Threonine as a Carbon Source for *Escherichia coli*

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Threonine can be used aerobically as the sole source of carbon and energy by mutants of *Escherichia coli* K-12. The pathway used involves the conversion of threonine via threonine dehydrogenase to aminoketobutyric acid, which is further metabolized by aminoketobutyric acid ligase, forming acetyl coenzyme A and glycine. A strain devoid of serine transhydroxymethylase uses this pathway and excretes glycine as a waste product. Aminoketobutyric acid ligase activity was demonstrated after passage of crude extracts through Sephadex G100.

Escherichia coli K-12 in its usual form is not efficient in the catabolism of threonine. Threonine catabolism could, a priori, supply the cell with glycine, nitrogen, and carbon or energy or both, and it does so in many organisms (1, 3, 6, 7, 10, 15). However, in *E. coli* K-12, endogenously synthesized threonine does not supply glycine (6), and even exogenous threonine provides physiologically significant amounts of glycine only when the level of L-threonine dehydrogenase (EC 1.1.1.103) (TDH) has been raised severalfold by induction or mutation (12, 13).

Threonine does not serve as a nitrogen source even in strains with increased TDH levels unless the strain is also capable of using glycine as a nitrogen source (11). This supports the idea that any quantitatively important threonine catabolism proceeds via TDH to α-amino-β-ketobutyrate (AKB) and thence to acetyl coenzyme A (CoA) and glycine via AKB ligase, a pathway which is also used in *Arthrobacter* spp. (7) and *Pseudomonas* sp. (15).

If this pathway were correct, a strain which could use threonine as a nitrogen source should also be able to use it, via acetyl-CoA, as a source of carbon and energy. As shown in this paper, this is essentially correct. Our derivative of *E. coli* K-12 is deficient in serine transhydroxymethylase (STHM) and can, therefore, do very little with glycine. It is shown to contain ligase activity and to convert threonine to glycine and acetyl-CoA, using the acetate as a source of carbon and energy and excreting much of the glycine into the medium as a by-product of growth.

MATERIALS AND METHODS

Media and cultures. Strain JEV73R is a prototrophic strain of *E. coli* K-12 that is deficient in STHM and is able to derive its glycine from threonine (6). Strain AT2046, also deficient in STHM, was obtained

from L. I. Pizer. Strain K10, our prototrophic F⁺ strain of *E. coli* K-12, was obtained from A. Garen. All cultures were grown in the minimal medium previously described (6) supplemented with carbon sources as indicated in the text.

Enzyme assays. AKB ligase was assayed by the method of McGilvray and Morris (8), using 40 mM glycine and 0.4 mM acetyl-CoA as substrates and measuring the aminoacetone formed by decarboxylation of the AKB produced. This represents a lower concentration of glycine than usual. All preparations of glycine give a considerable color with Ehrlich reagent. The sensitivity of the assay is, therefore, greater at lower concentrations of glycine even though these are below the saturating level. Phosphotransacetylase was determined as the glycine-independent deacylation of acetyl-CoA, measuring the CoA formed by its reaction with 5,5'-dithio-bis-(2-nitrobenzoic acid) (4).

TDH. TDH was assayed in crude extracts by the colorimetric method of McGilvray and Morris (7) as described previously (13). In partially purified preparations in which endogenous NADH oxidase activity could be avoided, a spectrophotometric method based on the threonine-dependent reduction of NAD was used (1).

STHM was measured by the Folk and Berg (5) modification of the method of Taylor and Weissbach (14) as previously described (9). Desalting of culture filtrates was carried out by the method of K. Thitlow (personal communication). Culture filtrate (25 ml; pH adjusted to 2) was applied to a Dowex-50 (H⁺) column (1.2 by 10 cm) equilibrated with 0.1 N acetic acid. The column was washed with 10 ml of 0.1 N acetic acid, and the amino acids were eluted with 0.1 M ammonium hydroxide.

RESULTS

Relation between acetate and threonine metabolism in *E. coli* K-12. Previous studies from this laboratory have suggested that the use of threonine as a source of glycine involves the action of an enzyme which simultaneously forms two products, acetyl-CoA and glycine. If this is true, strains able to use threonine as a source of

glycine should also be able to use it, via acetyl-CoA, as a source of carbon. One could test this by selecting acetate-nonutilizing mutants and showing that they do not use threonine. However, strain JEV73R (like many of our laboratory strains of *E. coli* K-12, data not shown) has the convenient property of being unable to use acetate as a carbon source and also cannot use threonine as a carbon source. It should thus be possible to isolate acetate-utilizing strains, and they should use threonine.

Ten independent acetate-utilizing strains (nine spontaneous, one UV treated) were isolated from strain JEV73R by plating on minimal medium with acetate as the sole source of carbon. All of these strains proved able to use threonine as a carbon source, with doubling times from 2.5 to 4 h.

In a reciprocal experiment, nine independent threonine-utilizing strains were isolated. Eight of these, including strain TE111, were able to use acetate. The other, strain TE11, was not. The most frequent mutational event leading to the use of threonine as a carbon source restored acetate metabolism. However, as in the case of strain TE11, the cell could gain the ability to use threonine by establishing some other pathway.

To further confirm this, strain TE111A, a TE111 derivative unable to use acetate as a carbon source, was isolated by penicillin selection. Since this strain was unable to use threonine as a carbon source, it seems clear that the use of threonine as a carbon source in *E. coli* K-12 involves its conversion to glycine and acetyl-CoA.

Excretion of glycine during growth with threonine as a carbon source. Strain TE111 was derived from an STHM-deficient parent strain, JEV73R, and is itself deficient in STHM, as would be expected (data not shown). If strain TE111 converts threonine to acetyl-CoA and glycine, it should accumulate a great deal of glycine and would presumably excrete it into the medium.

To examine this, strain TE111 was grown to the stationary phase, using threonine as the carbon source. The medium was separated from the cells by centrifugation, desalted on Dowex-50, concentrated by lyophilization, and chromatographed in the following three solvent systems: (i) (two-dimensional) *n*-butanol-acetone-ammonium hydroxide-water (10:10:5:2, vol/vol) followed by isopropanol-formic acid-water (20:1:5, vol/vol); (ii) (one-dimensional, silica gel thin-layer chromatography) ethanol-water (7:3, vol/vol); (iii) (one-dimensional, paper) phenol-ammonium hydroxide-water (80:0.3:20, vol/vol). In all cases, a ninhydrin-positive spot at the position of glycine was detected.

That the spot was glycine was also indicated by bioassay, using as the test organism strain AT2046, which responds to glycine but not to threonine (9). The supernatant of threonine-grown cultures of strain TE111 supported growth of strain AT2046.

The culture filtrate of mid-log-phase cultures was examined for amino acid content in the same way. In this case, two ninhydrin-positive spots were detected, corresponding to threonine and glycine in roughly equal amounts.

We conclude then that during growth on threonine, strain TE111 accumulated glycine and excreted it into the medium.

Yield of strain TE111 on different carbon sources. Strain TE111 can grow with glucose, threonine or acetate (among others) as the carbon source. With threonine as the carbon source, it should produce one molecule of acetyl-CoA, which it can use as the carbon and energy source, and one molecule of glycine, which it cannot. Its yield on threonine then should be only half as much per unit of carbon as that on acetate, although the two yields would be equivalent if expressed per mole of substrate used.

To confirm this, strain TE111 was grown with 0 to 0.5 mg of glucose per ml, 0 to 3 mg of threonine per ml, and 0 to 0.4 mg of acetate per ml, these amounts having been shown to be within the range that limits growth. Cultures were monitored until their turbidity was constant for at least 4 h, and the protein contents were determined on portions of trichloroacetic acid precipitates of cell material redissolved in NaOH.

Under these conditions, the protein content of the cells at the stationary phase was proportional to the amount of carbon source provided (Table 1). From this relationship, it can be calculated that for strain TE111 to produce 1 mg of protein, it had to use 93 μmol of threonine, 95 μmol of acetate, or 40 μmol of glucose.

Use of threonine by an STHM-positive derivative of TE111. Strain TE111 cannot use

TABLE 1. *Derivation of cell material of TE111 from limiting carbon sources*

Threonine		Acetate		Glucose	
μmol provided	mg of protein synthesized	μmol provided	mg of protein synthesized	μmol provided	mg of protein synthesized
0	0.6	0	0		
160	2.5	48.7	0.46	22	1.1
320	4.7	97.0	1.03	44	1.6
480	5.1	146.0	1.66	66	2.2
640	7.6	195.0	2.0	88	3.0
1,000	10.0			110	3.3

either threonine or glycine as the source of nitrogen because it is deficient in STHM (11). We selected an STHM-positive derivative of TE111, strain TE111RR, by looking for a colony which used glycine as the nitrogen source.

Strain TE111RR growing on threonine can metabolize the glycine it produces. To determine whether this ability to dispose of glycine affected the metabolic efficiency of the organism, the yield per micromole of threonine of strain TE111RR was compared with that of strain TE111. Whereas strain TE111 required 93 μmol of threonine to produce 1 mg of cell material, strain TE111RR required only 41. This increased yield, as compared with that for strain TE111, suggests that some glycine can be of general metabolic use when threonine is the principal carbon source, even though glycine alone does not support growth of either TE111 or TE111RR.

Demonstration of AKB ligase activity. The preceding evidence suggests that the use of threonine as the carbon source involves its conversion to acetyl-CoA and glycine by enzymes TDH and AKB ligase.

The conversion of threonine to glycine by *E. coli* has already been shown to involve TDH as the first step, producing AKB as its product (12). One would then expect that the next step would be the formation of glycine and acetate, catalyzed by AKB ligase (EC 2.3.1.29), cleaving threonine to form glycine and acetyl-CoA. This enzyme has been demonstrated in other organisms (7, 15), and its existence in *E. coli* has been predicted (12) but not directly demonstrated.

To show ligase activity, the assay was performed in the reverse direction with acetyl-CoA and glycine, forming CoA and AKB. Both CoA production and AKB production were assessed: CoA by the formation of a yellow color in the presence of 5,5'-dithio-bis-(2-nitrobenzoic acid) (4) and AKB via the reaction of its decarboxylation product, aminoacetone, with Ehrlich reagent (8).

The formation of aminoacetone from acetyl-CoA and glycine could not be demonstrated by these methods in crude extracts. However, acetyl-CoA is known to be readily degraded by enzymes such as phosphotransacetylase (E.C.2.3.1.8) (2). If this were present, it would deplete the acetyl-CoA concentration so rapidly that ligase activity might not be detected.

That this is, in fact, the case was demonstrated by chromatography of crude extracts on a Sephadex G100 column equilibrated with 0.1 M KCl in 0.05 M phosphate buffer (pH 7.2). Three relevant enzyme activities, ligase, TDH, and phosphotransacetylase, were detected (Fig. 1). TDH was found in fractions 35 through 40, ligase
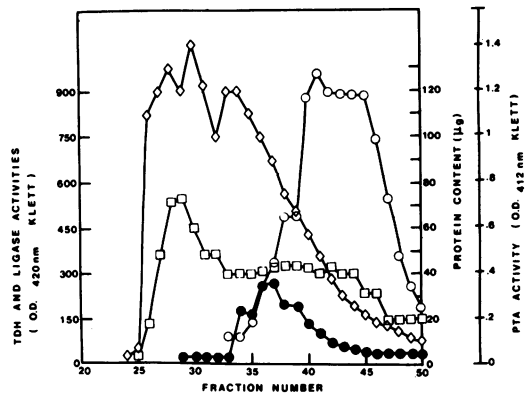

FIG. 1. *Elution of TDH and ligase. A crude extract of strain JEV73R was eluted from Sephadex G100 with 0.05 M phosphate buffer (pH 7.2) containing 0.1 M KCl. Fractions were assayed for ligase (O), TDH (●), glycine-independent deacylation of acetyl-CoA (◇), and protein (□). O.D.$_{412\,nm}$, Optical density at 412 nm.*

was found in fractions 40 through 50 (corresponding to a molecular weight of about 79,000), and an activity catalyzing the glycine-independent formation of a yellow 5,5'-dithio-bis-(2-nitrobenzoic acid) derivative, presumably phosphotransacetylase, appeared in the void volume.

It is clear then that extracts of strain JEV73R contained ligase activity. In crude extracts, this activity could not be detected by the particular methods used, presumably because there were at lease two enzymes which acted upon acetyl-CoA, the ligase, and the phosphotransacetylase. When both were present, the transacetylase depleted the acetyl-CoA concentration so rapidly that ligase activity could not be demonstrated. However, the two activities separated readily on the Sephadex G100 column, and ligase activity was then clearly demonstrable.

DISCUSSION

The experiments reported in this paper support the conclusion that an STHM-deficient derivative of *E. coli* K-12 can use threonine as the carbon source by converting it to glycine and acetyl-CoA via TDH and AKB ligase and by using acetate, but not glycine, as the source of carbon and energy for general cell metabolism (Fig. 2).

E. coli K-12 in its usual form does not degrade endogenously synthesized threonine. Even exogenously provided threonine will not serve as a source of glycine unless leucine is also provided, apparently as inducer of threonine cleavage (6).

However, a quantitatively significant degradation of endogenous threonine can be estab-
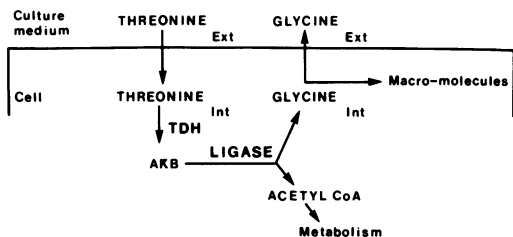
FIG. 2. *Proposed pathway for the use of threonine as carbon source by E. coli K-12. Ext, External; Int, internal.*

lished by a series of mutations, resulting in a strain (JEV73R) that makes its glycine entirely from endogenously synthesized threonine, and this without leucine being provided (6). Since JEV73R has a 100-fold increase in TDH activity (12), it seems clear that the strain synthesizes glycine from aspartate via threonine and TDH. JEV73R does not use either acetate or threonine as the carbon source. However, our demonstration that acetate-using derivatives also use threonine makes it clear that strain JEV73R degrades threonine by a two-enzyme pathway, forming glycine and acetyl-CoA.

The strain used in this study is deficient in STHM, so that glycine represents a metabolic dead end except insofar as it can be degraded to CO_2 and C_1 THF. A strain selected for its ability to use glycine as the nitrogen source (and therefore likely STHM positive [11]) derived considerably more material per micromole of threonine. Since TE111RR differs from TE111 only in its ability to derive nitrogen from glycine, it must be able to convert significant amounts of glycine carbon, perhaps via STHM and serine, into an energy-yielding set of reactions.

The TDH ligase pathway has also been demonstrated in *Pseudomonas cepacia* (15). This organism grows with threonine as the carbon source. However, mutants deficient in either TDH or ligase cannot use threonine (15). Wong and Lessie also showed that mutants deficient in L-serine deaminase are unable to use the TDH ligase pathway (15). It seems then that *E. coli* K-12, after several mutations, uses the same pathway as prototrophic *P. cepacia*.

The TDH ligase pathway is not the only pathway by which microorganisms degrade threonine. Indeed, one of our strains acquired the ability to use threonine by some as yet unidentified other pathway as judged by its using threonine without being able to use acetate. *Pseudomonas putida* grows on threonine by way of aldolase cleavage to acetaldehyde and glycine (10), as do various other *Pseudomonas* species

(1). Moreover, selection for the use of threonine as the nitrogen source resulted, in *Salmonella typhimurium*, in a threonine deaminase (biosynthetic) with altered regulatory properties (3).

LITERATURE CITED

1. Bell, S. C., and J. M. Turner. 1977. Bacterial catabolism of threonine. Threonine degradation initiated by 1-threonine acetaldehyde-lyase (aldolase) in species of *Pseudomonas*. Biochem. J. 166:209–216.

2. Brown, T. D. K., M. C. Jones-Mortimer, and H. L. Kornberg. 1977. The enzymic interconversion of acetate and acetyl-coenzyme A in *Escherichia coli*. J. Gen. Microbiol. 102:327–336.

3. Burns, R. O., J. G. Hoffer, and G. H. Luginbuhl. 1979. Threonine deaminase from *S. typhimurium*. Substrate-specific patterns of inhibition in an activator-site deficient form of the enzyme. J. Biol. Chem. 254:1074–1079.

4. Ellman, G. L. 1955. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82:70–77.

5. Folk, W. R., and P. Berg. 1970. Isolation and partial characterization of *Escherichia coli* mutants with altered glycyl transfer ribonucleic acid synthetase. J. Bacteriol. 102:193–203.

6. Fraser, J., and E. B. Newman. 1975. Derivation of glycine from threonine in *Escherichia coli* K-12 mutants. J. Bacteriol. 122:810–817.

7. McGilvray, D., and J. G. Morris. 1969. The utilization of L-threonine by a species of *Arthrobacter*: a novel catabolic role for "aminoacetone synthase." Biochem. J. 112:657–671.

8. McGilvray, D., and J. G. Morris. 1971. Aminoacetone synthase *(Arthrobacter)*. Methods Enzymol. 17B:585–589.

9. Miller, B. A., and E. B. Newman. 1974. Control of serine transhydroxymethylase in *Escherichia coli* K-12. Can. J. Microbiol. 20:41–47.

10. Morris, J. G. 1969. Utilization of L-threonine by a *pseudomonad*: a catabolic role for L-threonine aldolase. Biochem. J. 115:603–605.

11. Newman, E. B., G. Batist, J. Fraser, S. Isenberg, P. Weyman, and V. Kapoor. 1976. The use of glycine as nitrogen source by *Escherichia coli* K-12. Biochim. Biophys. Acta 421:97–105.

12. Newman, E. B., V. Kapoor, and R. Potter. 1976. Role of L-threonine dehydrogenase in the catabolism of threonine and synthesis of glycine by *Escherichia coli*. J. Bacteriol. 126:1245–1249.

13. Potter, R., V. Kapoor, and E. B. Newman. 1977. Role of threonine dehydrogenase in *Escherichia coli* threonine degradation. J. Bacteriol. 132:385–391.

14. Taylor, R. T., and H. Weissbach. 1965. Radioactive assay for serine transhydroxymethylase. Anal. Biochem. 13:80–84.

15. Wong, H. C., and T. G. Lessie. 1979. Hydroxy amino acid metabolism in *Pseudomonas cepacia*: role of L-serine deaminase in the dissimilation of serine, glycine, and threonine. J. Bacteriol. 140:240–245.