Metabolism of Glutamic Acid in a Mutant of *Escherichia coli*

JOYCE VENDER, KUNTHALA JAYARAMAN, AND H. V. RICKENBERG

Department of Bacteriology, Indiana University, Bloomington, Indiana

Received for publication 8 July 1965

ABSTRACT

VENDER, JOYCE (Indiana University, Bloomington), KUNTHALA JAYARAMAN, AND H. V. RICKENBERG. Metabolism of glutamic acid in a mutant of *Escherichia coli*. J. Bacteriol. 90:1304-1307. 1965.—A mutant strain of *Escherichia coli* W1485 was selected for its ability to utilize glutamic acid as the sole source of carbon. Growth of the mutant on glutamic acid led to the repression of glutamic acid dehydrogenase formation. The mutant differed from the wild-type strain in that glutamic decarboxylase activity was absent from the mutant under conditions of growth which supported the formation of this enzyme in the parent strain. Evidence is presented which suggests that loss of the decarboxylase activity results in the acquisition of the ability to utilize glutamic acid as sole source of carbon; a pathway of glutamate utilization via transamination is proposed.

Formation of glutamic acid by the reductive amination of α -ketoglutarate is generally accepted as the main pathway of incorporation of ammonia into α -amino nitrogen in coliform bacteria (see, for example, Umbarger and Davis, 1962). Because of its pivotal role in the formation of most of the nitrogen-containing constituents of *Escherichia coli*, it was of interest to determine how glutamate metabolism was controlled and whether glutamic dehydrogenase was involved in the catabolism as well as in the biosynthesis of glutamate. Adler et al. (1938) found only nicotinamide adenine dinucleotide phosphate (NADP)-linked glutamic dehydrogenase in *E. coli*; this finding was confirmed by us in preliminary studies.

Halpern and Umbarger (1961), in studying ammonia metabolism in *E. coli*, noted that wild-type strains of this organism could not grow on glutamate as sole source of carbon. These authors selected mutants capable of utilizing glutamate as sole source of carbon, and concluded that the mutation had enhanced the permeability of the mutants to glutamate; they also noted that the mutants had partially or completely lost glutamic decarboxylase activity.

The isolation of similar mutants is reported in the present study, and evidence is presented which suggests that the mutation leading to the loss of glutamic decarboxylase activity endows the bacteria with the ability to utilize glutamate as sole source of carbon. Findings reported here also indicate that two distinct pathways are employed in the biosynthesis and the degradation of glutamate by *E. coli*.

MATERIALS AND METHODS

Bacteria and conditions of growth. The strains of *E. coli* employed in this investigation were W1485 (Lederberg, 1951) and W1485^G. The mutant W1485^G, which could utilize glutamate as sole source of carbon, was isolated from W1485 by continued incubation of W1485 in the mineral salts medium containing glutamate as sole source of carbon; no mutagenic treatment was employed. Bacteria were grown in Monod's medium 56 (Monod, Cohen-Bazire, and Cohn, 1951); glutamate, when used, was autoclaved together with the medium at a concentration of 10^{-2} M; glycerol and glucose were autoclaved separately and added aseptically to medium 56 to final concentrations of 2×10^{-2} and 10^{-2} M, respectively. Cultures were grown with aeration at 37°C. Bacterial density was measured turbidimetrically in a Klett-Summerson colorimeter with the use of the no. 66 filter. Routinely, bacteria were harvested in the exponential phase of growth.

Preparation of cell-free extracts. Washed bacteria suspended in distilled water were treated for 20 min in a Raytheon 10-kc magnetostrictive oscillator.

Enzyme assays. Glutamic dehydrogenase activity was determined spectrophotometrically by following the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH₂) at 340 m μ at room temperature. To a 1-ml cuvette were added 90 μ moles of tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.5), 40 μ moles of (NH₄)₂SO₄, 6.7 μ moles of α -ketoglutarate, 0.15 μ mole of NADPH₂, and bacterial extracts containing between 5 and 500 μ g of protein. Aspartase activity was assayed by incubating, at 37°C in a volume of 2 ml, 100 μ moles of Tris (pH 7.5), 5 μ moles of L-aspartate, 5 μ g of MnCl₂, and bacterial extracts containing between 50 and 100 μ g of pro-

tein. The reaction was stopped by the addition of 0.2 ml of 50% trichloroacetic acid, and the ammonia produced was assayed colorimetrically by use of Nessler's reagent. Glutamic decarboxylase activity was determined manometrically at 30 C. Each Warburg vessel contained 45 μ moles of L-glutamate, 0.30 mg of pyridoxal phosphate, 180 mmoles of acetate buffer (pH 4.8), and approximately 2 mg of bacterial extract protein, in a total volume of 3.2 ml. Control flasks contained no glutamate. Glutamic-aspartic transaminase was assayed according to the procedure of Gunsalus and Stamer (1955). The ability of γ -aminobutyric acid (γ -ABA) to transaminate with oxaloacetate was determined by substituting a mixture of 0.5 mmole of γ -ABA, 0.01 mmole of oxaloacetate, and 0.5 μ mole of pyridoxal phosphate for the aspartate in the aspartase assay mixture and determining the amount of ammonia formed as described above. Protein was determined as described by Lowry et al. (1951).

Uptake of glutamate by bacteria. The uptake of glutamate was measured in an incubation mixture consisting of 5×10^8 bacteria per milliliter suspended in 0.05 M sodium phosphate buffer (pH 7.0) containing 50 μ g of chloramphenicol per ml. The suspension was shaken for 10 min at 37 C, and uniformly labeled L-glutamic acid (Isotopes Specialties Co., Burbank, Calif.) was added to a final concentration of 5×10^{-4} M. Samples were withdrawn at various time intervals, and were filtered rapidly through membrane filters (Millipore HA, 0.45 μ , 25 mm). The bacteria on the filter were washed with five 0.2-ml portions of ice-cold buffer, and the filters were glued to planchets for counting. The radioactivity was determined in a Nuclear-Chicago gas flow counter.

Paper chromatographic analyses. At intervals, 2.0-ml portions of the bacterial suspension were filtered, and the filtrates were collected individually. The bacteria on the filters were then extracted repeatedly with hot 75% ethyl alcohol, and the extracts were evaporated to dryness. The dry residues were taken up in water, and were desalted by passage through Dowex-50 (H^+ form, 6 by 1 cm); the amino acids were eluted with 2 N ammonium hydroxide. The eluates were concentrated and chromatographed on Whatman no. 1 paper by use of two solvent systems: *n*-butanol-acetic acid-water (4:1:1, v/v) and water-saturated *n*-butanol-formic acid (95:5, v/v). The amino acids were identified by ninhydrin and the keto-acids, by semicarbazide. After development of the chromatograms, 1.27 cm² pieces were cut out and eluted with 75% ethyl alcohol, and the radioactivities were determined. The filtrates were similarly desalted and analyzed by paper chromatography.

RESULTS

Glutamic dehydrogenase and aspartase activities. The presence of glutamate in the growth medium stimulated the formation of aspartase and repressed glutamic dehydrogenase formation (Table 1). Thus, when the mutant was grown in

TABLE 1. *Growth rates, and glutamic dehydrogenase and aspartase activities of strains W1485 and W1485^G*

Strain	Source of carbon	Generation time	Glutamic dehydrogenase activity*	Aspartase activity†
W1485	Glycerol	90	670	19
W1485	Glutamate	No growth	—	—
W1485	Glycerol and glutamate	75	300	45
W1485 ^G	Glycerol	90	650	48
W1485 ^G	Glutamate	72	120	620

* Expressed as millimicromoles of NADPH₂ oxidized per minute per milligram of protein.

† Expressed as millimicromoles of NH₃ formed per minute per milligram of protein.

TABLE 2. *Glutamic decarboxylase activity of strains W1485 and W1485^G*

Strain	Source of carbon	Specific activity*
W1485	Glycerol	317
W1485	Glucose	270
W1485 ^G	Glycerol	None detected
W1485 ^G	Glutamate	None detected

* Expressed as millimicromoles of CO₂ evolved per minute per milligram of protein.

the presence of glutamate, aspartase activity was 13-fold higher than after growth in the absence of glutamate. Growth in the presence of glutamate, however, repressed glutamic dehydrogenase activity fivefold. In the wild-type strain, the effect of glutamate was less pronounced with respect to both enzymes than in the mutant.

Glutamic decarboxylase. Table 2 shows that no glutamic decarboxylase activity was found in the mutant strain when grown under conditions which led to the formation of this enzyme in the wild-type bacteria.

Glutamic-aspartic transaminase. Both mutant and wild-type strains were found to contain glutamic-aspartic transaminase activity. Neither mutant nor wild-type extracts were capable of utilizing the γ -amino group of γ -ABA for the amination of oxaloacetate to aspartate.

Uptake of glutamate. Uptake of uniformly labeled glutamate by the bacteria of the wild-type and mutant strains is shown in Fig. 1. The accumulation of radioactivity was greater in the mutant than in the wild-type strain.

The distribution of the intracellular radioactivity derived from the labeled glutamate was analyzed in both strains. It did not change sig-

nificantly over a 20-min period of incubation. In the extracts of wild-type bacteria, only glutamate and γ -ABA were found to be labeled, whereas in the extracts of the mutant bacteria little, if any, γ -ABA was detected; but, in addition to glutamate, radioactivity was found to be associated with a number of other amino acids (Fig. 2). Furthermore, significant amounts of labeled α -ketoglutarate were detected in the mutant but

FIG. 1. Accumulation of radioactivity derived from uniformly labeled glutamate present in the incubation mixture at a concentration of 5×10^{-4} M; 600,000 counts/min correspond to 0.5 μ mole of the original L-glutamate. Symbols: Δ , strain W1485^G grown in medium containing glutamic acid as sole source of carbon; $\bullet$, strain W1485 grown in medium containing glycerol as sole source of carbon.

FIG. 2. Distribution of radioactivity derived from glutamate in extracts of strain W1485 (wild type) and W1485^G (mutant). The experimental procedure is described in the text; 600,000 count/min correspond to 0.5 μ mole of the original L-glutamate. The values given correspond to the radioactivity of extracts derived from 5×10^8 bacteria.

TABLE 3. Radioactivity in bacteria and medium after incubation with uniformly labeled glutamate*

Strain	Time of incubation	Radioactivity in bacteria	Radioactivity in medium		
			Total	Glutamate	γ -ABA
1485	min				
	3	1,160	133,000	114,000	2,000
	10	1,560	123,500	75,000	29,900
1485 ^G	20	2,100	135,500	31,000	34,800
	3	12,920	112,000	72,000	206
	10	22,200	93,250	57,000	600
	20	36,600	55,250	48,000	830

* The concentration of glutamate in the medium was 0.5 μ mole/ml, and the radioactivity was 600,000 counts per min per ml. Results are expressed as counts per minute per 10^8 bacteria and as counts per minute per 2 ml of medium.

not in the wild-type extracts (not shown in figure).

The distribution of total radioactivity in the bacteria and medium is given in Table 3. γ -ABA appeared in significant amounts only in the medium in which the wild-type strain had been grown.

DISCUSSION

The conclusions drawn from the findings reported here on the role of NADP-linked glutamic dehydrogenase and of aspartase in glutamate metabolism in *E. coli* are in general agreement with the conclusions of Halpern and Umbarger (1960). The fact that growth on glutamate stimulated aspartase and repressed glutamic dehydrogenase activity suggests that aspartase has a degradative, and glutamic dehydrogenase a biosynthetic, role. Aspartase can, however, also participate in biosynthesis. This is shown by the existence of a mutant of *E. coli* (Vender and Rickenberg, 1964) which could utilize ammonia as sole source of nitrogen, although it had lost glutamic dehydrogenase.

The results of the experiment on the uptake of glutamate (Fig. 1) show that glutamate is taken up by both wild-type and mutant strains. The differences in the rates of accumulation of radioactivity are most readily explained on the basis of differences in the rates of metabolism of glutamate and not on the basis of accumulation. This interpretation is strengthened by the fact that, even after only 1 min of incubation with glutamate, there are already significant differences between wild-type and mutant strains with respect to the distribution of the intracellular radioactivity.

The finding (in both this investigation and in Halpern and Umbarger's) that the strains which utilized glutamate as sole source of carbon lacked or had reduced levels of glutamic decarboxylase activity strongly suggests that the functioning of the decarboxylase prevents the utilization of glutamate as sole source of carbon. Halpern and Umbarger (1959) considered the possibility that glutamic decarboxylase prevented the utilization of glutamate either by producing an inhibitor or by lowering the effective intracellular concentration of glutamate. This hypothesis was later abandoned by the same authors in favor of the permeability hypothesis (Halpern and Umbarger, 1961).

Our data are compatible with the hypothesis that the utilization of glutamate as sole source of carbon is prevented by the presence of glutamic decarboxylase activity. In the wild-type strain, glutamate is converted to γ -ABA which cannot be utilized as sole source of carbon by this strain. [Succinic semialdehyde, one of the products of the transamination between γ -ABA and a keto acid, is, however, oxidized by a constitutive succinic semialdehyde dehydrogenase (Schubert and Rickenberg, *unpublished data*).] It has been observed (Vender and Rickenberg, *unpublished data*) that *E. coli* extracts have the ability to transaminate γ -ABA with α -ketoglutarate but not with oxaloacetate. The transamination of γ -ABA with α -ketoglutarate yields glutamic acid which is then irreversibly decarboxylated to regenerate γ -ABA. Evidently this sequence of reactions will not lead to any net gain of Krebs cycle intermediates.

It is proposed that in the mutant, which lacks glutamic decarboxylase activity, glutamate is catabolized by transamination with oxaloacetate to form α -ketoglutarate, and aspartate. The aspartate is then deaminated to fumarate. This interpretation is strengthened by the finding that aspartase activity is induced as a result of growth in the presence of glutamate. Conversely, the fact that growth in the presence of glutamate represses glutamic dehydrogenase activity suggests that the role of that enzyme is indeed biosynthetic. Finally it is worthy of note that in the mutant described here a *loss* mutation (loss of glutamic decarboxylase) has led to the *gain* of

a new metabolic potential (ability to utilize glutamate as sole source of carbon).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant 05868 from the National Institute of Arthritis and Metabolic Diseases. J. V. was a Public Health Service Trainee (Training Grant 20-503) from the Division of General Medical Science, Public Health Service.

The competent technical assistance of Antonina Adams and Eileen Smith is gratefully acknowledged.

LITERATURE CITED

- ADLER, E., V. HELLSTROM, G. GÜNTHER, AND H. v. EULER. 1938. Über den enzymatischen Abbau und Aufbau der Glutaminsäure. III. In *Bacterium coli*. Z. Physiol. Chem. **255**:14-26.
- GUNSALUS, I. C., AND J. R. STAMER. 1955. Transaminases in bacteria, p. 170-177. In S. P. Colowick and N. O. Kaplan [ed.], *Methods in enzymology*, vol. 2. Academic Press, Inc., New York.
- HALPERN, Y. S., AND H. E. UMBARGER. 1959. Glutamic decarboxylase, the limiting factor in the utilization of glutamate as a carbon source by *Escherichia coli*. Bacteriol. Proc., p. 122.
- HALPERN, Y. S., AND H. E. UMBARGER. 1960. Conversion of ammonia to amino groups in *Escherichia coli*. J. Bacteriol. **80**:285-288.
- HALPERN, Y. S., AND H. E. UMBARGER. 1961. Utilization of L-glutamic and 2-oxoglutaric acid as sources of carbon by *Escherichia coli*. J. Gen. Microbiol. **26**:175-183.
- LEDERBERG, E. M. 1951. Lysogenicity in *Escherichia coli* K-12. Genetics **36**:560.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. **193**:265-275.
- MONOD, J., G. COHEN-BAZIRE, AND M. COHN. 1951. Sur la biosynthèse de la β -galactosidase (lactase) chez *Escherichia coli*. La spécificité de l'induction. Biochim. Biophys. Acta **7**:585-599.
- UMBARGER, E., AND B. D. DAVIS. 1962. Pathways of amino acid biosynthesis, p. 167-251. In I. C. Gunsalus and R. Y. Stanier [ed.], *The bacteria*, vol. 3. Academic Press, Inc., New York.
- VENDER, J., AND H. V. RICKENBERG. 1964. Ammonia metabolism in a mutant of *Escherichia coli* lacking glutamic dehydrogenase. Biochim. Biophys. Acta **90**:218-220.