Occurrence of Taurine: α-Ketoglutarate Aminotransferase in Bacterial Extracts[1]

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High activity of taurine: α-ketoglutarate aminotransferase was found exclusively in cell-free extracts of *Achromobacter superficialis* and *A. polymorph*. The former was chosen for characterization of the enzymatic reaction. The enzyme activity was enhanced by addition of β-alanine to the growth medium. The product from α-ketoglutarate was identified as L-glutamate. Another product has been isolated, purified, and identified as sulfoacetaldehyde (2-oxoethanesulfonate), a deamination product from taurine, by comparison between the 2,4-dinitrophenylhydrazones of the synthetic and enzymatic products on the basis of studies by paper chromatography, by visible, infrared, and nuclear magnetic resonance spectrophotometries, and by elemental analysis. This enzymatic transamination was found to proceed stoichiometrically and reversibly as follows: $NH_2 \cdot CH_2 \cdot CH_2 \cdot SO_3H + HOOC \cdot CH_2 \cdot CH_2 \cdot CO \cdot COOH \rightleftharpoons OHC \cdot CH_2 \cdot SO_3H + HOOC \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$.

Taurine (2-aminoethanesulfonate) occurs free, generally in high concentration, in various plants and animal tissues, e.g., liver, brain, muscle, and kidney of cats (17), and also in the bound forms, e.g., taurocholic acid in bile. Evidence for the urinary excretion of guanidinotaurine (18), carbamyltaurine (18), and quinaldylglycyltaurine (6) after administration of taurine (18) and quinaldic acid (6) to rats has been presented. Taurine is formed generally as an end product in the animal metabolism of cysteine, as reviewed by Meister (9). The metabolism of taurine has not been fully elucidated, though a few studies have been reported (2, 5, 7).

We present here evidence for the occurrence of a bacterial enzyme that catalyzes transamination of taurine with α-ketoglutarate. The isolation and identification of the reaction products are reported.

MATERIALS AND METHODS

Chemicals. Taurine and β-alanine were obtained from Ajinomoto Co., Tokyo, Japan. α-Ketoglutaric acid, sodium L-glutamate and pyridoxal 5′-phosphate were products of Kyowa Hakko Kogyo Co., Tokyo, Japan. Aluminium oxide (W-80) and 2,4-dinitrophenylhydrazine were purchased from Wako

[1] Dedicated to Alexander E. Braunstein on the occasion of his 70th birthday.

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Pure Chemical Industries Co., Osaka, Japan. Bisulfite adduct of sulfoacetaldehyde (2-oxoethanesulfonate) was synthesized from 2-bromo-1,1-diethoxyethane and sodium bisulfite in the presence of NaOH according to the procedure of Kondo et al. (7). The other chemicals were analytical-grade reagents.

Microorganisms and conditions of culture. *Achromobacter superficialis* ICR B-89 and certain other organisms were grown in a medium containing (per liter): β-alanine, 3.0 g; peptone, 2.0 g; glycerol, 1.0 g; KH_2PO_4, 1.0 g; K_2HPO_4, 1.0 g; NaCl, 1.0 g; yeast extract, 0.1 g; and $MgSO_4 \cdot 7H_2O$, 0.1 g. The pH of the medium was adjusted to 7.2 with 2 N NaOH. The bacteria were grown aerobically in 2-liter flasks containing 700 ml of the medium, with vigorous shaking on a reciprocating shaker, at 28 C for 22 hr. The cells were harvested by centrifugation and were washed twice with 0.85% (w/v) NaCl.

Enzyme preparation. The cells were washed again with 0.02 M potassium phosphate buffer, pH 7.2, containing 10 μM pyridoxal 5′-phosphate and 0.01% (w/v) 2-mercaptoethanol, disrupted by grinding in a mortar with levigated aluminum oxide, and extracted with the same buffer. The supernatant solution obtained by centrifugation was dialyzed against the same buffer and employed as the crude enzyme. The crude enzyme was brought to 30% saturation with solid ammonium sulfate, and the precipitate was removed by centrifugation. To the supernatant fraction was added ammonium sulfate to achieve 65% saturation. The precipitate obtained by centrifugation was dissolved in 0.01 M potassium phosphate buffer, pH 7.2, containing 10 μM pyridoxal 5′-phosphate and 0.01% 2-mercaptoethanol, and was

dialyzed against the same buffer. The inactive precipitate formed during dialysis was removed by centrifugation. All operations were carried out at 0 to 5 C. This enzyme preparation, unless otherwise specified, was used in the experiments.

Analytical methods. Glutamate or certain other amino acids formed in the reaction mixture were separated by paper chromatography and determined with ninhydrin as described previously (14). Amino acids were also analyzed with a Yanagimoto LC-5S automatic amino acid analyzer (Yanagimoto Seisakusho, Kyoto, Japan) by the method of Spackman, Stein, and Moore (15). α-Ketoglutarate was determined spectrophotometrically after its conversion to 2,4-dinitrophenylhydrazone according to the procedure of Friedemann and Haugen (3). Absorption spectra were determined with a Shimadzu MPS;50L recording spectrophotometer (Shimadzu Seisakusho, Kyoto, Japan), and infrared spectra were determined with a Hitachi EPI-S$_2$ spectrometer (Hitachi Seisakusho, Tokyo, Japan). Nuclear magnetic resonance spectra were determined with a Varian Associates recording spectrometer (A60) at 60 MHz in deuterated methanol with tetramethylsilane as internal standard. Chemical shifts are reported in δ values (parts per million).

Enzyme assay. Aminotransferase activity was routinely assayed by determining glutamate formed. The standard reaction system consisted of 20 μmoles of taurine, 20 μmoles of α-ketoglutarate, 1 μmole of pyridoxal 5'-phosphate, 40 μmoles of potassium phosphate buffer, pH 8.0, and enzyme, in a final volume of 1.0 ml. In the blank, the amino donor was replaced by water. Incubation was carried out at 30 C for 30 min, and the reaction was terminated by addition of 0.1 ml of 20% (w/v) perchloric acid. After centrifugation, glutamate formed was determined as described above.

Protein was determined by the procedure of Lowry et al. (8). An enzyme unit was defined as the amount of enzyme required to form 1 μmole of glutamate per min. The specific activity was expressed as units per milligram of protein.

RESULTS

Distribution of taurine aminotransferase activity. The activity of taurine aminotransferase in various strains of bacteria was investigated by measuring the formation of glutamate with the crude enzymes. As shown in Table 1, high activity was found only in extracts of *A. superficialis* ICR B-89 and *A. polymorph* ICR B-88. *A. superficialis*, in which taurine aminotransferase occurs most abundantly, was used in the following experiments.

Under the experimental conditions, the β-alanine:pyruvate aminotransferase (L-alanine: malonate-semialdehyde aminotransferase, EC 2.6.1.18) activity was also investigated by measuring the formation of alanine with the crude enzymes. High β-alanine:pyruvate ami-

notransferase activity was found in all strains of *Pseudomonas* tested, but no activity of taurine:α-ketoglutarate aminotransferase was detected. The cell-free extracts of *A. superficialis* and *A. polymorph* did not contain β-alanine:pyruvate aminotransferase.

Effect of added taurine and β-alanine in the growth medium on enzyme activity. Table 2 presents data concerning the inducibility of taurine aminotransferase. Cells grown in the medium containing peptone and glycerol without β-alanine yielded minimally detectable enzyme activity. β-Alanine served as a good inducer. Addition of β-alanine at an initial concentration of 0.5% (w/v) resulted in a 10-fold increase in activity. When taurine was substituted for β-alanine, the organism grew poorly and the enzyme activity was increased only slightly, though the reason is at present unknown.

Taurine:α-ketoglutarate aminotransferase reaction. As shown in Table 3, both taurine and α-ketoglutarate were required as an amino donor and an amino acceptor in the presence of enzyme and pyridoxal 5'-phosphate to produce glutamate. The increase in the rate of transamination was linear over a sixfold range of enzyme concentrations (0.5 to 3 mg of protein) under the standard conditions. The enzymatic formation of glutamate proceeded linearly as a function of incubation time within 60 min, when 3 mg of enzyme protein was used. The omission of pyridoxal 5'-phosphate from the reaction system caused a slight decrease in the activity of the enzyme, but further work is needed to elucidate the role of the cofactor.

Identification of L-glutamate formed. The standard reaction mixture incubated as described above was analyzed with an automatic amino acid analyzer, after deproteinization and neutralization with NaOH solution. The amino acid formed was identified as glutamate. Co-chromatography of the enzymatic product with authentic glutamate also confirmed their identity. The enzymatic product was quantitatively decarboxylated, as well as authentic L-glutamate, with L-glutamate 1-carboxy-lyase (EC 4.1.1.15) from *Escherichia coli* when analyzed manometrically according to the procedure of Najjar (10).

Identification of sulfoacetaldehyde by paper chromatography of 2,4-dinitrophenylhydrazone of a product. To the reaction mixture incubated for 40 min and deproteinized as described above was added 0.5 ml of 0.5% (w/v) 2,4-dinitrophenylhydrazine solution dissolved in 2 N HCl to yield the hydra-

zones. After incubation at 37 C for 30 min, 10 ml of ethyl acetate was added to the solution, and air was bubbled through the mixture for 3 min. The aqueous layer was washed again with ethyl acetate in the same manner, and the washings (ethyl acetate layer) were added to the first ethyl acetate layer. The combined ethyl acetate layers were washed twice with water and mixed with 1.0 ml of 10% (w/v) Na_2CO_3 solution; air was then bubbled through the mixture for 3 min. Some portion of the Na_2CO_3 layer was chromatographed on Toyo filter paper no. 51 with butan-1-ol-

TABLE 1. *Distribution of taurine aminotransferase activity in various strains of bacteria[a]*

Strain	Specific activity
Achromobacter superficialis ICR B-89	0.019
A. polymorph ICR B-88	0.016

[a] The growth medium contained 0.2% (w/v) β-alanine. The conditions of culture and enzyme assay are given in Materials and Methods. No activity was found in the following organisms: *Achromobacter liquidum* IFO 3084, *Pseudomonas aeruginosa* IFO 3080, *P. riboflavina* IFO 3140, *P. fluorescens* IFO 3081, *P. fragi* IFO 3458, *P. dacunhae* IFO 12047, *P. marginalis* IFO 3925, *P. ovalis* IFO 3738, *P. polycolor* IFO 3918, *P. striata* ICR B-320, *P. synxantha* IFO 30906, *Aerobacter aerogenes* IFO 3320, *Alcaligenes faecalis* ICR B-80, *Agrobacterium radiobacter* IAM 1526, *A. tumefaciens* IFO 3058, *Bacillus cereus* IFO 3001, *B. sphaericus* IFO 3525, *B. subtilis* IFO 3009, *B. subtilis* ICR B-36, *Bacterium cadaveris* IFO 3731, *Brevibacterium acetylicum* IFO 12146, *B. ammoniagenes* IFO 12071, *Escherichia coli* ICR B-1, *E. coli* ICR B-3, *Flavobacterium suaveolens* IFO 3752, *Proteus mirabilis* IFO 3849, *P. vulgaris* IFO 3167.

TABLE 2. *Effect of added β-alanine in the medium on enzyme activity*

β-Alanine added[a] (%, w/v)	Specific activity
0.1	0.016
0.2	0.020
0.3	0.028
0.5	0.032
1.0	0.031
None	0.003

[a] The growth medium contained, in addition to the indicated concentrations of β-alanine, the following (w/v): 0.2% peptone, 0.1% glycerol, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.1% NaCl, 0.01% yeast extract, and 0.01% $MgSO_4 \cdot 7H_2O$. The organisms were grown and the activity of the dialyzed crude enzyme was determined as described in Materials and Methods.

TABLE 3. *Aminotransferase reaction between taurine and α-ketoglutarate*

Reaction system[a]	Glutamate formed (μmoles)
Complete system	0.71
Minus pyridoxal 5'-phosphate	0.69
Minus taurine	0.0
Minus α-ketoglutarate	0.0
Boiled enzyme used	0.0

[a] Glutamate formed was determined as described in Materials and Methods; 1 mg of enzyme was used.

ethanol-water (7:1:2, v/v) as the solvent. In addition to 2,4-dinitrophenylhydrazone of α-ketoglutarate (R_F, 0.12), the hydrazone of an unknown compound (R_F, 0.31) was observed. The R_F of this compound was the same as that of 2,4-dinitrophenylhydrazone of synthetic sulfoacetaldehyde as described below.

Isolation of 2,4-dinitrophenylhydrazone of the product. A large-scale reaction (final volume, 100 ml; incubation time, 1 hr) was carried out, followed by formation of 2,4-dinitrophenylhydrazone and extraction with ethyl acetate as stated above. The ethyl acetate layer, which contained most of the hydrazone of α-ketoglutarate and a small amount of that of the product, was washed with water. The washings (aqueous layer) were added to the first aqueous layer, in which the unreacted reagent, a large part of the hydrazone of the product, and a small amount of that of α-ketoglutarate were found. The combined aqueous layers were washed once with ethyl acetate to remove the hydrazone of α-ketoglutarate, and this ethyl acetate layer was discarded. Then the aqueous layer was repeatedly extracted with ethyl acetate. The resultant ethyl acetate fractions, which contained almost all of the hydrazone of the product, were evaporated to dryness. The residue was dissolved in a small volume of warm water, followed by neutralization with Na_2CO_3 solution. The crystals were obtained by addition of ethanol. Repeated recrystallizations from water and ethanol gave yellow needles. The hydrazone of synthetic sulfoacetaldehyde was also prepared from the bisulfite adduct of sulfoacetaldehyde in a similar way.

Characterization of the 2,4-dinitrophenylhydrazone of the product. The 2,4-dinitrophenylhydrazone of the product had a melting point of 238 to 240 C (decomposition), and the mixture with an authentic sample (melting point, 239 to 240 C, decomposition) prepared

as described above had a melting point of 239 to 240 C (uncorrected).

Analysis. Calculated for $C_8H_7N_4O_7SNa \cdot H_2O$ (2,4-dinitrophenylhydrazone of sodium sulfoacetaldehyde): C, 27.91; H, 2.62; N, 16.33; H_2O, 5.2%. Found: C, 27.38; H, 2.35; N, 15.89; loss at 100 C, 5.1%.

The nuclear magnetic resonance spectrum of the hydrazone of the enzymatic product (Fig. 1) gave signals at δ 3.89 (2H, doublet, $C_1 <^H_H$), at δ 7.80 (1H, triplet, C_2—H), at δ 7.98 (1H, doublet, C_3—H), at δ 8.35 (1H, doublets of doublet, C_4—H), and at δ 9.01 (1H, splitted doublet, C_5—H). These results suggest strongly that the compound is the 2,4-dinitrophenylhydrazone of sodium sulfoacetaldehyde.

An infrared absorption spectrum of the hydrazone of the enzymatic product was demonstrated to be identical with that of 2,4-dinitrophenylhydrazone of synthetic sodium sulfoacetaldehyde as shown in Fig. 2. The 2,4-dinitrophenylhydrazones of synthetic and enzymatic products exhibited identical visible spectra (λ_{max}, 435 nm).

2,4-Dinitrophenylhydrazones of the enzymatically formed and synthetic sulfoacetaldehyde dissolved in 10% (w/v) Na_2CO_3 solution were examined by paper chromatography with the use of four solvent systems. The two compounds were shown to be identical by comparison of their R_F values (Table 4).

Stoichiometry of the reaction. After the standard reaction mixture was incubated for 60 and 180 min, all of the reactants and the products were determined. The reaction mixture to which perchloric acid was added before initiation of the reaction was used as a control. This enzymatic transamination was found to proceed stoichiometrically, as shown in Table 5. The formation of sulfoacetaldehyde never resulted from incubation of taurine with the enzyme in the absence of α-ketoglutarate. Oxygen uptake was not observed when taurine was incubated with the enzyme.

Reverse reaction. The reverse reaction, i.e., transamination between L-glutamate and sulfoacetaldehyde, was investigated. As shown in Table 6, taurine and α-ketoglutarate were formed in almost equimolar amounts in the complete system, whereas the reaction never occurred in a system lacking the amino donor, the amino acceptor, or enzyme.

DISCUSSION

Although considerable effort has been devoted to the characterization of ω-amino acid aminotransferases in recent years (1, 4, 11, 13, 16), little attention has been paid to the aminotransferase catalyzing the transamination of taurine. Ikeda, Yamada, and Tanaka (5) reported the degradation of taurine to sulfate, ammonia, and carbon dioxide by a species of *Agrobacterium*. Their data suggest that deamination precedes release of sulfate, though it is
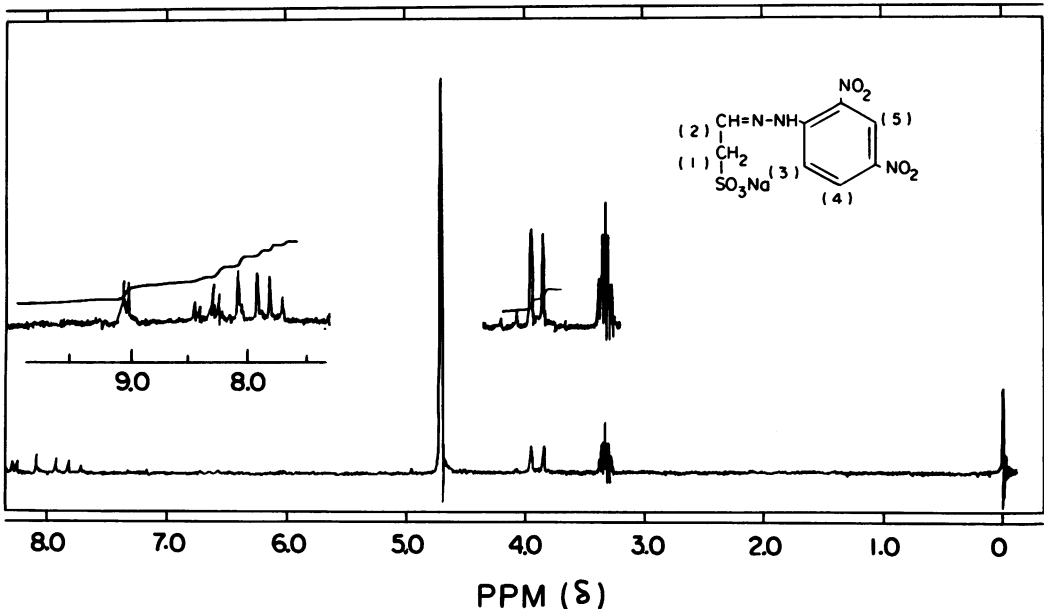

FIG. 1. *Nuclear magnetic resonance spectrum of 2,4-dinitrophenylhydrazone of enzymatically formed sulfoacetaldehyde.*
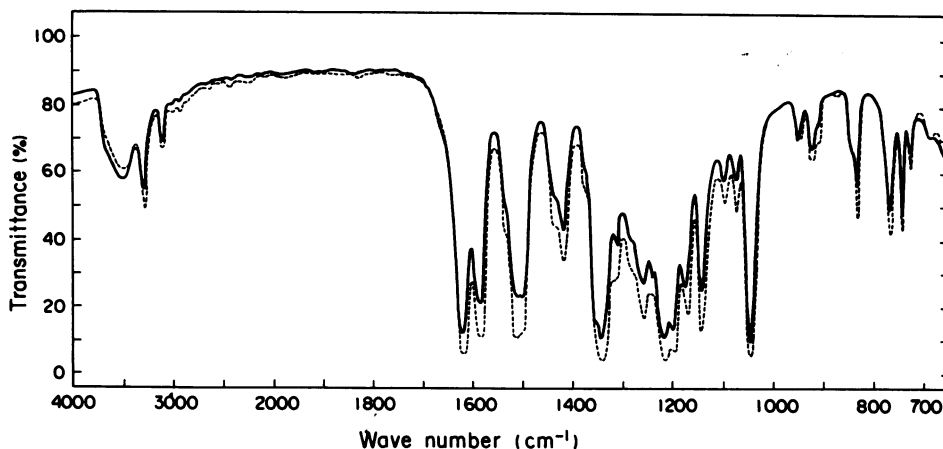
FIG. 2. *Infrared absorption spectra of 2,4-dinitrophenylhydrazones of synthetic and enzymatically formed sulfoacetaldehyde. Solid line, enzymatically formed; dotted line, synthetic.*

TABLE 4. *Paper chromatography of 2,4-dinitrophenylhydrazones of enzymatically formed and synthetic sulfoacetaldehyde*

2,4-Dinitrophenylhydrazone[a]	R_F			
	A	B	C	D
Enzymatically-formed sulfoacetaldehyde	0.31	0.79	0.81	0.68
Synthetic sulfoacetaldehyde	0.31	0.78	0.80	0.68

[a] The 2,4-dinitrophenylhydrazones were dissolved in 10% (w/v) Na₂CO₃ solution, and chromatographed on Toyo filter paper, no. 51, at 20 C, with the following solvents: A, butan-1-ol-ethanol-water (7:1:2, v/v); B, propan-2-ol-water-27% ammonia (20:2:1, v/v); C, 0.1 M sodium carbonate buffer, pH 10.7-butan-1-ol-ethanol (2:5:1, v/v, lower layer); D, propan-2-ol-3-methylbutan-1-ol-pyridine-water (20: 4:1:5, v/v).

TABLE 5. *Stoichiometry of the reaction*

Incubation time (min)[a]	Taurine decreased (μmoles)[b]	α-Keto-glutarate decreased (μmoles)[c]	Sulfoacet-aldehyde formed (μmoles)[c]	Glutamate formed (μmoles)[b]
60	1.98	2.00	2.02	2.00
180	3.75	3.90	3.80	3.80

[a] Enzyme used was 1.3 mg.

[b] Taurine and glutamate were determined with an amino acid analyzer.

[c] α-Ketoglutarate and sulfoacetaldehyde were determined spectrophotometrically after their conversion to 2,4-dinitrophenylhydrazones, followed by their paper chromatographic separation with solvent A (Table 4).

not certain whether isethionate is formed as an intermediate. Deamination of taurine to isethionate has been demonstrated in *Aspergillus niger* grown on a medium containing taurine as the sole source of sulfur (2). Formation of isethionate from taurine was also demonstrated in the rat brain by Peck and Awapara (12). They suggested a two-step sequence to account for the product, i.e., a transamination of taurine and a reduction of the resultant sulfoacetaldehyde, though no evidence has been obtained for the occurrence of these enzymatic reactions. Recently, Kondo et al. (7) reported that sulfoacetaldehyde is formed enzymatically with the consumption of oxygen, when taurine is incubated with the bacterial cell-free extract.

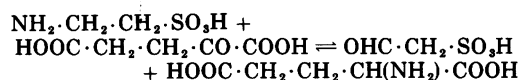The present investigation has revealed that

TABLE 6. *Aminotransferase reaction between L-glutamate and sulfoacetaldehyde*

Reaction system[a]	α-Ketoglutarate formed (μmoles)	Taurine formed (μmoles)
Complete system	0.83	0.79
Minus L-glutamate	0.0	0.0
Minus sulfoacetaldehyde ...	0.0	0.0
Boiled enzyme used	0.0	0.0

[a] The complete reaction mixture contained 20 μmoles of sodium L-glutamate, 20 μmoles of sulfoacetaldehyde as bisulfite adduct, 1 μmole of pyridoxal 5'-phosphate, 40 μmoles of potassium phosphate buffer, pH 8.0, and 2.0 mg of enzyme, in a final volume of 1.0 ml. After incubation at 30 C for 60 min, α-ketoglutarate and taurine were determined as described in Table 5.

sulfoacetaldehyde and L-glutamate are formed stoichiometrically from taurine and α-ketoglutarate in the presence of an enzyme isolated from *A. superficialis*. The reverse reaction also proceeds enzymatically. The possibility that sulfoacetaldehyde may be produced from taurine through an oxidative deamination is excluded, because the consumption of oxygen was not observed during the reaction, and sulfoacetaldehyde was never formed in the absence of α-ketoglutarate.

Thus, good evidence has been presented for the occurrence of the enzyme catalyzing the reversible transamination between taurine and α-ketoglutarate in the cell-free extract of *A. superficialis* as follows:

$$NH_2 \cdot CH_2 \cdot CH_2 \cdot SO_3H +$$
$$HOOC \cdot CH_2 \cdot CH_2 \cdot CO \cdot COOH \rightleftharpoons OHC \cdot CH_2 \cdot SO_3H$$
$$+ HOOC \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

The further metabolic fate of sulfoacetaldehyde has not been established. The finding that the activity of taurine aminotransferase was stimulated by addition of β-alanine to the growth medium seems to suggest that the enzyme may also catalyze the transamination of β-alanine with α-ketoglutarate.

The crude enzyme from β-alanine-adapted cells of *Pseudomonas fluorescens* has high β-alanine:pyruvate aminotransferase activity as reported by Hayaishi et al. (4). However, no taurine:α-ketoglutarate aminotransferase activity was found in cell-free extracts of several strains of *Pseudomonas* tested. On the other hand, extracts of *A. superficialis* and *A. polymorph* contain no activity of β-alanine:pyruvate aminotransferase. These observations suggest that the taurine:α-ketoglutarate aminotransferase reported here is probably different from the β-alanine:pyruvate aminotransferase of pseudomonads, although both enzyme activities are enhanced by addition of β-alanine to the growth medium.

Purification of the enzyme to homogeneity is needed to resolve these problems. The enzymological aspects of this transamination are now under investigation to shed light on the properties and reaction mechanism of the new aminotransferase.

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