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# Role of Threonine Dehydrogenase in *Escherichia coli* Threonine Degradation

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Threonine was used as nitrogen source by *Escherichia coli* K-12 through a pathway beginning with the enzyme threonine dehydrogenase. The 2-amino-3-ketobutyrate formed was converted to glycine, and the glycine was converted to serine, which acted as the actual nitrogen donor. The enzyme formed under anaerobic conditions and known as threonine deaminase (biodegradative) is less widespread than threonine dehydrogenase and may be involved in energy metabolism rather than in threonine degradation per se.

Threonine catabolism has been studied in a variety of microorganisms for which threonine serves as a good carbon source. For example, aerobic growth with threonine as the sole source of carbon, as well as of nitrogen and energy, has been described for three *Fusarium* strains (11). Threonine is also used as both a carbon and energy source by *Pseudomonas multivorans* (2). In both of these cases, the first catabolic enzyme is threonine dehydrogenase (TDH). The 2-amino-3-ketobutyrate formed in the first step is then converted by a coenzyme A-linked lyase to glycine and acetyl-coenzyme A.

Threonine does not serve as the sole carbon and energy source for *Escherichia coli* K-12 and is not even a good nitrogen source. To the extent that it is degraded at all, two enzymes have been implicated: a deaminase known as biodegradative threonine deaminase (TD-B) (12) and a TDH (5).

TD-B has been extensively studied from a biochemical point of view, but relatively little is known about its function in cells (10). It is formed only in cells grown in very particular growth conditions (anaerobic, rich medium without glucose), requires adenosine 5'-monophosphate for activity, and is subject to catabolite repression (8, 12).

TDH is formed in cells grown in aerated synthetic medium with glucose; its activity is increased in cells grown with L-leucine as well. It can serve as the first enzyme in a pathway that forms glycine (1), but its actual function is thought to be degradative rather than biosynthetic (5).

The experiments in this paper are intended to assess the physiological roles of TDH and TD-B. To the extent that either enzyme is involved in threonine degradation, one might expect it to be involved in the provision of nitrogen from threonine. The paper then begins with a study of the use of threonine as a nitrogen source during growth in aerated synthetic medium. It then surveys anaerobic conditions under which each enzyme can be found in a variety of *E. coli* strains. The results indicate that TDH is properly considered to be a threonine-degrading enzyme, whereas the role of TD-B cannot be specified at present.

## MATERIALS AND METHODS

**Cultures.** Strains K10, AT2046, JEV73, and JEV73R are all strains of *E. coli* K-12 and have been previously described (1). Strain JEV73RR was isolated from strain JEV73R as described in this paper. Two prototrophic strains of *E. coli* K-12, strains CU4 and CU1, were obtained from H. E. Umbarger, Purdue University. Strain CU1008, containing an *ilvA* deletion, was obtained from L. S. Williams, Purdue University. *E. coli* Crookes was obtained from W. A. Wood, Michigan State University; and *E. coli* B was from D. Goldthwaite, Case Western Reserve University. Strain W3828 *cya-19* is a *cya* derivative of strain W3828, an *E. coli* K-12 prototroph, both obtained from E. McFall, New York University; strain *cya-238* is a *cya* derivative of *E. coli* K-12 isolated by J. Beckwith, Harvard University, and obtained from T. Newman, Purdue University.

**Media.** Cultures in synthetic medium were grown in the medium previously described (1) supplemented with 0.2% glucose for aerobic cultures and 0.5% glucose for anaerobic cultures. Cultures were also grown in a "rich medium" consisting of 2% tryptone, 1% yeast extract, and 0.5% dibasic potassium phosphate (13), with 0.2% glucose added where noted. Anaerobic conditions were assured by growing the cultures in filled 35-ml screw-cap vials.

**Enzyme assays.** TDH was assayed in extracts as

previously described (5). For a whole-cell assay, early-log-phase cells were harvested, washed, and suspended in 0.01 M tris(hydroxymethyl)aminomethane buffer (pH 8.5) at a turbidity of 450 Klett units (540 filter). To 0.3 ml of this cell suspension were added 0.1 ml of 0.3 M threonine, 0.1 ml of 0.025 M nicotinamide adenine dinucleotide, and 0.02 ml of toluene. This mixture was incubated for 20 min at 37°C. The reaction was stopped by adding 0.05 ml of 30% trichloroacetic acid, and the determination of aminoketones was carried out as in the assay in extracts (5).

TD-B was assayed by the method of Shizuta and Tokushige (9) in the presence of 0.01 M adenosine 5'-monophosphate. A whole-cell assay used log-phase cells suspended at 50 Klett units (540 filter) in 0.005 M potassium phosphate buffer (pH 7.4) containing 0.01 M adenosine 5'-monophosphate. A 0.3-ml portion of this suspension was incubated with 0.02 ml of toluene plus 0.1 ml of 0.5 M threonine for 20 min, when dinitrophenylhydrazine in hydrochloric acid was added, and the assay was continued as for the extracts (9). The assay was performed in triplicate, using controls with substrate and isoleucine ($2 \times 10^{-4}$ M) to avoid measuring biosynthetic TD as well as controls without substrate.

## RESULTS

**Use of threonine as a nitrogen source during aerobic growth. (i) Use of a combination of L-threonine and L-leucine as a nitrogen source for *E. coli* K-12.** *E. coli* K10 will use a combination of threonine and leucine as a nitrogen source but will not use either of them alone. Thus, when *E. coli* K10 was grown in glucose-synthetic medium with a slight excess of ammonium sulfate (500 μg/ml) and subcultured into a medium with a little ammonium sulfate (5 μg/ml) and either threonine (650 μg/ml) or leucine (100 μg/ml), no growth other than that attributable to ammonium sulfate was seen. When both threonine and leucine were provided, the cells grew slowly, with a doubling time of about 300 min.

The organism does not use equimolar amounts of threonine and leucine. To determine the optimal amounts of each, cells of *E. coli* K10 were grown with ammonium sulfate (500 μg/ml) and subcultured into a medium with various concentrations of threonine and leucine and with 0.2% glucose and allowed to grow until the culture turbidity remained constant for 12 h. As seen in Table 1, both amino acids are required. When either amino acid was provided at a constant concentration and the concentration of the other was varied, the final turbidity was proportional to the concentration of the one varied. The exception to this (threonine, 600 μg/ml; leucine, 100 μg/ml) may indicate that a balance between threonine and leucine is necessary. When leucine was provided at constant concentration and threonine was varied, the turbidity was proportional to the total nitrogen available. However, when threonine was provided at constant concentration and leucine was varied, it was not. It seems then that leucine is needed to permit the use of threonine but that most nitrogen comes from threonine.

**(ii) Ability of *E. coli* mutants to use threonine or leucine as a nitrogen source.** The preceding results are consistent with the hypothesis that the principal role of leucine in these cultures is to facilitate the derivation of nitrogen from threonine. Leucine is known to induce TDH, permitting the conversion of threonine to 2-amino-3-ketobutyrate (5). If the derivation of nitrogen from threonine required its conversion to glycine, the presence of leucine would be required for this conversion to take place. Since leucine is itself slowly deaminated during growth in the absence of ammonium sulfate (3), growth would stop when leucine was decreased by deamination to a concentration at which TDH was no longer induced.

If this hypothesis were true, one would expect that mutants altered either in the use of glycine as a nitrogen source or in the regulation

TABLE 1. *Effect of variations in threonine and leucine concentrations on the turbidity of E. coli cultures*

| L-Threonine (μg/ml) | L-Leucine (μg/ml) | (A) Total N provided (μg/ml) | (B) Final turbidity (Klett units) | B/A |
|---|---|---|---|---|
| 600 | 100 | 81.1 | 145 | 1.8 |
| 400 | 100 | 57.6 | 134 | 2.3 |
| 200 | 100 | 34.1 | 107 | 3.1 |
| 50 | 100 | 16.5 | 62 | 3.7 |
| 25 | 100 | 13.5 | 38 | 2.8 |
| 0 | 100 | 10.6 | NG[a] | |
| 600 | 200 | 91.7 | 198 | 2.1 |
| 600 | 50 | 75.8 | 125 | 1.6 |
| 600 | 10 | 71.6 | 55 | 0.8 |
| 600 | 0 | 70.5 | NG | |

[a] NG, No growth.

of TDH would also be altered in their use of threonine as a nitrogen source. That this is true can be seen, as follows.

(i) In *E. coli* K-12, glycine is used as nitrogen source exclusively by conversion to serine (4). This involves cleavage of some glycine to $C_1$ units, the condensation of these $C_1$ units with other molecules of glycine via the enzyme serine transhydroxymethylase to form serine, and the deamination of serine (Fig. 1). Strain AT2046, which lacks serine transhydroxymethylase and strain LESV3, which cannot convert glycine to $C_1$ units, are unable to use glycine as a nitrogen source (4). Neither strain uses threonine as a nitrogen source, with or without leucine (Table 2). Thus, a complete pathway for deriving nitrogen from glycine must function for threonine to serve as a nitrogen source.

(ii) Strain JEV73R, a derivative of strain AT2046, produces high levels of TDH in the absence of leucine but does not synthesize serine transhydroxymethylase (1). The high TDH level allows production of glycine from threonine, so that the strain does not require leucine to facilitate glycine production. However, this strain does not use threonine as a nitrogen source (Table 2), presumably due to the absence of serine transhydroxymethylase.

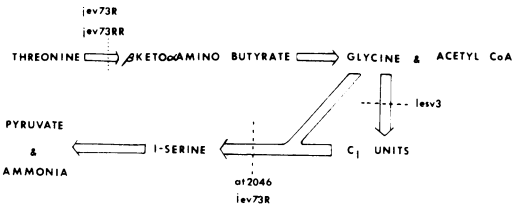


FIG. 1. *Proposed pathway for use of threonine as a nitrogen source. Mutations abolishing enzyme activity are indicated by dashed lines followed by the designation of strains carrying the particular mutation. The enzymes affected are glycine cleavage enzymes (lesv3) and serine transhydroxymethylase (AT2046, jev73R). Mutations increasing the activity of TDH are indicated by dotted lines followed by strain designations.*

From strain JEV73R, a strain, JEV73RR, that does synthesize serine transhydroxymethylase was selected (by plating with glycine as the sole nitrogen source after ultraviolet irradiation). It showed close to normal serine transhydroxymethylase levels (11 nmol of $H^{14}CHO$ per min per mg of protein as compared to 17 nmol in *E. coli* K10 and no detectable activity in strain JEV73R). This strain uses both glycine and threonine as a nitrogen source and uses the threonine without added leucine. It seems then that, when TDH activity is increased, leucine is no longer needed.

It can be concluded then that the use of threonine or leucine as a nitrogen source involves the degradation of threonine by TDH, an enzyme induced by L-leucine. To be absolutely certain that the biosynthetic, isoleucine-inhibited TD is not involved in the provision of nitrogen from threonine, strain CU1008, which carries a deletion in *ilvA* and cannot form that enzyme, was shown to be able to use threonine as a nitrogen source—again exclusively in the presence of leucine.

**(iii) Use of radioisotopes to determine the pathway of nitrogen derivation from threonine.** If the pathway by which threonine is used as a nitrogen donor involves its conversion to glycine and the use of glycine as nitrogen donor via serine, this should be clearly indicated by the appropriate isotope experiments. To confirm this, *E. coli* K10 was grown in glucose synthetic medium in the following conditions (Table 3): with threonine and ammonium sulfate; with threonine, leucine, and no other nitrogen source. These experiments were carried out in two sets, one with [*U*-$^{14}$C]threonine ($1.13 \times 10^4$ cpm/μmol; 650 μg/ml) (experiments 1 to 3) and the other with [*U*-

TABLE 2. *Ability of certain E. coli K-12 strains to use glycine and threonine as nitrogen sources*

| *E. coli* strain | Physiological alteration | Enzymatic alteration | Amino acid used as nitrogen source | | |
|---|---|---|---|---|---|
| | | | Threonine + leucine | Threonine alone | Glycine alone |
| K10 | | | + | − | +[a] |
| AT2046 | No glycine biosynthesis | STHM[−][b] | − | − | +[a] |
| LESV3 | No glycine cleavage | ? | − | − | −[a] |
| JEV73R | Alternative glycine biosynthesis | STHM[−], TDH | − | − | −[a] |
| JEV73RR | Normal glycine biosynthesis | STHM[+], TDH | + | + | + |
| CU1008 | No isoleucine biosynthesis | TD | + | − | + |

[a] Data taken from reference 4.
[b] STHM, Serine transhydroxymethylase.

TABLE 3. *Derivation of cell components from radioactive precursors*[a]

| Expt | Nitrogen source | Radioactive nutrilite | Sp act (cpm/μmol) | $C_{12}$ nutrilite(s) | Adenine sp act (cpm/μmol) | No. of C from $^{14}C$ | Labeled amino acid in hydrolysate | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Thr | Gly | Ser | Glu | Asp | Ala | Ilv |
| 1 | $(NH_4)_2SO_4$ | Threonine | 11,300 | Glucose | 2,025 | 0.7 | ++ | + | – | – | – | – | ++ |
| 2 | $(NH_4)_2SO_4$ | Threonine | 11,300 | Glucose, leucine | 4,850 | 1.7 | ++ | ++ | Tr | Tr | Tr | Tr | ++ |
| 3 | Thr, Leu | Threonine | 11,300 | Glucose, leucine | 11,800 | 4.1 | ++ | ++ | ++ | + | + | + | ++ |
| 4 | $(NH_4)_2SO_4$ | Glucose | 29,500 | Threonine | 18,350 | 3.7 | – | + | + | ++ | ++ | ++ | ++ |
| 5 | $(NH_4)_2SO_4$ | Glucose | 29,500 | Threonine, leucine | 14,975 | 3.0 | – | + | + | ++ | ++ | ++ | ++ |
| 6 | Thr, Leu | Glucose | 29,500 | Threonine, leucine | 6,450 | 1.3 | – | – | – | ++ | ++ | ++ | ++ |

[a] Tr, Trace. Hydrolysates were prepared as indicated in reference 4. "ilv" refers to an unresolved mixture of isoleucine, leucine, and valine.

$^{14}C$]glucose ($2.95 \times 10^4$ cpm/μmol; 0.2%) (experiments 4 to 6). In both cases nucleic acids and proteins were isolated from the cells, the specific activity of the adenine was determined, and radioautograms of the protein hydrolysates were examined visually.

These experiments gave a clear picture of threonine metabolism. As is indicated by a comparison of experiments 1 and 2 or 4 and 5, when ammonium sulfate was available as a nitrogen source, leucine addition somewhat increased the conversion of threonine to glycine. This would be expected from previously reported data from experiments with lower concentrations of threonine and leucine (1). Thus, leucine increased the derivation of adenine carbon from threonine (1.7 versus 0.7 carbons) and decreased the derivation from glucose (3.0 versus 3.7).

When ammonium sulfate was not available as a nitrogen source, a great increase in the derivation of adenine carbon from threonine occurred (4.1 versus 1.7). The maximum number of adenine carbons that can be derived from threonine is four (two as glycine and two as $C_1$). It seems then that all of the glycine is derived from threonine when threonine is serving as the nitrogen source. This is also indicated by the reciprocal experiments in which the contribution of glucose to the adenine carbon is drastically decreased in cells grown in the absence of ammonium sulfate (3.0 to 1.3).

Examination of radioautograms of the protein hydrolysates gave corresponding results. When ammonium sulfate and threonine were both present, glycine and serine were both derived from glucose (experiments 1 and 4). When leucine was also provided, but still with ammonium sulfate, some glycine was derived from threonine; however, all serine and some of the glycine was derived from glucose (experiments 2 and 5). When threonine and leucine were present as sole nitrogen sources, no radioactivity from glucose could be found in either serine or glycine, but both were heavily labeled from threonine (experiments 3 and 6).

In summary, these experiments indicate that, when threonine and leucine are not nitrogen sources, leucine induces some conversion of threonine to glycine, but most of the glycine is derived from glucose, presumably via serine. Where threonine and leucine are nitrogen sources, all of the glycine and all of the serine are derived from threonine. Thus, the isotope experiments, taken with the mutant studies, indicate that the use of threonine as nitrogen source requires the conversion of threonine through glycine to serine (Fig. 1).

**Role of TDH during anaerobic growth. (i) TDH and TD-B levels in various *E. coli* strains.** The previous sections have dealt with the pathway for the provision of nitrogen from threonine in aerobic cultures grown with glucose as a carbon source and have implicated TDH as the first enzyme in this pathway. However TD-B formation would not be expected in these growth conditions. It has been described only for cells grown under a very restricted set of conditions: anaerobic, rich medium without glucose. TDH on the other hand was never assayed in such cells. Therefore, various *E. coli* strains were grown anaerobically to mid-log phase in rich medium with and without glucose and assayed for both enzymes, using the whole-cell assay in both cases.

Where glucose was omitted from the medium, TD-B was made by four of the six strains tested (Table 4) and in rather high amounts (27 to 146 μmol of keto acid/20 min per mg of protein) with considerable variation in independent assays. That this is, in fact, TD-B activity is indicated by its dependence upon the presence of adenosine 5'-monophosphate, by its lack of sensitivity to isoleucine, and by the fact that none of the strains formed the enzyme when grown with glucose.

However, two *E. coli* prototrophs, CU4 and K10, did not produce TD-B even in the absence

TABLE 4. *Activity of TDH and TD-B in various E. coli strains grown in rich medium without aeration (whole-cell assay)*

| *E. coli* strain | TD-B (μmol of keto acid/20 min per mg of protein) | | TDH (μmol of amino ketone/20 min per mg of protein) | |
|---|---|---|---|---|
| | −Glucose | +Glucose | −Glucose | +Glucose |
| B | 37, 45, 146 | NDA[a] | 0.09, 0.06 | 0.12, 0.09 |
| W | 30, 47, 27 | NDA | 0.06, 0.12 | 0.08 |
| Crookes | 29, 80, 58 | NDA | 0.07, 0.11, 0.19 | 0.08, 0.08, 0.05 |
| K-12 | | | | |
| K-10 | NDA | NDA | 0.13, 0.07 | 0.09, 0.09 |
| CU4 | NDA | NDA | 0.13, 0.21 | 0.15, 0.19 |
| CU1 | 29, 79, 91 | NDA | 0.13, 0.11 | 0.11, 0.13 |

[a] NDA, No detectable activity.

of glucose. To be sure that this was not an artifact of the whole-cell assay, the enzyme was assayed in extracts of strains CU1 and CU1008, which showed TD-B activity, and strains CU4 and K10, which did not.

Every strain tested showed TDH activity when grown anaerobically in rich medium either with or without glucose. The amounts varied little (0.05 to 0.21 μmol of amino ketone per 20 min per mg of protein), with an average of 0.11 for cells grown with glucose and 0.12 for those without. These are slightly higher than enzyme levels in cells grown aerobically with synthetic medium and glucose but slightly lower than those in aerobic cultures induced with leucine (Table 5).

It seems then that the cell makes TDH to about the same extent under all growth conditions tested, even those in which TD-B is induced. The presence of glucose, which completely represses TD-B, has no effect on TDH. Thus, TDH is more widely distributed among organisms than TD-B, and its synthesis is regulated very differently. However, the activities of TDH are much lower than those of TD-B.

**(ii) Use of TDH in rich medium.** Anaerobic growth in rich medium without glucose clearly does not depend on the ability of the cell to make TD-B, even though the enzyme is present in large amounts under these conditions in the strains that do make it. Indeed, it is not obvious why the cell would degrade threonine at all in a medium plentifully supplied with other nutrients that it can degrade more readily.

It is difficult to elucidate the role of each enzyme in a mixture as complex and undefined as this. However, it is possible to determine whether TDH is actually used in the rich medium cultures, since its product is glycine. To the extent that TDH is used, carbon from threonine should be found in the glycine of cell protein.

TABLE 5. *Activity of TDH in three E. coli K-12 strains grown in synthetic medium aerobic with glucose (whole-cell assay) with and without leucine*

| Strain | TDH (μmol of amino ketone/mg of protein per 20 min) | |
|---|---|---|
| | −Leucine | +Leucine |
| K10 | 0.07 | 0.22 |
| CU4 | 0.06 | 0.14 |
| CU1 | 0.05 | 0.20 |

To test this, cells of three strains (CU4, CU1, and K10) were grown to mid-log phase in anaerobic rich medium without glucose and subcultured for 4.5 doubling times in 35 ml of rich medium to which 16.7 μCi of undiluted [*U*-$^{14}$C]threonine (specific activity, ca. 10 μCi/μmol) was added. The extent to which this threonine was diluted in the medium was not determined. Protein hydrolysates from harvested cells were subjected to chromatography and radioautography.

In all three cases, only two amino acids were radioactive, threonine and glycine. No radioactivity was seen in serine. Thus, whether TD-B was present or not, TDH was functioning. What use this was to the cell is not clear.

**(iii) Use of threonine as a nitrogen source in anaerobic synthetic medium.** If TDH were also involved in the provision of nitrogen from threonine in cells grown in anaerobic synthetic medium, one might expect that leucine would be required to allow the use of threonine anaerobically as well as aerobically. That this is so is indicated by the following experiment.

Two strains, CU1 and K10, were grown aerobically in glucose synthetic medium with ammonium sulfate in slight excess (500 μg) and

inoculated into anaerobic conditions with low amounts of ammonium sulfate (3 μg/ml) under the following five conditions: with threonine at 650 μg/ml; with threonine at 650 μg/ml and leucine at 100 μg/ml; with threonine and with glucose at 0.2%; with leucine and glucose; and with threonine, leucine, and glucose. Of these conditions, only the last permitted growth of either strain.

Without glucose, neither strain grew, whether leucine was present or not. With glucose present, both strains grew when provided both threonine and leucine; neither grew when leucine was omitted. Since neither strain could use leucine alone as nitrogen source, it seems clear that both relied on TDH to obtain nitrogen.

To verify this, mid-log-phase cells of both strains grown anaerobically with glucose, threonine, and leucine in synthetic medium with and without ammonium sulfate were assayed for TD-B and TDH activities. None showed TD-B activity; all showed TDH activity in amounts between 0.04 and 0.11 μmol of amino ketone per 20 min per mg of protein. That TD-B is not made is not surprising in view of the presence of glucose in the medium. In any case it is clear that in synthetic anaerobic medium, as in rich anaerobic medium, TDH is made and functions.

**Attempts to determine TD-B and TDH in mutants deficient in adenyl cyclase.** We have previously indicated that TDH does not seem to be subject to catabolite repression (5). To confirm this, strain *cya-238*, a *cya* mutant, was grown aerobically in minimal medium with and without leucine; it produced TDH inducible by leucine, as did all other strains tested.

The enzyme TD-B, however, has been reported to be subject to catabolite repression (8), and one would therefore expect it to be missing in a *cya* mutant. To test this, strain W3828 *cya-19* and its parent W3828 were grown in the usual medium for producing TD-B. Strain W3828, the *cya*$^+$ parent, grew well and formed the usual amounts of TD-B. However strain W3828 *cya-19*, the *cya* derivative, did not grow in the anaerobic rich medium without glucose. Overnight cultures in this medium invariably turned out to consist of *cya*$^+$ revertants as judged by fermentation of several sugars on MacConkey agar. The *cya* mutant was repeatedly reisolated in pure culture and retested with the same result. We conclude therefore that the absence of cyclic adenosine 5′-monophosphate prevents the use of whatever reactions are needed for anaerobic fermentations.

## DISCUSSION

This paper is concerned with two subjects: the elucidation of the pathway by which *E. coli* K-12 derives nitrogen from threonine; and the assessment of the physiological roles of two threonine-degrading enzymes, TD-B and TDH.

**Derivation of nitrogen from threonine.** Threonine serves as nitrogen source for *E. coli* only if leucine is also provided. The fact that leucine acts as inducer of TDH suggests that threonine serves as a nitrogen donor by conversion to glycine. Glycine would then serve as the actual nitrogen donor by the pathway described earlier (3), glycine conversion to serine and deamination of serine.

That this is indeed the pathway has been demonstrated by a consideration of the results of certain mutations on the use of threonine as nitrogen source and by radioactive tracer experiments. Threonine can be used as nitrogen source only if leucine is also provided. A mutant that cannot use glycine as a nitrogen source also cannot use threonine as a nitrogen source. Threonine then must be used by the same pathway as glycine. Furthermore, a mutant that synthesizes high levels of TDH without leucine in the growth medium can use threonine as a nitrogen source without leucine in the growth medium. This indicates that the principal role of leucine is to induce TDH. The same conclusions are reached by consideration of the isotope competition data. When the cell uses threonine as a nitrogen source, all of its glycine and all of its serine are derived from threonine. However, when ammonium sulfate is present as a nitrogen source, the cells obtain all of their serine and most of their glycine from glucose, with only a small amount of glycine being made from threonine. It seems then that the pathway from threonine through glycine to serine only becomes quantitatively important when threonine is used as nitrogen source. Threonine is not a very good source of nitrogen for *E. coli* K-12, but, to the extent that it serves as nitrogen source, it does so via conversion to glycine.

**Physiological roles of TDH and TD-B.** TD-B is a puzzling enzyme. Its role is particularly difficult to elucidate because it appears only in a complex, glucose-free, anaerobic medium. It is, however, not necessary for growth in this medium, since strains that do not form it (CU4, K10) grow just as well in it as strains that do (CU1).

On the other hand, the strains that do form TD-B make a great deal of it. An average value for TD-B would be 58 μmol of product

per 20 min per mg of protein as compared with 0.12 for TDH. This suggests that TD-B is more likely to be involved in energy metabolism or in nitrogen transfer than in handling threonine per se. The requirement of the enzyme for adenosine 5'-monophosphate and its sensitivity to catabolite repression would seem to support this. This idea for the role of TD-B was previously suggested by Umbarger, who viewed it as an enzyme that would provide a keto acid acceptor of amino groups that would be removed from other amino acids (10).

Whatever the role of TD-B may be, it is clear that the enzyme used to derive nitrogen from threonine is TDH. The first reaction in the pathway by which threonine is used as a nitrogen source, aerobically and anaerobically, is catalyzed by TDH. TDH is even made, and functions, in the one growth medium in which TD-B is made, and it is also made under these conditions in the two strains that do not make TD-B at all. It seems then that TDH is a constitutive enzyme in all *E. coli* strains studied, and its level changes little with growth conditions except insofar as its level increases when leucine is present in the growth medium or in certain mutants.

TDH is not controlled by the catabolite repression system as judged by the ability of adenylcyclase-deficient mutant (*cya*) to express TDH in normal amounts and to be induced by leucine normally. This was indicated earlier by the fact that TDH levels were not changed by growth in glycerol and succinate as opposed to glucose (5). TD-B seems to be sensitive to catabolite repression, since it is not made at all in the presence of glucose. However, the inability of a *cya* mutant to grow under conditions that induce TD-B made this impossible to test directly.

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