase; 3,glutamic-valine transaminase (pyridoxal phosphate was added); 4,glutamic-valine transaminase; 5,growth curve (right-hand scale).

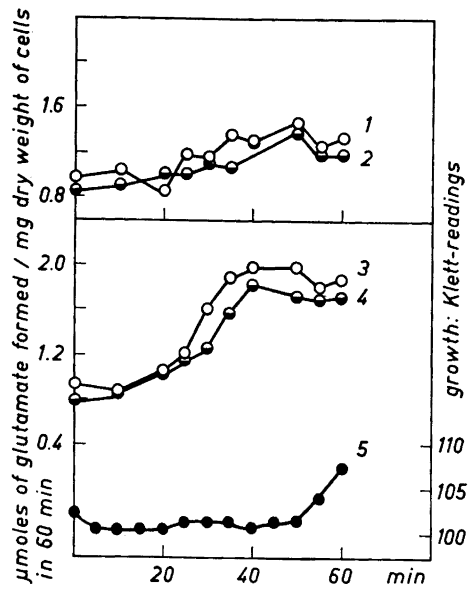

Fig. 6. Transaminase activity during the lag phase of growth of *E. coli*. 1,glutamic-*nor*-leucine transaminase (pyridoxal phosphate was added);2, glutamic-*nor*leucine transaminase; 3,glutamic-*nor*valine transaminase (pyridoxal phosphate was added); 4, glutamic-*nor*valine transaminase; 5, growth curve (right-hand scale).

also determined during the early acceleration growth phase. In the following, however, only the results pertaining to the lag phase are presented.

As can be seen from Fig. 3 the aspartic-glutamic transaminase activity remained constant throughout the lag phase. As seen from the same figure, the leucine-glutamic transaminase activity underwent a fourfold increase from the beginning of the lag phase.

No variations were noted in the activities of the phenylalanine-glutamic and tryptophan-glutamic transaminases (Fig. 4), but Fig. 5 demonstrates that the activities of the *iso*leucine-glutamic and the valine-glutamic transaminases increased considerably, nearly three times and one and a half times, respectively.

Fig. 6 shows that a 1.5-fold increase occurred in the activity of *nor*leucine-glutamic transaminase, and a twofold increase in the activity of the *nor*valine-glutamic transaminase.

Figs. 3—6 show data obtained in experiments where pyridoxal phosphate was present in the medium and in experiments where it was absent. As the results demonstrate, the transaminase activities were slightly higher when pyridoxal phosphate was present than when it was absent. In a few cases this difference was quite considerable, for instance, for the *iso*leucine-glutamic transaminase and for the valine-glutamic transaminase.

DISCUSSION

The present investigation has revealed that variations occur in certain transaminase activities during the lag phase of growth of *E. coli*. The study of the abilities of 25 amino acids to function in transamination reactions gave the same results as were obtained by Feldman and Gunsalus [4] and by Rudman and Meister [5] (Table 1).

On the basis of the results, those transaminases which clearly are present in *E. coli* cells can be divided into two groups. The transaminases (Group A) exhibiting clear variations in activity are the following: *iso*leucine-glutamic, valine-glutamic, leucine-glutamic, *nor*valine-glutamic, and *nor*leucine-glutamic. Those transaminases (Group B) for which no variations in activity were observed were the aspartic-glutamic, phenylalanine-glutamic, and tryptophan-glutamic transaminases.

Perhaps the most interesting result of this work is that the activities of certain transaminases, those included in Group A, increase during the lag phase. In these cases the length of the lag phase was approximately 50 min. As Figs. 5 and 6 show, the activities of the transaminases of Group A increased already at the beginning of the lag phase and reached a maximum about 40 min later. From this point onwards, the activities remained practically constant. With the leucine-glutamic transaminase, the activity decreased after the maximum had been reached. In this connection it is interesting to note that Rudman and Meister [5] obtained two fractions, A and B, when they fractionated an extract of *E. coli* cells. In fration B these authors found *iso*leucine-glutamic transaminase, valine-glutamic transaminase, and leucine-glutamic transaminase. In the present work the activities of these same transaminases were observed to increase during the lag phase, while the other transaminase systems that we found to undergo no change in activity are the same as those which Rudman and Meister found in their fraction A.

The variations we found in the transaminase activities were not caused by changes in the pH of the medium, because no changes in pH were observed during the experiments. Thus they cannot be explained by the finding of Gale and Epps [6] that the amount of enzyme per cell increases when the pH becomes unfavourable.

An interesting comparison can be made between the increases in the transaminase activities observed in this work and the increase in the rate of biosynthesis of vitamin B_6 demonstrated in one of our previous studies [2]. The rate of biosynthesis of vitamin B_6 increased throughout the lag phase of growth and reached a maximum during the acceleration phase, while the activities of the transaminases of Group A reached their maximal values already during the lag phase (providing that no other maxima occur during the later part of the growth cycle). These activities either remained constant or decreased when the growth progressed to the acceleration phase, where the rate of biosynthesis of vitamin B_6 seemed to be especially high.

At the present stage of our studies, the observation that the activities of certain transaminases increase while those of others remain practically constant cannot be easily explained. However, a plausible explanation may lie in the accepted fact that glutamic acid and glutamine are the first amino

acids synthesized by the assimilation of inorganic nitrogen (ammonia) [7], and that the other amino acids are synthesized from these. It may be that during the lag phase of growth the organism needs three branched-chain amino acids, *iso*leucine, leucine, and valine, in increasing amounts (*e.g.* for protein synthesis) before cell division can occur. If these amino acids are synthesized from glutamic acid by transaminase reactions, the rise in the enzyme activity of *iso*leucine-glutamic, leucine-glutamic, and valine-glutamic transaminases would be understandable.

Our opinion is that a close study of the variations in enzyme activity, especially during the first growth phase of an organism, will throw new light on the mechanism regulating the growth of cells. Experiments in this field are therefore being continued in this laboratory.

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REFERENCES

1. Nurmikko, V. and Laaksonen, S. *Suomen Kemistilehti. In press.*
2. Nurmikko, V. and Raunio, R. *Acta Chem. Scand.* **15** (1961) 856.
3. Mc Carty, B. J. *Proc. Roy. Soc. London* B **150** (1959) 410.
4. Feldman, L. I. and Gunsalus, J. C. *J. Biol. Chem.* **187** (1951) 821.
5. Rudman, D. and Meister, A. *J. Biol. Chem.* **200** (1953) 591.
6. Gale, E. F. and Epps, H. M. R. *Biochem. J.* **36** (1942) 600.
7. Roine, P. *Ann. Acad. Sci. Fennicae A II* **1947** No. 26.

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