

Taurine-Sulfur Assimilation and Taurine-Pyruvate Aminotransferase Activity in Anaerobic Bacteria

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We demonstrated the ability of strictly fermentative, as well as facultatively fermentative, bacteria to assimilate sulfonate sulfur for growth. Taurine (2-aminoethanesulfonate) can be utilized by *Clostridium pasteurianum* C1 but does not support fermentative growth of two *Klebsiella* spp. and two different *Clostridium* spp. However, the latter are able to assimilate the sulfur of a variety of other sulfonates (e.g., cysteate, 3-sulfo-pyruvate, and 3-sulfolactate) anaerobically. A novel taurine-pyruvate aminotransferase activity was detected in cell extracts of *C. pasteurianum* C1 grown with taurine as the sole sulfur source. This activity was not detected in extracts of other bacteria examined, in *C. pasteurianum* C1 grown with sulfate or sulfite as the sulfur source, or in a *Klebsiella* isolate assimilating taurine-sulfur by aerobic respiration. More common aminotransferase activities (e.g., with aspartate or glutamate as the amino donor and pyruvate, oxalacetate, or α -ketoglutarate as the amino acceptor) were present, no matter what sulfur source was used for growth. Partial characterization of the taurine-pyruvate aminotransferase revealed an optimal temperature of 37°C and a broad optimal pH range of 7.5 to 9.5.

Sulfonates contain a sulfur atom covalently linked to one of carbon; the sulfur has an oxidation state of +5 (24). These compounds are widely distributed in nature as a result of biosynthesis by quite diverse biota, as well as chemical syntheses for commerce. Examples of naturally occurring sulfonates are taurine in vertebrate eyes and hearts (9, 11); isethionate in the squid giant axon (13); methanesulfonate, which is widely distributed in the atmosphere and deposited on the planet by rainfall (12); sulfolactate in *Bacillus* spores (2); sulfonolipids in the gliding bacteria *Cytophaga* sp. and *Flexibacter* sp. (8); aeruginosin in *Pseudomonas aeruginosa*; and coenzyme M in methanogenic archaea (20). Synthetic sulfonates include, for example, sodium dodecyl sulfonate (a detergent), the buffers HEPES and MOPS (morpholinepropanesulfonate), and some aromatic sulfonates in shampoos and household soaps (1, 3). Since these compounds are present in the environment but do not accumulate, it is expected that they are used by organisms for growth or otherwise biotransformed and that this is part of the global sulfur cycle.

Only relatively recently has it become clear that the sulfur of sulfonates can be assimilated by aerobic and fermentative bacteria even when the entire molecule cannot be utilized as a sole source of carbon and energy for growth (4, 17, 22). Although the sulfur of quite diverse sulfonates can be assimilated as a sole sulfur source by different microorganisms, including strictly aerobic bacteria (17, 22), facultatively anaerobic bacteria, strictly fermentative bacteria, and some yeasts (4, 23), the details of the metabolic pathway(s) for assimilation of sulfonate sulfur and the enzyme(s) involved in these metabolisms have not been elucidated.

Stapley and Starkey (19) indicated that for some soil microorganisms, utilization of cysteate and taurine might proceed first by deamination of these compounds. Ikeda et al. (10) also reported that removal of taurine's amino group might first

occur when taurine was used as a carbon and energy source by a putative *Agrobacterium* sp. Although it cannot serve as a carbon, energy, and nitrogen source, taurine (2-aminoethanesulfonate) can be utilized as a sole sulfur source for growth by *Clostridium pasteurianum* C1 isolated from soil by enrichment culture (4). Whether deamination of taurine is involved in this anaerobic assimilation of taurine-sulfur is unknown. In this study, we examined *C. pasteurianum* C1 and other bacteria for the ability to assimilate the sulfur of taurine, cysteate, and potential deamination (or transamination) products for anaerobic growth. We also examined cell extracts of these bacteria for aminotransferase activity with taurine as the amino donor.

Among the isolates and bacteria examined (*C. pasteurianum* C1, *C. pasteurianum* ATCC 6013, *Clostridium* sp. strain MS-1, *Klebsiella* sp., and *Klebsiella oxytoca* M5A1), only *C. pasteurianum* C1 assimilated taurine-sulfur during anaerobic growth. It was also the only bacterium examined whose cell extracts exhibited taurine-pyruvate aminotransferase activity. The study reveals a possible relationship between taurine-pyruvate aminotransferase activity and the ability of *C. pasteurianum* C1 to assimilate taurine-sulfur.

MATERIALS AND METHODS

Bacterial strains. *C. pasteurianum* C1 and *Klebsiella* sp. were isolated from soil by enrichment culture as previously described (4); strain MS-1, also isolated from soil enrichment culture, is another anaerobic, endospore-forming bacterium we have tentatively identified as a *Clostridium* sp. L. O. Ingram, University of Florida, Gainesville, kindly provided *K. oxytoca* M5A1, and Susan Leschine, University of Massachusetts, Amherst, provided *C. pasteurianum* ATCC 6013.

Media and cultivation of bacteria. The minimal media for *Klebsiella* sp. and *K. oxytoca* M5A1 contained 55 mM glucose as the carbon and energy source, 20 mM NH₄Cl as the nitrogen source, 0.1 M potassium-sodium phosphate (pH 7), 1% (vol/vol) mineral base (modified from the "concentrated base" of Cohen-Bazire et al. [6] so that chloride salts replaced those listed as sulfate), and a sulfur source at 100 μ M. The media for *C. pasteurianum* C1 and ATCC 6013 and *Clostridium* strain MS-1 were the same as that described above, except that 55 mM sucrose replaced glucose as the carbon and energy source and biotin (20 μ g/liter) was added. The sulfur sources tested included sulfate, sulfite, cysteate, cysteine, isethionate, sulfoacetaldehyde (synthesized as described by Kondo et al. [14]), 3-sulfolactate (synthesized as described by Bensen et al. [2]), 3-sulfo-pyruvate (synthesized as described by White [25]), and taurine. The identities of sulfoacetaldehyde, 3-sulfo-pyruvate, and 3-sulfolactate were confirmed by gas chromatography-mass spectrometry (25). Sulfoacetaldehyde and isethionate are possible

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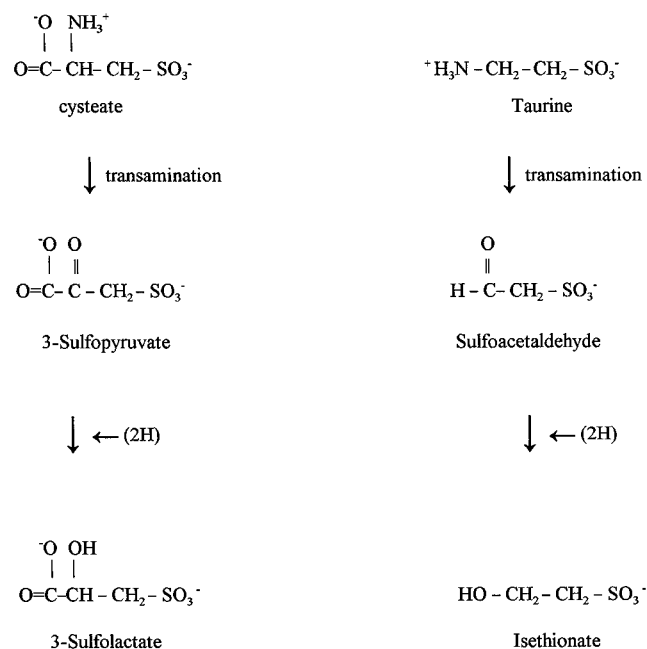


FIG. 1. Proposed transaminations of short-chain sulfonates (taurine and cysteate).

primary and secondary transamination products of taurine (Fig. 1), while 3-sulfofopryruvate and 3-sulfolactate are possible transamination products of cysteate (Fig. 1). All of the chemicals used in this study were of the highest purity available from Sigma Chemical Co., Fisher Scientific, Aldrich, or Eastman Kodak.

The ability of sulfonate sulfur to support fermentative growth of bacteria was assessed by measuring the growth of bacteria incubated in the minimal media described above in an anaerobic hood at 27°C. Anaerobiosis was monitored by using resazurin as an indicator.

Preparation of crude cell extracts. Bacteria were grown with different sulfur sources to mid-exponential phase (optical density at 650 nm [OD₆₅₀], 0.7) and harvested by centrifugation. Cells were resuspended, washed three times with 50 mM potassium phosphate buffer (pH 7.5), resuspended in the buffer (2 g [wet weight] of cells/5 ml of buffer), and disrupted in a French pressure cell at 15,000 lb/in². After centrifugation, the extract supernatant was dialyzed against 2 liters of the buffer overnight. Protein content of extracts was determined by a modified Lowry-sodium dodecyl sulfate method with bovine serum albumin as the standard (15).

Assessment of aminotransferase activities in crude cell extracts. Crude cell extracts were prepared from *C. pasteurianum* C1 grown with either taurine or sulfate as the sole sulfur source, *C. pasteurianum* ATCC 6013 grown with cysteate or sulfate, strain MS-1 grown with sulfoacetaldehyde or sulfate, *Klebsiella* sp. grown with cysteate or sulfate, and *K. oxytoca* M5A1 grown with sulfate as the sole source of sulfur, all under anaerobic conditions. The enzyme assay system contained 10 μmol of the amino donor (taurine, cysteate, glutamate, or aspartate), 20 μmol of the amino acceptor (pyruvate, oxalacetate, or α-ketoglutarate), 50 μmol of potassium phosphate (pH 8.5), 4 μg of pyridoxal 5-phosphate, and cell extract in a final volume of 400 μl. The reaction mixture was incubated at 37°C for 1 h, and the enzymes were then inactivated by heat (90°C for 3 min). Certain amino acids (alanine, aspartate, and glutamate) formed in the reaction mixture were separated by thin-layer chromatography on cellulose (solvent sys-

tem: isobutyric acid-water at 4:1 [vol/vol]) and detected by using acetate-buffered ninhydrin spray (0.5% ninhydrin in 95% ethanol containing 5% (vol/vol) 1 M sodium acetate [pH 5.5]).

Characterization and quantitative determination of taurine-pyruvate aminotransferase. The calculated activity of taurine-pyruvate aminotransferase was based on the fixed-time assay amount of alanine present (formed from pyruvate), which was quantitatively determined with commercial alanine dehydrogenase (27). The coupled assay system contained 1.7 μmol of NAD⁺, 200 μl of heat-inactivated aminotransferase assay mixture containing reaction products formed in the complete standard assay system described above, 1 U of alanine dehydrogenase, and 37.5 μmol of Na-CAPS [3-(cyclohexylamino)-1-propanesulfonate] (pH 10.5) in a final volume of 750 μl. In this latter assay system, 1 μmol of alanine yielded 1 μmol of NADH, which was determined by measuring the A₃₄₀.

For quantitation of aminotransferase activities by using glutamate, aspartate, and cysteate as amino donors and pyruvate as an amino acceptor, the same assay system was employed except that one of the three amino acids replaced taurine. To determine whether taurine-pyruvate aminotransferase is a constitutive or an inducible activity, extracts were prepared from *C. pasteurianum* C1 cells grown with one of several different sulfur sources—sulfate, sulfite, cysteine, isethionate, sulfoacetaldehyde, taurine, or sulfate-starved cells (i.e., sulfate-grown cells were resuspended in sulfur-free medium for 3 h, and then extracts were prepared as described above).

Reproducibility. All experiments were performed at least three times, and the values obtained were reproducible within ±5%. Replicates of the results presented in the tables and figures were essentially identical.

RESULTS

Ability of sulfonate sulfur to support fermentative growth of bacteria. Of the five strains tested, only *C. pasteurianum* C1 could assimilate the sulfur of taurine for anaerobic growth and could also use the sulfur of the potential transamination products of taurine (sulfoacetaldehyde and isethionate) as a sole sulfur source; *C. pasteurianum* C1 did not utilize the sulfur of cysteate, sulfofopryruvate, or sulfolactate. The other bacteria examined were unable to use taurine as a sole sulfur source (for fermentative growth) but could use the sulfur of some other sulfonates, e.g., cysteate, 3-sulfofopryruvate, 3-sulfolactate, sulfoacetaldehyde, and isethionate (Table 1). All of the bacteria tested could use the sulfur of sulfate, sulfite, and cysteine for fermentative growth.

Assessment of taurine-pyruvate aminotransferase activities in cell extracts. By using thin-layer chromatography, we detected different aminotransferase activities in cell extracts, as judged by amino compounds formed in combinations of amino donors and amino acceptors. The R_f values for different amino products determined by using the chromatography system described were as follows: Glu, 0.323; Ala, 0.420; Asp, 0.277; cysteate, 0.084; taurine, 0.187.

Of the bacteria examined, only *C. pasteurianum* C1 possessed taurine-pyruvate aminotransferase activity; it was also the only one that could assimilate taurine-sulfur. Enzyme activity was demonstrated by using taurine as an amino donor and either pyruvate or oxalacetate as an amino acceptor. Although none of the other bacteria examined possessed this activity, all possessed other, more common aminotransferase activities, regardless of the sulfur source used for growth. The aminotransferase activities demonstrated included those using aspartate or glutamate as amino donors and pyruvate, oxalac-

TABLE 1. Ability of sulfonate sulfur to support fermentative growth of bacteria^a

S source	Sulfate	Taurine	Sulfoacetaldehyde	Isethionate	Cysteate	Sulfofopryruvate	Sulfolactate
<i>C. pasteurianum</i> C1	+	+	+	+	-	-	-
<i>Klebsiella</i> sp.	+	-	-	-	+	+	+
<i>C. pasteurianum</i> ATCC 6013	+	-	-	-	+	+	+
<i>Clostridium</i> strain MS-1	+	-	+	+	+	+	+
<i>K. oxytoca</i> M5A1	+	-	ND ^b	-	-	ND	ND

^a +, growth (OD₆₅₀ > 1.0); -, no growth (OD₆₅₀ ≤ 0.1).

^b ND, not determined.

TABLE 2. Occurrence of aminotransferase activity in cell extracts^a

Enzyme	<i>C. pasteurianum</i> C1	<i>C. pasteurianum</i> ATCC 6013	<i>Clostridium</i> strain MS-1	<i>Klebsiella</i> sp.	<i>K. oxytoca</i> M5A1
Taurine-pyruvate aminotransferase	+	-	-	-	-
Taurine-oxalacetate aminotransferase	+ ^b	-	-	-	-
Taurine- α -ketoglutarate aminotransferase	-	-	-	-	-

^a Aspartate-pyruvate, aspartate- α -ketoglutarate, cysteate-pyruvate, cysteate-oxalacetate, cysteate- α -ketoglutarate, glutamate-pyruvate, and glutamate-oxalacetate aminotransferase activities were detected in all extracts. The sulfur sources used for growth of bacteria were as follows: *C. pasteurianum* C1, taurine; *C. pasteurianum* ATCC 6013, cysteate; *Clostridium* strain MS-1, cysteate; *Klebsiella* sp., cysteate; *K. oxytoca* M5A1, sulfate. +, aminotransferase activity detected; -, no aminotransferase activity detected.

^b The product of this reaction was identified as alanine, not aspartate.

etate, or α -ketoglutarate as amino acceptors. Crude cell extracts of all five bacteria showed aminotransferase activity with cysteate as the amino donor, as well. The transamination activities with cysteate were significantly lower than those obtained by using other amino acids as amino donors (Table 2).

Traits of taurine-pyruvate aminotransferase. Taurine-pyruvate aminotransferase activity in crude cell extracts appeared to be optimal at about 37°C with an optimal pH range of 8.5 to 9.5; no activity was detected at pH 10.5 (Fig. 2a and b). Activity was not detected when either taurine, pyruvate, or cell extract was absent from the complete standard assay system. The activity in the complete system minus pyridoxal 5-phosphate was essentially that seen when this cofactor was added, suggesting that if it was involved, it remained tightly bound to the aminotransferase. No activity was detected with boiled cell extract. Upon partial purification, this activity was separable from other aminotransferase activities (data not shown).

Quantitative comparison of different aminotransferase activities in cell extracts. The levels of aminotransferase activity obtained with glutamate, aspartate, cysteate, and taurine as individual amino donors and pyruvate as the amino acceptor were compared. In extracts of cells grown with taurine as the sole sulfur source, the amount of alanine formed by the reaction mixtures using different amino donors in the fixed-time assay was as follows (micromoles of alanine per milligram of protein): glutamate, 0.55; aspartate, 0.17; cysteate, 0.08; taurine, 0.14. When extracts prepared from cells grown with sulfate as the sole sulfur source were used, the activities of these aminotransferases were essentially identical except that taurine-pyruvate aminotransferase activity was barely detectable (less than 5% of full activity) in such extracts or in extracts prepared either from cells grown with sulfite as the sole source of sulfur or from sulfate-starved cells. Extracts of cells grown with sulfoacetaldehyde or isethionate as the sole sulfur source had taurine-pyruvate aminotransferase activity (about 60% of the activity noted in taurine-grown cells). Cysteine-grown cells possessed only 30 to 40% of the aminotransferase activity of taurine-grown cells. Activities of other aminotransferases (i.e., aspartate and glutamate) were comparable to those of sulfate-grown cells.

DISCUSSION

In contrast to the recognition of aerobic utilization of sulfonates (12, 16, 18, 19, 28), until recently it was not recognized that aliphatic sulfonates, particularly their sulfur component,

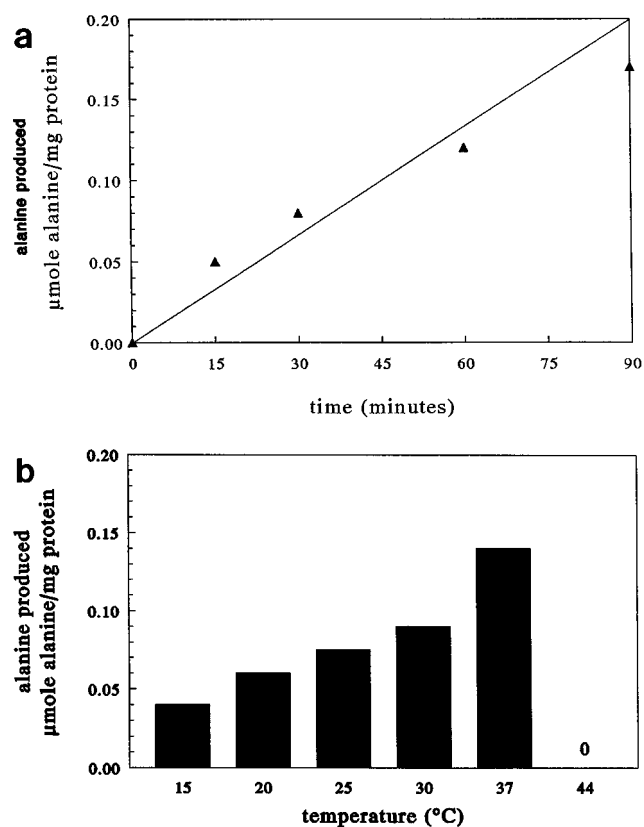


FIG. 2. (a) Alanine formed via taurine-pyruvate aminotransferase as a function of time. The protein content in the assay system was ca. 6 mg of protein/ml of reaction mixture. (b) Alanine formed via taurine-pyruvate aminotransferase as a function of temperature. The protein content in the assay system was ca. 4 mg of protein/ml of reaction mixture.

are subject to attack and biodegradation under anaerobic conditions. We demonstrated, for the first time, that the sulfur of some sulfonates, including taurine, can be assimilated by *C. pasteurianum* C1 for strictly anaerobic growth (4). However, knowledge of the details of assimilation of such sulfur, as well as information about attack on the C-S bond under anoxic conditions, is very incomplete.

Since it was suggested (10) that some aerobic bacteria utilize taurine by first removing the amino group, we asked whether such an event might be an essential step in assimilation of the sulfur of cysteate or taurine by anaerobic bacteria as well. The patterns of sulfonate sulfur utilization noted in growth experiments provided evidence consistent with such a possibility. Our *Klebsiella* isolate utilized not only cysteate but also sulfo-pyruvate—a likely direct transamination product of cysteate—and sulfolactate, which could be formed from sulfo-pyruvate. Neither taurine nor sulfoacetaldehyde nor isethionate served as a sole source of sulfur for growth. In contrast to this spectrum of organosulfur utilization was that noted for *C. pasteurianum* C1: sulfur of taurine, sulfoacetaldehyde, and isethionate was utilized, while that of cysteate, sulfo-pyruvate, and sulfolactate was not.

It is the demonstration of an apparently inducible taurine-pyruvate aminotransferase activity, however, that provides the strongest suggestion of a role for transamination in utilization of taurine-sulfur by *C. pasteurianum* C1. Three facts, i.e., (i) that this activity was not detected in other bacteria that could not grow on taurine-sulfur, (ii) that more common aminotrans-

ferase activities were present irrespective of the sulfur source employed for growth, and (iii) that this activity was not detected in aerobic, respiring *Klebsiella* cells grown on taurine-sulfur, are consistent with a special role for taurine-pyruvate aminotransferase activity in anaerobic utilization of taurine-sulfur.

This enzyme activity appears not to have been detected in anaerobic bacteria until now. Curiously, the taurine- α -ketoglutarate and ω -amino acid-pyruvate aminotransferase activities of, respectively, *Achromobacter superficialis* (21) and a pseudomonad (26) were not noted to be involved in taurine metabolism, since they were induced in alanine-grown cells. In *P. aeruginosa* TAU-5 (18), however, a taurine-pyruvate aminotransferase was induced when taurine served as the sole source of energy, carbon, nitrogen, and sulfur for growth of this strictly respiratory bacterium. Certain differences among these activities are apparent: the strain TAU-5 activity employs α -ketoglutarate as an amino acceptor, while the *C. pasteurianum* C1 preparation does not, and the " ω -aminotransferase" activity of strain TAU-5 (seen with ethylamine or ethanalamine as the amino donor) is not seen in strain C1 (data not shown).

It seemed surprising, at first, to find cysteate aminotransferase activity with either α -ketoglutarate, oxalacetate, or pyruvate as the amino acceptor, regardless of whether the test organism is able to utilize cysteate sulfur. However, it was argued that either an aspartate aminotransferase (5) or a cysteate aminotransferase (7) could be responsible for utilization of cysteate as an amino donor in extracts of pig heart muscle.

Since the expected transamination product (aspartate) from use of oxalacetate as an amino acceptor was not detected in our study, while alanine was, it seems possible that an active oxalacetate decarboxylase or aspartate decarboxylase activity was present in the crude extract.

Further studies on purified preparations of the taurine-pyruvate aminotransferase activity of *C. pasteurianum* C1 should be of value in delineating the precise roles of this activity in taurine-sulfur metabolism and, by extension, provide testable insights into sulfonate sulfur utilization in other bacteria.

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