JOURNAL OF BACTERIOLOGY, June 1976, p. 1245-1249
Vol. 126, No. 3

Printed in U.S.A.

Role of L-Threonine Dehydrogenase in the Catabolism of Threonine and Synthesis of Glycine by *Escherichia coli*

E. B. NEWMAN,* V. KAPOOR, AND R. POTTER

Department of Biological Sciences, Concordia University, Sir George Williams Campus, Montreal, Quebec, Canada

Received for publication 25 March 1976

The enzyme L-threonine dehydrogenase was demonstrated in extracts of *Escherichia coli* K-12, and was shown to be the first enzyme of the pathway converting threonine to glycine. The enzyme was induced by L-leucine, but not by its substrate, L-threonine. The metabolic significance of leucine as a catabolic signal for amino acid degradation is considered.

Escherichia coli K-12 normally derives its glycine from L-serine. However, in certain conditions it can derive some or all of its glycine from L-threonine (5). Though this conversion of threonine to glycine has been demonstrated by mutant and tracer studies, the enzyme(s) involved has not yet been characterized.

Four threonine-metabolizing enzymes have been described in microorganisms. Two of these, the biosynthetic and degradative threonine deaminases (TDs), convert threonine to ketobutyrate (20). Threonine aldolase cleaves it to form acetaldehyde and glycine (2). L-Threonine dehydrogenase (TDH), the fourth enzyme, oxidizes threonine in a nicotinamide adenine dinucleotide (NAD)-linked reaction to form α-amino-β-ketobutyrate (AKB) (4). The AKB can then be cleaved by a second enzyme known variously as aminoacetone synthase (10) or glycine-acetyl coenzyme A ligase (22) to form glycine and acetyl coenzyme A.

In this paper, we demonstrate that it is in fact the fourth enzyme, TDH, which is involved in the formation of glycine in *E. coli* K-12. The enzyme is demonstrated in our strain, and the amounts of enzyme synthesized are shown to coincide with the extent of derivation of glycine from threonine in the various nutritional conditions previously described (5). Factors in the regulation of TDH activity are also considered.

MATERIALS AND METHODS

Cultures. Strains K-10, AT2046, AT2046T, JEV73, JEV73R, and CU1008 are all strains of *E. coli* K-12 (Table 1) and have been previously described (5), as have growth conditions. All cultures were grown in synthetic medium with 0.2% glucose unless otherwise stated. Other additions are noted in the text.

Enzyme assays. TD was assayed according to the method of Pardee and Prestidge (15), with slight modifications described earlier (7). Threonine aldolase was assayed by the method of Yamada et al. (23), and the aldehyde formed was measured by the colorimetric method of Paz et al. (16). TDH assays were made with mid-log phase cells, harvested by centrifugation, and washed once with cold, unsupplemented medium. The cells were suspended at 20% (wt/vol) in 0.01 M potassium phosphate (pH 7.2) and treated for five 30-s periods with an MSE 100-W ultrasonic disintegrator. Extracts were clarified by centrifugation at 7,000 $\times$ *g* for 20 min. The enzyme was assayed by the method of McGilvray and Morris (11) in 3-ml volumes using L-threonine at 0.03 M and NAD at 5×10^{-4} M. The reaction was started with the addition of enzyme and stopped with 0.8 ml of cold 30% trichloroacetic acid. Protein was removed by centrifugation, and 1-ml samples were assayed for aminoacetone by the rapid determination method of Urata and Granick (21) with the modified Ehrlich reagent of Mauzerall and Granick (9) using delta-aminolevulinic acid as a standard. Aminoacetone is not a direct product of the TDH reaction but is formed by the decarboxylation in acid of the actual product, AKB.

RESULTS

Demonstration of TDH in *E. coli* K-10. Since previous studies have indicated that threonine is converted to glycine only when both threonine and leucine are present in the growth medium (5), TDH was assayed in extracts of cells grown with glucose, threonine (650 μg/ml), and leucine (200 μg/ml). Such extracts catalyzed the formation of an aminoketone in the presence of both threonine and NAD (Table 2, lines 1, 2, 3). Aminoketone formation was proportional to the amount of extract added (Table 2, lines 1, 4, 5) and proceeded linearly with time for more than 60 min (data not shown). The substrates L-threonine and NAD were shown to be saturating at the concentrations provided.

To determine the optimum pH for aminoketone formation, an extract prepared at pH

TABLE 1. *Strains of E. coli K-12 used*

Strain	Description	Growth factors required
K-10	Prototroph	None
AT2046	Serine transhydroxymethylase-deficient auxotroph	Glycine; or threonine and leucine (see text)
AT2046T	Threonine-utilizing derivative of AT2046	Glycine or threonine
JEV73	Leucine-utilizing derivative of AT2046T	Glycine or threonine or leucine
JEV73R	Prototrophic derivative of JEV73	None
CU1008	*ilvA* mutant	Isoleucine

TABLE 2. *Aminoketone formation by extracts of E. coli K-10 grown with threonine and leucine*

Expt	Substrates added[a]	Amt extract added (ml)	μmol aminoketone formed/10 min
1	L-threonine, NAD	0.04	0.087
2	L-threonine	0.04	0.004
3	NAD	0.04	0.001
4	L-threonine, NAD	0.02	0.045
5	L-threonine, NAD	0.01	0.023

[a] L-threonine was supplied at 0.03 M; NAD was added at 5×10^{-4} M.

7.2 in 0.05 M phosphate buffer was diluted in phosphate, tris(hydroxymethyl)aminomethane, and diethanolamine buffers at pH 6.5 to 10.0, and incubated for 10 min. The enzyme proved to be active over a wide range of pH with an optimum above pH 9. Assays were routinely performed at pH 8.5.

To assess the reliability of the assay, several different extracts were prepared from different batches of cells grown with threonine and leucine, and the specific activity was determined, using delta-aminolevulinic acid as a standard. On eight different extracts, the specific activity varied from 0.016 to 0.021 μmol of aminoketone formed per min per mg of protein, or an average of 0.0186 μmol.

Various compounds were tested as possible effectors of the enzyme. These were L-leucine, L-isoleucine, L-glutamic acid, L-glutamine, glycine, L-serine, and L-valine, all tested at one-tenth the concentration of the substrate, L-threonine. L-leucine and glycine were also tested at the same concentration as threonine. None of these compounds altered the activity measured.

Regulation of TDH synthesis in *E. coli* K-10. According to the ^{14}C data presented in an earlier paper (5), threonine is converted to glycine only when both threonine and leucine are present in the growth medium. To see whether TDH as measured in vitro follows the same pattern as [U-^{14}C]threonine conversion to glycine in vivo, extracts were made from cells grown in minimal medium with glucose (i) with no further supplement; (ii) with threonine, 650 μg/ml; (iii) with leucine, 200 μg/ml; and (iv) with both threonine and leucine.

As can be seen in Table 3, TDH is induced by the presence of leucine in the medium; this induction is somewhat increased if threonine is also present. Threonine alone does not induce. The in vivo and in vitro results are consistent except insofar as leucine-grown cells have increased levels of TDH but do not appear to derive glycine from threonine.

In these studies, TDH is viewed as the first enzyme of a pathway converting threonine to glycine, and thus might be subject to control. It has already been seen that glycine does not inhibit the enzyme (see above). To test for repression of enzyme synthesis, cells were grown with threonine (650 μg/ml), leucine (200 μg/ml), and glycine (200 μg/ml). Extracts of these cells showed the usual enzyme levels (0.018 μmol/min per mg of protein). Since *E. coli* K-10 does not readily transport glycine (12), the cells were also grown with a higher level of glycine (2 mg/ml) and the usual levels of threonine and leucine. TDH level again fell within the usual induced range (0.021 μmol/min per mg of protein).

TDH in mutants of *E. coli* strain AT2046. In our previous study, we demonstrated that the ability of cells to derive glycine from threonine could be altered not only by nutrition, but also by mutation. To see whether the ability to synthesize TDH followed the phenotypes described, each mutant was grown in various media and assayed for TDH.

It can be seen from Table 4 that the variations in TDH activity are sufficient to explain the nutritional phenotypes of the strains. Strain AT2046T makes increased "constitutive" levels of TDH: the activity in this strain when grown with glycine is higher than that seen in

TABLE 3. *Activity of TDH in cells of E. coli K-10 grown with possible inducers*

Amino acid supplement	TDH sp act (μmol aminoketone/min per mg protein)	Avg
Threonine, leucine	0.0207, 0.0184, 0.019, 0.017 0.016, 0.018, 0.021, 0.019	0.0186
Leucine	0.015, 0.015	0.015
Threonine	0.0025, 0.0032	0.0028
None	0.0025, 0.003, 0.0028	0.0028

strain K-10 grown with leucine and threonine. Strain JEV73 has the same levels as AT2046T, but the activity is even higher during growth with leucine. Strain JEV73R has high levels of TDH even when grown on minimal medium, though leucine induces yet higher levels.

It is clear then that the changes in TDH are those predicted on the basis of the phenotypes described earlier. Indeed, the enzyme level in glycine-grown JEV73R is about 60-fold higher than in the starting strain AT2046.

Now strain AT2046 does not grow with threonine as a source of glycine. If TDH is induced by leucine in all strains of *E. coli* K-12, one would expect AT2046 to grow with a combination of threonine and leucine in place of glycine. This was tested and found to be true.

TDH in a mutant unable to synthesize TD. That TDH is the enzyme responsible for glycine synthesis from threonine is clearly indicated by the preceding data. However, to be absolutely certain that the enzyme assayed here is different from the biosynthetic TD, a strain carrying a deletion in *ilvA*, the gene for TD, strain CU1008, was grown with isoleucine, or with isoleucine, threonine, and leucine, and assayed for TDH. The strain showed activity of 0.0054 μmol/min per mg when grown with isoleucine only, and 0.012 μmol/min per mg when grown with isoleucine, threonine, and leucine. It thus clearly does synthesize TDH. Activity of TD was undetectable in both cases.

Attempts to assay threonine aldolase. In the course of this work, many attempts have been made in our laboratory to demonstrate threonine aldolase, most of them with strain JEV73. None has been successful. We wish to mention in particular the assay based on the use of *N*-methylbenzothiazolone hydrazone (MBTH) (16). Though this assay has been used in screening for threonine aldolase (23), it should be noted that it is not specific for aldehydes, but reacts also with other carbonyl groups, including keto acids. Thus, when extracts of leucine-grown strain JEV73 were assayed for threonine-dependent formation of MBTH-reactive material, a considerable amount of activity was seen. However, 99+% of this activity could be inhibited by isoleucine and was attributed to TD. No significant isoleucine-resistant activity could be detected. It seems then that our strains do not produce threonine aldolase, and that the MBTH assay with isoleucine incorporated into it might be useful for a strain that did have threonine aldolase.

Other possible factors in TDH regulation. To test whether TDH is subject to catabolite repression, *E. coli* K-10 was grown with glycerol as carbon source, with and without threonine and leucine, and assayed for TDH. As can be seen in Table 5, the levels of TDH in glycerol-grown cells are little different from those in glucose-grown cells. The extent of increase in the presence of inducers is also unaltered.

The activity of L-serine deaminase is increased by growth without inorganic nitrogen (7). To see if the same is true for TDH, cells were grown in minimal medium with threonine and leucine as sole sources of nitrogen. This gave the same TDH activity (0.021 μmol/min per mg) as cells grown with ammonium sulfate (0.0186 μmol/min per mg, Table 5).

TABLE 4. *Activity of TDH in mutants of E. coli K-12*

Strain	Growth conditions	TDH sp act (μmol aminoketone/min per mg protein)	Avg
AT2046	Glycine	0.0023, 0.0039	0.0031
AT2046T	Glycine	0.025, 0.013, 0.015, 0.01	0.0157
	Threonine	0.026, 0.030	0.028
JEV73	Glycine,	0.023, 0.029	0.026
	Threonine	0.034, 0.035	0.035
	Leucine	0.194, 0.188	0.191
JEV73R	Glycine	0.156, 0.144	0.150
	Threonine	0.156, 0.127	0.142
	Leucine	0.382, 0.284	0.333
	No additions	0.217, 0.186	0.202

DISCUSSION

This paper consists of a demonstration that the enzyme responsible for the derivation of glycine from threonine is TDH and a description of some of the factors involved in setting the level of TDH activity in *E. coli* K-12.

Role of TDH in glycine production. That *E. coli* K-10 does synthesize TDH is indicated

TABLE 5. *Activity of TDH in cells of E. coli K-10 grown with various nutritional conditions*

Carbon source	Amino acids	Ammonium sulfate	TDH sp act (μmol aminoketone/min per mg protein)	Avg
Glucose		+		0.0028[a]
Glycerol		+	0.005, 0.003	0.004
Glycerol	Threonine, leucine	+	0.025, 0.021	0.023
Glucose	Threonine, leucine	+		0.0186[a]
Glucose	Threonine, leucine	−	0.017, 0.025	0.021

[a] Values taken from Table 3.

by the ability of extracts to catalyze formation of an aminoketone dependent on the presence of both L-threonine and NAD. This indicates that TDH converts threonine to AKB, which is decarboxylated in acid to form aminoacetone. This is the standard assay for TDH (19).

That this is the enzyme responsible for glycine production is indicated by two lines of evidence: (i) its induction pattern in prototrophic *E. coli* K-10 as contrasted with data published earlier on the metabolism of [^{14}C]-aspartate and [^{14}C]threonine, and (ii) its activity in a series of mutants of *E. coli* K-12.

(i) TDH activity is induced in *E. coli* K-10 by L-leucine; threonine does not induce, and its presence along with L-leucine increases TDH activity only slightly. In the earlier experiments (5), it was reported that cells grown with [^{14}C]threonine and leucine converted threonine to glycine; those grown with [^{14}C]threonine alone did not, nor did cells grown with [^{14}C]aspartate and leucine. These two sets of data are consistent if it is assumed that TDH will be active in vivo only when internal threonine pools are higher than in prototrophic cells grown without supplement. Thus, an increase in TDH is necessary but not sufficient for in vivo conversion of threonine to glycine.

(ii) The TDH activities measured in mutants of strain AT2046 are consistent with the ^{14}C data, and with the hypothesis made earlier as to the nature of the mutations (5). Viewed overall, it is clear that wherever glycine is derived from exogenously provided threonine, TDH levels are increased; wherever glycine is derived from endogenously synthesized threonine, TDH levels are increased even more, and L-leucine induces further increases in all strains.

Thus, TDH activity follows the pattern expected for an enzyme producing glycine from threonine, both in the prototroph, and in a series of mutants. TDH activity can be demonstrated in a deletion mutant unable to make TD. Furthermore, no attempt to demonstrate threonine aldolase in these strains has been successful. We conclude, therefore, that TDH is the first enzyme in the conversion of threonine to glycine. We think it likely that the second enzyme of the pathway would be acetyl coenzyme A-glycine ligase (10, 22), but this enzyme has not been assayed in our strains.

This is not the first report of TDH in *E. coli*. It was demonstrated in extracts of *E. coli* B/2 (6) and further characterized in another strain of *E. coli* as an NAD-linked enzyme with a optimum pH of 9.6 (19). However, no clear idea of the metabolic role of this enzyme in *E. coli* has emerged. It has been postulated to be the first step in the biosynthesis of vitamin B12 (14, 18). It has also been considered as part of a degradative pathway for both threonine and glycine, indeed, as part of a cycle for glycine oxidation (4).

Factors in the control of threonine catabolism. So far in this discussion, TDH has been examined primarily in its biosynthetic role as a source of glycine. We suggest, however, that this is not its major role, and that it actually functions as the first step in a threonine degradation pathway, its product AKB being cleaved to form glycine, and the glycine degraded by conversion to serine (E. B. Newman, G. Batist, J. Fraser, S. Isenberg, P. Weyman, and V. Kapoor, Biochim. Biophys. Acta, in press). That this is so is indicated by the fact that glycine neither inhibits TDH nor represses its synthesis, and that threonine serves as a nitrogen source for *E. coli* only via the agency of TDH and the glycine degradation pathway (E. B. Newman, S. Azudis, R. Potter, and V. Kapoor, manuscript in preparation). The use of TDH for threonine catabolism is seen in several other microorganisms that use threonine as sole carbon and energy source, including an *Arthrobacter* (10), *Pseudomonas oxaliticus* (1), *P. multivorans* (8), and three strains of fusaria (22).

There are now three threonine-degrading enzymes known in *E. coli*: TD, biosynthetic, TD, catabolite repressed, and TDH, as described here. TD, biosynthetic, is inhibited totally by small amounts of isoleucine, and therefore cannot serve to degrade threonine to any quantitatively significant extent. TD, catabolite repressed, is used for threonine degradation by cultures grown without glucose, but is not made in the presence of glucose. Nonetheless, *E. coli* can degrade threonine even in the presence of glucose—as long as leucine is also present. The cell apparently finds it expedient to elaborate two enzymes for threonine catabolism, one that functions anaerobically in the absence of glucose, and the other that functions aerobically in the presence of glucose and leucine.

It has been known for some time that leucine signals for serine degradation (7, 15) and it also greatly stimulates glycine degradation (E. B. Newman, T. Adley, R. Potter, and V. Kapoor, manuscript in preparation). In *E. coli*, then, the presence of L-leucine signals the cell to degrade at least three amino acids, and it does so in the presence not only of glucose but also of ammonium sulfate. It seems then that *E. coli* has keyed its amino acid-degrading

ability not to its need for carbon, energy, or nitrogen, but to the availability of exogenous amino acids, as indicated by the results with external leucine. We suggested earlier that leucine may be the signal for this system precisely because it is one of the most metabolically inert amino acids, and lies at the end of a metabolic pathway, acting as precursor to no other cell component (except protein). This may be related to the origins of *E. coli* as an intestinal organism living in an environment with a good deal of organic material, amino acids included.

There is so far no evidence as to whether this increase in amino acid degradation is due to increased transcription or is post-transcriptional, or indeed post-translational in mechanism. It seems likely that the leucine signal is read entirely or in part by the enzyme leucyl-, phenylalanyl-transfer ribonucleic acid (tRNA)-protein transferase (transferase) (17). A mutant deficient in transferase has abnormal regulation of three enzymes now shown to be subject to leucine control: serine deaminase, TD, and TDH (C. Deutch, E. B. Newman, R. Scarpula, V. Kapoor, and R. L. Soffer, manuscript in preparation), and this regulation is restored to normal in a transferase-positive revertant. Similar results for another amino acid-degrading enzyme, proline oxidase, have also been reported (3).

This transferase enzyme may be postulated to function in either or both of two ways. The synthesis of amino acid-catabolizing enzymes may be under the control of a leucyl-tRNA signal, the amount of charged and uncharged tRNA being governed by transferase activity. On the other hand, the actual form of the enzymes may be covalently modified by acylation with leucine. The fact that the enzyme also recognizes phenylalanyl-tRNA indicates that a second class of enzymes under control of a phenylalanine signal may also exist.

ACKNOWLEDGMENTS

This work has been supported by grant no. A6050 of the NRC (Canada), for which we are profoundly grateful. The help of S. Azudis with many experiments is greatly appreciated. The contribution of D. W. and S. Heywood is thankfully noted by E. B. Newman.

LITERATURE CITED

1. **Blackmore, M. A., and J. M. Turner.** 1971. Threonine metabolism via two-carbon compounds by *Pseudomonas oxaliticus*. J. Gen. Microbiol. 67:243–246.
2. **Dainty, R. H., and J. L. Peel.** 1970. Biosynthesis of amino acids in *Clostridium pasteurianum*. Biochem. J. 240:3361–3371.
3. **Deutch, C. E., and R. L. Soffer.** 1975. Regulation of proline catabolism by leucyl, phenylalanyl-tRNA-protein transferase. Proc. Natl. Acad. Sci. U.S.A. 72:405–408.
4. **Elliott, W. H.** 1960. Aminoacetone formation by *Staphylococcus aureus*. Biochem. J. 74:478–485.
5. **Fraser, J., and E. B. Newman.** 1975. Derivation of glycine from threonine in *Escherichia coli* K-12 mutants. J. Bacteriol. 122:810–817.
6. **Green, M. L., and W. H. Elliott.** 1964. The enzymic formation of aminoacetone from threonine and its further metabolism. Biochem. J. 92:537–549.
7. **Isenberg, S., and E. B. Newman.** 1974. Studies on L-serine deaminase in *Escherichia coli* K-12. J. Bacteriol. 118:53–58.
8. **Lessie, T. G., and H. R. Whiteley.** 1969. Properties of threonine deaminase from a bacterium able to use threonine as sole source of carbon. J. Bacteriol. 100:878–889.
9. **Mauzerall, D., and S. Granick.** 1956. The occurrence and determination of delta-aminolevulinic acid and porphobilinogen in urine. J. Biol. Chem. 219:435–446.
10. **McGilvray, D., and J. G. Morris.** 1969. Utilization of L-threonine by a species of *Arthrobacter*: a novel catabolic role for "aminoacetone synthase." Biochem. J. 112:657–671.
11. **McGilvray, D., and J. G. Morris.** 1971. L-Threonine dehydrogenase, p. 580–584. *In* H. Tabor and C. W. Tabor (ed.), Methods in enzymology, vol. 17, part B. Academic Press Inc., New York.
12. **Miller, B., and E. B. Newman.** 1974. Control of serine transhydroxymethylase in *Escherichia coli* K12. Can. J. Microbiol. 20:41–47.
13. **Morris, J. G.** 1969. Utilization of L-threonine by a pseudomonad: a catabolic role for L-threonine aldolase. Biochem. J. 115:603–605.
14. **Neuberger, A.** 1961. Aspects of the metabolism of glycine and porphyrins. Biochem. J. 78:1–10.
15. **Pardee, A. B., and L. S. Prestidge.** 1955. Induced formation of serine and threonine deaminases by *Escherichia coli*. J. Bacteriol. 70:667–674.
16. **Paz, M. A., O. O. Blumenfeld, M. Rojkind, E. Henson, C. Furfine, and P. M. Gallop.** 1965. Determination of carbonyl compounds with *N*-methylbenzothiazole hydrazone. Arch. Bioch. Biophys. 109: 548–559.
17. **Soffer, R. L.** 1974. Aminoacyl-tRNA transferase, p. 91–139. *In* A. Meister (ed.), Advances in enzymology, vol. 40. John Wiley & Sons, Inc. New York.
18. **Turner, J. M.** 1966. Microbial metabolism of aminoketones: aminoacetone formation from 1-aminopropan-2-ol by a dehydrogenase in *E. coli*. Biochem. J. 99:427–432.
19. **Turner, J. M.** 1967. Microbial metabolism of aminoketones: 1-aminopropan-2-ol dehydrogenase and L-threonine dehydrogenase in *E. coli*. Biochem. J. 104:112–121.
20. **Umbarger, H. E., and B. Brown.** 1957. Threonine deaminases in *E. coli*. II. Evidence for two L-threonine deaminases. J. Bacteriol. 73:105–112.
21. **Urata, G., and S. Granick.** 1963. Biosynthesis of -aminoketones and the metabolism of aminoacetone. J. Biol. Chem. 238:811–820.
22. **Willets, A. J.** 1972. Metabolism of threonine by fusaria: growth on threonine as the sole carbon and nitrogen source. Antonie von Leeuwenhoek; J. Microbiol. Serol. 38:591–603.
23. **Yamada, H., H. Kumagai, T. Nagata, and H. Yoshida.** 1971. Formation of threonine aldolase by bacteria and yeasts. Agric. Biol. Chem. 35:1340–1345.