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CATABOLISM OF TAURINE IN *PSEUDOMONAS AERUGINOSA*

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Summary

Cell-free extracts of taurine-grown *Pseudomonas aeruginosa* catalyze the transamination of taurine and pyruvate resulting in the formation of L-alanine and sulfoacetaldehyde. The enzyme responsible for this activity has been partially purified in order to demonstrate its participation in a pathway of taurine degradation. Ethyl methane sulfonate treatment of *Ps. aeruginosa* yielded a mutant deficient in taurine transaminase and incapable of growing on taurine indicating that the enzyme is of physiological significance in this organism.

Introduction

Taurine (2-aminoethanesulfonate) is an amino acid analogue of β-alanine and occurs in nature as a result of cysteine catabolism [1]. In animal tissues and in urinary excretions taurine is present in both free and bound forms [2]. Despite its presence in many animal tissues, animals are unable to metabolize taurine. Therefore, its physiological significance is unknown although some hypothetical roles have been put forward [3]. On the other hand, a select few microorganisms have been found capable of utilizing taurine as the sole source of carbon, nitrogen, sulfur, and energy [4]. In *Agrobacterium* [5] and in an unidentified Gram-negative rod [6] it appears that the first step in taurine degradation is oxidative deamination, while in *Pseudomonas sp.* F-126 [7] and in *Achromobacter superficialis* [8] the initial reaction occurs by transamination. The amino acceptor in *Pseudomonas sp.* F-126 and *Achromobacter superficialis* is pyruvate and α-ketoglutarate, respectively. There are also implications

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that *Pseudomonas fluorescens* may possess a taurine : pyruvate aminotransferase [9], but on the basis of relative activity for various amino donors this enzyme may be different than that reported for *Pseudomonas sp.* F-126. Concurrent with the transamination investigations with pseudomonads has been our findings that a *Pseudomonas aeruginosa* strain, isolated by enrichment culture from manure, also possesses a taurine : pyruvate aminotransferase which catalyzes the formation of L-alanine and sulfoacetaldehyde from pyruvate and taurine. We have further shown this enzyme to be of physiological significance to the organism since a mutant deficient in this activity is incapable of growing on taurine as the sole source of carbon, nitrogen, or sulfur. It may also be possible that this enzyme is different from that of *Pseudomonas sp.* F-126 and *Ps. fluorescens* since β-alanine could not induce taurine : pyruvate aminotransferase in *Ps. aeruginosa*, but could act as an inducer in the other two strains.

Materials and Methods

Cultural. The organism used in this study was a *Ps. aeruginosa* strain, designated TAU-5, which could grow on taurine as the sole source of carbon, nitrogen, sulfur, and energy. The organism was isolated by the enrichment culture technique [10] using minimal medium containing (w/v): 1% taurine (filter-sterilized)/0.45% K_2HPO_4/0.10% KH_2PO_4/0.05% $MgCl_2 \cdot 6H_2O$/0.05% NaCl/ 10^{-6}% $FeCl_3 \cdot 6H_2O$ and $2 \cdot 10^{-7}$ $CaCl_2 \cdot 2H_2O$ (pH 7.5). This medium (T medium) was also used for routine growth of the bacteria. Cells were grown by inoculation from agar slopes containing T medium into 2-l Erlenmeyer flasks containing 1 l of T medium. The cultures were incubated vigorously at 37° C on a rotary shaker. Exponential-phase cultures were harvested, washed, and cell-free extracts prepared by passage through a French pressure cell as previously described [11] except that cells were extracted into 0.02 M potassium phosphate buffer (pH 8.0) containing 10^{-5} M pyridoxal 5′-phosphate and 0.01% (w/v) 2-mercaptoethanol. This buffer was also used for partial enzyme purification and dialysis of cell-free extracts. For enzyme assays, mutant strains (derived from TAU-5) unable to grow on taurine were grown initially in nutrient broth; then exponential-phase cultures were diluted with an equal volume of T medium and were incubated on a shaker at 37° C for 8 h. This time period was found to be optimal for induction of taurine : pyruvate aminotransferase in the parental strain. In studies testing the induction of the enzyme by compounds other than taurine, a concentration of 1% (w/v) of the test compound was used in place of taurine as the sole carbon source in T medium. For compounds not containing sulfur and/or nitrogen, the T medium was supplemented with 0.05% (w/v) $MgSO_4$ and/or 0.5% (w/v) NH_4Cl.

Mutagenesis. Mutagenesis of the parental strain, TAU-5, was accomplished by use of the ethyl methane sulfonate method of Fargie and Holloway [12]. Mutant enrichment was done using a final concentration of 4 mg/ml of carbenecillin and 10^7 cells/ml in T medium. Cells were screened from differential agar plates containing T medium with 0.1% (w/v) nutrient broth, 0.5% (w/v) taurine, 0.002% (w/v) Bromthymol blue, 0.002% (w/v) Bromcresol purple, and a 30-fold reduction of KH_2PO_4 and K_2HPO_4 (pH 7.5). Prototrophic colonies became yellow after 48 h at 37° C due to acid production from taurine metab-

olism [13] and the subsequent color change of the pH indicators from blue to yellow [14]. Mutant colonies, growing on the nutrient broth, are translucent with a blue background. Mutant strain TR-3, taurine : pyruvate transaminase negative, had the same biotype as the parental strain by API criteria [15]. Strain TR-3R, a spontaneous revertant of strain TR-3, was obtained by plating large numbers of mutants on the differential medium lacking nutrient broth and then selecting yellow colonies after 48 h.

Analytical. L-Alanine was assayed and identified using L-alanine dehydrogenase [16] and by paper chromatography [17]. Sulfoacetaldehyde was assayed and identified using o-aminobenzaldehyde [18] and by paper chromatography before and after derivation to the 2,4-dinitrophenyl-hydrazone [19,6]. Taurine was assayed by the method of Sörbo [20] and by paper chromatography [17]. Pyruvate was assayed using lactate dehydrogenase [21]. Protein assays were done by the Bio-Rad method [22] using bovine serum albumin as a standard. Taurine : pyruvate aminotransferase was partially purified 5-fold with 75% recovery by batchwise treatment of cell-free extracts with DEAE-cellulose (0.02 M potassium phosphate buffer, pH 8.0, containing 10^{-5} pyridoxal 5′-phosphate and 0.01% (w/v) 2-mercaptoethanol) and eluting the activity stepwise with NaCl from 0.15 M to 0.20 M in the same buffer.

Results

Enzyme activity in cell-free extracts

In a previous study we demonstrated taurine-dependent oxygen consumption and sulfate formation by resting cells of *Ps. aeruginosa* TAU-5 induced by growth on taurine as the sole source of carbon, nitrogen, sulfur, and energy [13]. However, subsequent experiments to detect the ability of cell-free extracts from taurine-grown organisms to catalyze oxidation of taurine in the

TABLE I

FORMATION OF L-ALANINE AND SULFOACETALDEHYDE BY TAURINE : PYRUVATE AMINOTRANSFERASE FROM TAURINE-GROWN *PSEUDOMONAS AERUGINOSA* TAU-5

The complete reaction mixture (1.0 ml) contained 100 μmol of potassium phosphate buffer (pH 8.0), 50 μmol of taurine, 50 μmol sodium pyruvate, 1 μmol pyridoxal 5′-phosphate, and 1.5 mg of enzyme. The missing reagent in controls was replaced by water. After 30 min at 30°C, the reaction was terminated by heating at 100°C for 5 min and the denatured protein removed by centrifugation. The amount of L-alanine and sulfoacetaldehyde formed were then assayed using L-alanine dehydrogenase and o-aminobenzaldehyde, respectively. In the case of paper chromatographic assays the reaction was terminated by addition of 0.1 ml of 25% trichloroacetic acid. One unit of activity is defined as that amount of enzyme required to catalyze formation of 1 μmol of product per min.

Assay	Product formed (μmol/ml)	
	L-Alanine	Sulfoacetaldehyde
Complete	3.15	3.10
Minus taurine	0.00	0.00
Minus pyruvate	0.00	0.00
Minus pyridoxal 5′-phosphate	3.12	3.10
2 × [Enzyme]	6.10	6.14
Boiled or acid denatured enzyme	0.00	0.00

absence and presence of various electron acceptors failed using manometric and oxygen electrode methods. However, cell-free extracts did contain a taurine : pyruvate aminotransferase activity. This was demonstrated by incubating dialyzed cell-free extracts with taurine and pyruvate in the presence of pyridoxal 5′-phosphate followed by assay for the L-alanine and sulfoacetaldehyde produced (Table I). The activity was dependent upon cell-free extract, pyruvate, and taurine. Glyoxylate and α-ketoglutarate could not substitute for pyruvate although L-alanine was detected when oxalacetate was substituted for pyruvate. Presumably this occured because of the presence of oxaloacetate decarboxylase activity (detected manometrically) which resulted in pyruvate formation. The reaction showed no dependence upon the presence of pyridoxal 5′-phosphate. The assay was linear under the time period and substrate concentrations used since a 2-fold increase in the enzyme concentration yielded twice as much product formation. The stoichiometry of the reaction was established using partially purified enzyme. The ratio of reactants to products was 1 : 1 : 1 : 1 of taurine : pyruvate : L-alanine : sulfoacetaldehyde.

Mutant analysis

Intracellular metabolite and taurine : pyruvate aminotransferase levels for various strains of *Ps. aeruginosa* are shown in Table II. Mutant TR-3, unable to grow on taurine, lacked the transaminase activity while revertant TR-3R concomitantly regained this activity and the ability to grow on taurine as sole source of carbon, nitrogen, sulfur, and energy. ATCC strain 19660 could not grow on taurine as a source of carbon, nitrogen, or sulfur and did not accumulate intracellular taurine or sulfoacetaldehyde, but did show a small amount of intracellular alanine present. Mutant TR-3 accumulated intracellular taurine at significantly higher levels than in the other strains showing that

TABLE II

LEVELS OF TAURINE : PYRUVATE AMINOTRANSFERASE AND METABOLITES IN VARIOUS STRAINS OF *PSEUDOMONAS AERUGINOSA*

Determination of intracellular levels of taurine, L-alanine, and sulfoacetaldehyde was performed using cells harvested from 4 l of overnight nutrient broth cultures and inoculating them into 1 l of T medium without taurine followed by incubation for 5 h at 37°C on a rotary shaker. Taurine was then added to a concentration of 1% (w/v) and incubation continued for an additional 8 h at which time the cells were harvested, washed with 0.85% NaCl, and weighed (all operations were conducted at 0—5°C). The cells were then extracted with 10 ml of 80% ethanol per gram of packed cells. The suspension was centrifuged, the pellet washed twice with water, and the supernatant fluids pooled and then evaporated to dryness with a rotary vacuum evaporator. The resulting residue was dissolved in 0.5 ml of water and then clarified by centrifugation. The amount of taurine, L-alanine, and sulfoacetaldehyde were then determined. Taurine : pyruvate aminotransferase was assayed as described in Table I.

Strain	Growth on taurine *	Taurine : pyruvate aminotransferase spec. act. (U/mg protein)	Metabolite (μmol/g (wet wt.) cells)		
			Taurine	L-Alanine	Sulfoacetaldehyde
TAU-5	+	0.070	0.208	0.054	0.025
TR-3	—	0.000	6.210	0.006	0.000
TR-3R	+	0.065	0.224	0.056	0.019
ATCC Strain 19660	—	0.000	0.000	0.006	0.000

* +, wild-type growth in 24 h; —, no growth in 72 h.

taurine can still enter the cell, but is not transaminated due to the mutation. The mutant also possessed lower levels of alanine, but no sulfoacetaldehyde as compared to the parental and revertant strains which is consistent with a loss of the transaminase in the mutant strain. A second class of mutants has also been isolated which exhibits taurine : pyruvate aminotransferase activity, but do not grow on taurine (possibly due to a defect in taurine transport).

Induction of enzyme activity

Various compounds were tested as inducers of the transaminase. Of the following compounds only taurine acted as an inducer: β-alanine, citrate, acetate, glyoxylate, pyruvate, taurine, DL-alanine, cysteine, serine, asparagine, aspartic acid, and nutrient broth. Cells grown in the presence of β-alanine exhibited β-alanine : pyruvate aminotransferase activity similar to that reported for *Pseudomonas sp.* F-126 [7], but not taurine : pyruvate aminotransferase. On the other hand, cells grown on taurine showed no β-alanine : pyruvate aminotransferase activity.

Discussion

These data provide evidence for the presence of taurine : pyruvate aminotransferase in cell-free extracts of taurine-grown *Ps. aeruginosa* TAU-5 that is essential for taurine catabolism. This activity required the addition of both taurine and pyruvate to the reaction mixture containing enzyme. Pyridoxal 5′-phosphate did not enhance enzyme activity although this cofactor may have already been bound to the enzyme. The inability to detect taurine oxidase activity in cell-free extracts is consistent with the absence of oxidative deamination of taurine and with the transaminase having a primary function in taurine catabolism.

Taurine : pyruvate aminotransferase activity was observed both with crude cell-free extracts and partially purified enzyme. The stoichiometry of the reaction showed an equal molar ratio of reactants to products. The further catabolism of the reaction products, L-alanine and sulfoacetaldehyde, is under current investigation and will be reported elsewhere. It is possible that the further metabolism of sulfoacetaldehyde and/or alanine account for the taurine-dependent oxygen consumption observed in earlier studies with resting cells of taurine-grown organisms.

A mutant strain (TR-3) missing the transaminase was unable to grow on taurine and had accumulated taurine intracellularly, whereas a revertant of this strain (TR-3R) regained the ability to grow on taurine and regained the transaminase activity, but did not accumulate taurine intracellularly. This data confirms the participation of taurine-pyruvate aminotransferase in taurine catabolism and establishes that it catalyzes a physiologically significant step of taurine degradation in this organism.

Since growth of *Ps. aeruginosa* TAU-5 in the presence of β-alanine did not induce taurine : pyruvate aminotransferase and growth on taurine did not induce β-alanine : pyruvate aminotransferase, it is possible that the enzyme we are reporting is different from that of ω-amino acid : pyruvate aminotransferase described by Yonaha et al. [7] and that of Hayaishi et al. [9] even

though pyruvate appears to be the exclusive amino acceptor.

In summary, the data of this report provides evidence for an instrumental step of taurine catabolism in *Ps. aeruginosa* TAU-5 that procedes as follows: taurine + pyruvate → L-alanine + sulfoacetaldehyde. Although other reports have appeared concerning this reaction in strains other than *Ps. aeruginosa*, this is the first report that provides genetic evidence to support its physiological significance. It is also possible that the enzyme catalyzing this reaction is different than similar ones described in previous reports. Future studies will concentrate on the metabolic fate of the sulfoacetaldehyde and L-alanine reaction products.

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