Homotaurine Metabolized to 3-Sulfopropionate in *Cupriavidus necator* H16: Enzymes and Genes in a Patchwork Pathway[†]

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Homotaurine (3-aminopropanesulfonate), a natural product and an analogue of GABA (4-aminobutyrate), was found to be a sole source of nitrogen for *Cupriavidus necator* (*Ralstonia eutropha*) H16, whose genome sequence is known. Homotaurine nitrogen was assimilated into cell material, and the quantitative fate of the organosulfonate was sulfopropionate, which was recovered in the growth medium. The first scalar reaction was shown to be inducible homotaurine:2-oxoglutarate aminotransferase, which released 3-sulfopropional from homotaurine. This aminotransferase was purified to homogeneity and characterized. Peptide mass fingerprinting yielded locus tag H16_B0981, which was annotated *gabT*, for GABA transaminase (EC 2.6.1.19). Inducible, NAD(P)⁺-coupled 3-sulfopropional dehydrogenase, which yielded 3-sulfopropionate from 3-sulfopropional, was also purified and characterized. Peptide mass fingerprinting yielded locus tag H16_B0982, which was annotated *gabD1*, for succinate-semialdehyde dehydrogenase (EC 1.2.1.16). *GabT* and *GabD1* were each induced during growth with GABA, and cotranscription of *gabTD* was observed. In other organisms, regulator *GabC* or *GabR* is encoded contiguous with *gabTD*: candidate *GabR'* was found in strain H16 and in many other organisms. An orthologue of the GABA permease (*GabP*), established in *Escherichia coli*, is present at H16_B1890, and it was transcribed constitutively. We presume that *GabR'*PTD are responsible for the inducible metabolism of homotaurine to intracellular 3-sulfopropionate. The nature of the exporter of this highly charged compound was unclear until we realized from the sodium dodecyl sulfate-polyacrylamide gel electrophoresis data that sulfoacetaldehyde acetyltransferase (EC 2.3.3.15; H16_B1872) was strongly induced during growth with homotaurine and inferred that the sulfite exporter encoded at the end of the gene cluster (H16_B1874) has a broad substrate range that includes 3-sulfopropionate.

Homotaurine (3-aminopropanesulfonate) (Fig. 1A) is a natural product of marine red algae (26, 36), a component (sometimes derivatized) of accredited and experimental pharmaceuticals (e.g., see reference 49), an over-the-counter “memory enhancer,” and a moiety of Good buffer CAPS [3-(cyclohexylamino)-1-propanesulfonate]. The enrichment and isolation of organisms to degrade homotaurine yielded terrestrial bacteria (35); largely of relevance here is the utilization of the nitrogen moiety and excretion of sulfopropionate. One of the isolates was identified as *Cupriavidus* sp. (J. Mayer, unpublished), so we tested whether the genome-sequenced strains of *Cupriavidus necator* (*Ralstonia eutropha*) (e.g., see reference 40) also expressed this phenotype. *C. necator* H16 did so (Fig. 1A; also see Fig. S1 in the supplemental material).

Homotaurine is not only a homologue of taurine (2-aminoethanesulfonate), which has undefined roles in brain function (e.g., see reference 25), but also an analogue of 4-aminobutyrate (γ -aminobutyric acid; GABA), which was recognized as a neurotransmitter by 1960 (e.g., see reference 41). Much effort was then invested in elucidation of the bacterial degradation of GABA, which could be metabolized via 4-hydroxybutyrate (24) or via succinate semialdehyde (14), depending on the organism concerned. The latter pathway was established in

Escherichia coli K-12, in which a four-gene cluster was identified (2, 15, 37, 48). The putative proteins are the controller *GabC* (b2664 in *E. coli* K-12 substrain MG1665); the permease *GabP* (TC 2.A.3.1.4; b2663); the transaminase *GabT* (GABA: 2-oxoglutarate aminotransferase; GABA transaminase) (EC 2.6.1.19; b2662), whose structure has been published (33); and the dehydrogenase *GabD* [succinate-semialdehyde dehydrogenase; NAD(P)⁺] (EC 1.2.1.16; b2661), whose structure is available online (<http://www.rcsb.org/pdb/cgi/explore.cgi?pdbId=3ETF>). In other organisms, an alternative regulator, *GabR*, is found (3).

The substrate range of enzymes in GABA metabolism in *Pseudomonas fluorescens* was shown to include the sulfonate analogues of GABA (homotaurine) and of succinate semialdehyde (3-sulfopropional) (9). GABA aminotransferase converted homotaurine to putative sulfopropional, whose further oxidation was attributed to succinate-semialdehyde dehydrogenase.

Here, we present an inducible patchwork pathway for the assimilation of homotaurine nitrogen in *C. necator* H16 (Fig. 1A and B), in which three of the four relevant enzymes were found to be shared with the regulated metabolism of GABA (*GabPTD*), whereas the sulfonate exporter function was attributed to an apparent sulfite exporter (*TauE*) (54). We thus attribute a set of known enzymes to novel functions in the assimilatory pathway of homotaurine nitrogen. The enzymes for the scalar reactions were characterized and identified, and the genes encoding the transporters were deduced from reverse transcription (RT)-PCR data.

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FIG. 1. Pathway of assimilatory deamination of homotaurine nitrogen in *C. necator* H16 (A), and the genes proven or believed to encode the proteins involved (B). 2-OG, 2-oxoglutarate; Glu, L-glutamate.

MATERIALS AND METHODS

Materials. The sodium salt of 3-sulfo-3-oxopropanoate was synthesized from propanoic acid and sulfonyl chloride in the presence of the radical starter azoisobutyronitrile as described previously (35). The bisulfite addition complex of 3-sulfo-3-oxopropanal was generated (19, 29, 55), but it was not a substrate for the 3-sulfo-3-oxopropanal dehydrogenase, and we could not convert it to the free aldehyde by published methods (23, 29, 55). Some 3-sulfo-3-oxopropanal was generated from homotaurine with homotaurine transaminase (see below), but we were unable to separate 3-sulfo-3-oxopropanal from the reaction mixture. Commercial chemicals were of the highest purity available from Sigma-Aldrich, Fluka, Roth, Merck, or Biomol. *Taq* DNA polymerase, Moloney murine leukemia virus reverse transcriptase, and RNase-free DNase were from Fermentas.

Organisms, their growth, and preparation of cell extracts. *C. necator* (*R. eutropha*) H16 (DSM 428) (40), *C. necator* JMP134 (DSM 4058), *Burkholderia xenovorans* LB400 (5), *Sinorhizobium meliloti* 1021 (18), *Delftia acidovorans* SPH-1 (46), and *Comamonas testosteroni* KF-1 (46) were grown aerobically at 30°C in a phosphate-buffered mineral salts medium, pH 7.2 (52). *Chromohalobacter salexigens* DSM 3043, *Neptuniibacter caesariensis* MED92^T (CCUG 52065; previously *Oceanospirillum* sp.) (1), *Roseovarius* sp. strain 217 (45), and *Sagittula stellata* E-37 (22) were cultured in Tris-buffered artificial seawater (30). *Roseovarius nubinhibens* ISM (21) and *Ruegeria* (*Silicibacter*) *pomeroyi* DSS-3 (DSM 15171) were grown in modified *Silicibacter* basal medium (11). Strains 217 and E-37 required the addition of vitamins (39), and strain ISM needed a supplement of 0.05% yeast extract (10). Two to 3 mM homotaurine, GABA, or ammonium was added to the appropriate medium as the sole source of nitrogen; 10 mM succinate served as the carbon source. Precultures (3 ml) were grown in 30-ml screw-cap tubes in a roller. Cultures for enzyme assays (50 ml in 300-ml Erlenmeyer flasks) and for protein purification (1 liter in 5-liter Erlenmeyer flasks) were grown on a shaker and harvested at an optical density at 580 nm (OD_{580}) of ~0.6 by centrifugation (20,000 × *g* for 20 min at 4°C). Cells were washed with 50 mM potassium phosphate buffer, pH 7.2, containing 5 mM magnesium chloride and resuspended therein to give 50- to 250-fold concentrated suspensions. Disruption was done by four to five passages through a chilled French pressure cell at 140 MPa (27) in the presence of DNase (50 μg ml⁻¹), and cell debris was removed by centrifugation (11,000 × *g* for 3 min at 4°C). Cultures for total RNA preparation were harvested in the early exponential growth phase at OD_{580} values between 0.2 and 0.27.

Analytical methods. Growth was followed as turbidity at 580 nm (OD_{580} = 1.0 = 156 μg protein ml⁻¹) or quantified as protein in a Lowry-type reaction (7). Sulfate was quantified turbidimetrically as a suspension of BaSO₄ (50). Ammonium ion was assayed colorimetrically by the Berthelot reaction (20). Homotaurine, GABA, glutamate, alanine, glycine, and 2-aminobutyrate were determined after derivatization with 2,4-dinitrofluorobenzene (DNFB) (44) and separation by reversed-phase high-pressure liquid chromatography as described previously (35). 3-Sulfo-3-oxopropanal and other aldehydes were separated by reversed-phase high-pressure liquid chromatography after derivatization with 2-(diphenylacetyl)indane-1,3-dione-1-hydrazone (DIH) as detailed elsewhere (35). Alternatively, a colorimetric assay after derivatization with *o*-aminobenzaldehyde was used to detect 3-sulfo-3-oxopropanal (and other aldehydes) with succinate semialdehyde as the standard (53). This method was modified to obtain a quick (but nonquantitative) activity test to identify active fractions during the purification of homotaurine:2-oxoglutarate aminotransferase: samples (200 μl) from aminotransferase reactions were stopped with 3 M trichloroacetic acid (20 μl), and the protein precipitate was removed by centrifugation (11,000 × *g* for 2 min). The supernatant fluid (200 μl) was added to 900 μl of derivatization mixture (12 mM *o*-aminobenzaldehyde, 450 mM glycine-KCl, 75 mM KOH) in a cuvette. 3-Sulfo-3-oxopropanal formation in reaction mixtures with active fractions was immediately visible as an absorption increase at 440 nm. 3-Sulfo-3-oxopropanoate was quantified by ion chromatography as described for sulfoacetate (12). Protein in extracts was assayed by protein dye binding (4). Denatured proteins were separated on 13% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and stained with Coomassie brilliant blue R250 (31). Peptide mass fingerprint analyses were done under contract by TopLab (Martinsried, Germany).

RT-PCR experiments were done as described previously (30), with the primers listed in Table S1 in the supplemental material. Before reverse transcription, RNA preparations were tested for residual DNA using primer pair H16-gabT-F1/H16-gabT-R1 (see Table S1 in the supplemental material). Chromosomal DNA of *C. necator* H16, which was used as a positive control for PCR, was isolated as described elsewhere (13). PCR products were visualized on 1.5% agarose gels.

Enzyme assays. Homotaurine:2-oxoglutarate aminotransferase activity was quantified by the discontinuous assay of glutamate formation (or homotaurine depletion), with reaction conditions described elsewhere (35). Kinetic constants were determined with reaction mixtures containing various homotaurine or

GABA concentrations and 10 mM 2-oxoglutarate or with various 2-oxoglutarate concentrations and 10 mM homotaurine. Protein concentrations used in this assay ranged from 5 $\mu\text{g ml}^{-1}$ (purified enzyme) to 250 $\mu\text{g ml}^{-1}$ (crude cell extracts). During the purification process, fractions containing homotaurine:2-oxoglutarate aminotransferase were routinely identified by detection of 3-sulfo-3-propanal with the modified aldehyde assay (see above). The pH optimum of the aminotransferase was examined using the following buffers (each at 50 mM): 2-(*N*-morpholino)ethanesulfonate (pH 6.0), potassium phosphate (pH 7.2), Tris (pH 8.0 and 9.0), and CAPS (pH 10). 3-Sulfo-3-propanal dehydrogenase was assayed photometrically (340 nm) at 30°C as the generation of NAD(P)H. The reaction mixture contained 0.1 mM succinate semialdehyde, 5 mM NAD(P)⁺, and 1 μg to 200 μg protein ml^{-1} in 50 mM Tris-HCl, pH 9.0. Occasionally, succinate semialdehyde was replaced by 3-sulfo-3-propanal produced by a homotaurine:2-oxoglutarate aminotransferase reaction (0.4 mg ml^{-1} soluble fraction, 1.5 h of incubation, boiling for 10 min, and centrifugation at 11,000 $\times g$ for 3 min to remove denatured protein). The resulting sulfonated aldehyde (in concentrations of 3 to 5 mM, estimated from glutamate formation) was used without further purification. The kinetic constants of 3-sulfo-3-propanal dehydrogenase were determined using the photometric assay described above by varying the concentrations of succinate semialdehyde at a NAD⁺ concentration of 5 mM or by varying the NAD⁺ or NADP⁺ concentration at a succinate semialdehyde concentration of 0.1 mM (for GabD1) or 1 mM (for GabD3). The pH optimum was determined in a buffer system with constant ionic strength, composed of *N*-(2-acetamido)-2-aminoethanesulfonate, Tris, and ethanolamine (17). Glutamate dehydrogenase (Gdh) was assayed photometrically (47).

Enzyme purification. The membrane/particulate fraction was removed from the crude extract by ultracentrifugation (170,000 $\times g$ for 30 min at 4°C). The supernatant fluid was designated the soluble fraction. All concentration steps were done with Vivaspins concentrators (10-kDa cutoff, polyethersulfone membrane; Sartorius), and PD10 columns (Sephadex G-25; Pharmacia) were used for all buffer exchanges.

Soluble fractions of homotaurine-grown *C. necator* H16 were subjected to anion-exchange chromatography (Mono Q HR 10/10; Pharmacia) with a flow rate of 1 ml min^{-1} . A two-step gradient of sodium sulfate (0 to 75 mM in 10 min followed by 75 to 150 mM in 45 min) in 50 mM potassium phosphate buffer, pH 7.2, was applied, in which homotaurine:2-oxoglutarate aminotransferase eluted at about 40 mM sodium sulfate. Active fractions were combined, rebuffed in 50 mM Tris-HCl, pH 8.1, concentrated, and reloaded on to the anion-exchange chromatography column. The two-step gradient of sodium sulfate described above was now applied in Tris-HCl buffer, pH 8.1, which eluted homotaurine:2-oxoglutarate aminotransferase at a sodium sulfate concentration of about 110 mM. Concentrated active fractions were subjected to gel filtration (Superose 12 HR 10/30; Pharmacia) in 35 mM Tris-HCl, pH 8.1, with 150 mM sodium sulfate at a flow rate of 0.4 ml min^{-1} .

3-Sulfo-3-propanal dehydrogenase was purified from the soluble fraction of homotaurine-grown *C. necator* H16 after an initial ammonium sulfate fractionation. Portions of 3.4 M ammonium sulfate, pH 6.4, were added dropwise with stirring; the protein solution was kept on ice. After addition of the precipitant, the protein solution was incubated for 15 min, and the precipitate was pelleted (11,000 $\times g$ for 3 min at 4°C). The pellet was immediately resuspended in the same volume of potassium phosphate buffer, pH 7.2, whereas the supernatant fluid was subject to further fractionation. 3-Sulfo-3-propanal dehydrogenase activity was found either in the fractions precipitated in 1.1 to 1.3 M ammonium sulfate (GabD1) or in solution (1.8 M ammonium sulfate; GabD3). Active fractions were augmented with 10 mM dithiothreitol (DTT). The second purification step for GabD1 or GabD3 was anion-exchange chromatography (Mono Q HR 10/10; Pharmacia) with 50 mM Tris-HCl (pH 8.6, containing 5 mM DTT) as the eluent: active fractions were concentrated and rebuffed into this eluent. The two-step sodium sulfate gradient described above was applied; the flow rate was 1 ml min^{-1} . 3-Sulfo-3-propanal dehydrogenase eluted at a sodium sulfate concentration of about 100 mM. Concentrated active fractions of GabD1 were subjected to gel filtration (Superose 12 HR 10/30; Pharmacia) in 50 mM potassium phosphate buffer, pH 7.2, with 150 mM sodium sulfate and 5 mM DTT at a flow rate of 0.4 ml min^{-1} .

Bioinformatic analyses. Analyses of the genome sequence of *C. necator* H16 (accession no. AM260479 [chromosome 1], AM260480 [chromosome 2], and AY305378 [megaplasmid pHG1]) were done using the BLAST algorithm on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>). Subroutines from the LASERGENE program package (DNASTAR, Madison WI) were used for handling sequence data, which were in the public domain before 1 May 2009. Alignments were made using ClustalX and plotted in NJPlot (51). Primers for RT-PCR were designed with the program Amplify (version 1.2).

RESULTS AND DISCUSSION

Homotaurine as a sole source of nitrogen for the growth of several genome-sequenced bacteria. The marine alphaproteobacteria *Roseovarius nubinhibens* ISM, *Roseovarius* sp. strain 217, *Ruegeria pomeroyi* DSS-3, and *Sagittula stellata* E-37 (but not the terrestrial *Sinorhizobium meliloti* 1021); the terrestrial betaproteobacteria *Burkholderia xenovorans* LB400, *Cupriavidus necator* H16, and *Delftia acidovorans* SPH-1 (but not *Comamonas testosteroni* KF-1 or *C. necator* JMP134); and the marine gammaproteobacteria *Chromohalobacter salexigens* DSM 3043 and *Neptuniibacter caesariensis* MED92 utilized homotaurine as a sole source of nitrogen for growth. We chose to use *C. necator* H16 because it is a nonpathogenic organism that is easy to cultivate (e.g., see reference 54) and because it showed the same phenotype as *Burkholderia* sp. strain N-APS2, with which the project was initiated (35).

Growth physiology. *C. necator* H16 grew exponentially in the presence of 3 mM homotaurine and 10 mM succinate, with a specific growth rate (μ) of 0.23 h^{-1} (see Fig. S1A in the supplemental material). The molar growth yield with homotaurine nitrogen [40 g protein (mol N)⁻¹] (see Fig. S1B in the supplemental material) was comparable to the value for growth with ammonium as the nitrogen source [38 g protein (mol N)⁻¹], which indicated complete substrate utilization (6). The specific degradation rate for homotaurine was calculated from these data to be 1.7 mkat (kg protein)⁻¹. Homotaurine was utilized quantitatively and concomitantly with both growth and the excretion of 3-sulfo-3-propanoate. There was no release of sulfate or ammonium ions (see Fig. S1B in the supplemental material) or of 3-sulfo-3-propanal (not shown). The metabolic product, 3-sulfo-3-propanoate, was identified by cochromatography (ion chromatography) with authentic material. These results correspond with data from *Burkholderia* sp. strain N-APS2 (35). 3-Sulfo-3-propanoate was utilized as a sole source of carbon and energy for growth by *Roseovarius* sp. 217 and by the *Cupriavidus* sp. isolate, indicating the biodegradability of the excretion product.

Strain H16 was also found to utilize GABA ($\mu = 0.37 \text{ h}^{-1}$) and β -alanine as sole sources of carbon and of nitrogen for growth, characteristic for this species (then named *Alcaligenes eutrophus*) (28). The organism was already known to utilize taurine (2-aminoethanesulfonate) via sulfoacetaldehyde acetyltransferase (Xsc; EC 2.3.3.15) (e.g., see reference 54).

Enzymes involved in the degradative pathway. As anticipated (35), the activity of homotaurine:2-oxoglutarate transaminase [8.3 mkat (kg protein)⁻¹] (Table 1) was detected in crude cell extracts of *C. necator* H16 grown with homotaurine as the sole nitrogen source. There was negligible activity of the enzyme in extracts of ammonium-grown cells (Table 1), so the enzyme was considered inducible. The reaction products L-glutamate (as the dinitrophenyl derivative) and sulfo-3-propanal (as the *o*-amino-benzaldehyde derivative) were detected. The latter was identified as the DIH derivative by cochromatography with authentic material (35). GABA was also subject to transamination, and the reverse reaction could also be measured (Table 1).

Also as predicted (35), activity of 3-sulfo-3-propanal dehydrogenase [~ 5 mkat (kg protein)⁻¹] was detected in extracts of homotaurine-grown cells of strain H16 (Table 1). The reaction product was 3-sulfo-3-propanoate, whose identity was confirmed

TABLE 1. Specific activities of three enzymes in cell extracts from *C. necator* H16 grown with different sole sources of nitrogen

Enzyme	Substrate(s)	Enzyme sp act [mkat (kg protein) ⁻¹] ^a in extracts from cells grown with:		
		Homotaurine	GABA	Ammonium
Homotaurine:2-oxoglutarate aminotransferase	Homotaurine	8.3	6.5	0.02
	GABA	9.9	8.2	0.03
	Succinate semialdehyde	8.0	Not done	Not done
3-Sulfopropional dehydrogenase	Sulfopropional, NAD ⁺	~5	~3	~0.6
	Sulfopropional, NADP ⁺	~10	~2.5	~0.3
	Succinate semialdehyde, NAD ⁺	7.3	5.0	0.5
	Succinate semialdehyde, NADP ⁺	7.0	0.7	0.5
Glutamate dehydrogenase	2-Oxoglutarate, NADH	1.3	Not done	0.8
	2-Oxoglutarate, NADPH	0.5	Not done	1.3

^a Some values (–) are approximate, because the concentrations of 3-sulfopropional were approximate.

by cochromatography (ion chromatography). Both NAD⁺ and NADP⁺ served as electron acceptors (Table 1). A significant level of activity [0.6 mkat (kg protein)⁻¹] was detected in ammonium-grown cells (Table 1), so constitutive expression of the enzyme was present, together with an inducible component. Succinate semialdehyde was also subject to NAD(P)⁺-dependent oxidation (Table 1).

The activity of an NAD(P)⁺-dependent Gdh was detected at similar specific activities [0.5 to 1.3 mkat (kg protein)⁻¹] in extracts from both homotaurine- and ammonium-grown cells (Table 1). Regeneration of the amino group acceptor 2-oxoglutarate for the deamination of homotaurine was thus largely constitutive.

Extracts of *C. necator* H16 grown with GABA as the sole source of nitrogen contained essentially the same enzyme activities as found in homotaurine-grown cells (Table 1). A preliminary indication that not only the same activities but also the same enzymes were involved was observed with the sulfopropional dehydrogenases, where the enzyme in ammonium-grown cells displayed hyperbolic kinetics with succinate semialdehyde, whereas the enzyme in homotaurine- or GABA-grown cells showed substrate inhibition (see below).

Purification and some properties of homotaurine:2-oxoglutarate aminotransferase. The enzyme was purified to apparent homogeneity (Fig. 2A) from homotaurine-grown cells in three steps (see Table S2 in the supplemental material). The purification factor, about 160, was achieved with about 20% recovery of activity; the apparent loss of activity in the soluble fraction may represent the presence of an inhibitory compound which was subsequently removed.

The molecular mass of the denatured enzyme was about 44 kDa (Fig. 2A). A peptide mass fingerprint of this material indicated no ambiguity (not shown), so a single gene product was present, and it was established as being encoded at locus H16_B0981. The calculated molecular mass of the derived protein was 45.0 kDa, in good agreement with experimental data (Fig. 2A). The molecular mass of the native enzyme, assayed by gel filtration chromatography, was 120 kDa (not shown), so the aminotransferase is a homomultimer. Many aminotransferases are homodimers (16), so given the errors in the assay method ($\pm 50\%$) (32), homotaurine:2-oxoglutarate aminotransferase might be homodimeric. GabT from *E. coli* is tetrameric (33).

Locus tag H16_B0981 is annotated *gabT* (GABA transami-

nase; see the introduction). The possible alternative ω -amino acid substrate influenced our choice of compounds to be examined as amino-group donors. The specific activities, normalized to that with homotaurine [100%; 271 mkat (kg protein)⁻¹], were as follows: 6-aminocaproate (25%), 5-aminovalerate (226%), GABA (270%), 3-aminopropanoate (none detected), taurine (none detected), (S)-cysteate (none detected), 3-aminopropanol (none detected), and 4-aminobutanol (none detected). The K_m^{app} values (V_{max}) for homotaurine, 8.5 mM [310 mkat (kg protein)⁻¹], and GABA, 9.9 mM [340 mkat (kg protein)⁻¹], were very similar. At least five amino-group acceptors were observed with homotaurine as the donor. The specific activities, normalized to that with 2-oxoglutarate [100%; 46 mkat (kg protein)⁻¹], were as follows: pyruvate (63%), glyoxylate (59%), oxaloacetate (52%), and 2-oxobutyrate (9%). The corresponding amino acid (except aspartate) was detected in each case. The K_m^{app} value (V_{max}) for 2-oxoglutarate was 6.3 mM [300 mkat (kg protein)⁻¹].

Enzyme activity was detected between pH values of 7.2 and

FIG. 2. Electropherograms (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of fractions taken during the purification of homotaurine:2-oxoglutarate aminotransferase (A) and of purified sulfopropional dehydrogenase (B) from *C. necator* H16. The impurity at 44 kDa in panel B was shown to be homotaurine:2-oxoglutarate aminotransferase by its enzyme activity and its peptide mass fingerprint. Lanes 1, 7, and 8, molecular mass markers (kDa); lane 2, soluble fraction of ammonium-grown cells; lane 3, soluble fraction of homotaurine-grown cells; lane 4, active fraction after first anion-exchange chromatography; lane 5, active fraction after second anion-exchange chromatography; lane 6, purified enzyme after gel filtration chromatography; lane 9, purified enzyme after gel filtration chromatography.

at least 10.0, with an optimum at pH 9.0 (not shown). The UV-visible spectrum, compared with that of pyridoxal 5'-phosphate (PLP), showed that the cofactor remained bound during purification. Correspondingly, there was no effect of adding PLP to assay mixtures. An experiment to follow reaction stoichiometry indicated the disappearance of 1.6 mM homotaurine and the formation of 1.4 mM glutamate, which we interpret as unit stoichiometry; the formation of sulfopropanal was detected (not shown). This latter material was used as a substrate for the sulfopropanal dehydrogenase discussed in the next section. However, the suspicion that the homotaurine pathway was also, in part, the GABA pathway led us to use succinate semialdehyde as a commercially available substrate.

Purification and some properties of 3-sulfopropanal dehydrogenase(s). The soluble fraction of extract from homotaurine-grown cells of strain H16 was subject to ammonium sulfate fractionation, which precipitated a major portion of the sulfopropanal dehydrogenase activity and left some activity in solution. The activity from each fraction was purified, and each was found to represent a unique gene product. The minor component was examined by peptide mass fingerprinting and found to be encoded by the gene with locus tag H16_B1537 (annotated *gabD3*). This gene was transcribed constitutively (see below) and was presumed to encode the low level of sulfopropanal dehydrogenase observed in ammonium-grown cells (Table 1). This enzyme was unstable. It showed hyperbolic enzyme kinetics (not shown), and it eluted from the anion-exchange column at the same retention time as the major portion of sulfopropanal dehydrogenase.

The major portion of the sulfopropanal dehydrogenase could be extensively purified (Fig. 2B), whereby an impurity of about 44 kDa remained. This was identified as homotaurine transaminase by its activity and its peptide mass fingerprint. The purification resulted in a 28-fold increase in specific activity, with a 1% yield (see Table S3 in the supplemental material). The enzyme was labile (50% loss of activity overnight) but could be largely stabilized (in crude extract only) by the addition of 10 mM DTT. The protein was identified by peptide mass fingerprinting as being encoded by the gene with locus tag H16_B0982 (annotated *gabD1*) and shown to be inducible (see below). The presence of succinate-semialdehyde dehydrogenases which can be separated by ammonium sulfate fractionation has been observed in *E. coli* (8) and *Pseudomonas* sp. (38), and in the latter case, they also represented constitutive and inducible isoenzymes. The molecular mass of 51 kDa deduced from the amino acid sequence of GabD1 was in good agreement with the physical data of the denatured enzyme (49 kDa) (Fig. 2B). Estimates of the molecular mass of the native protein varied from 50 to 100 kDa and might indicate a homodimer. A dimeric succinate-semialdehyde dehydrogenase consisting of 53-kDa subunits is known in *Pseudomonas putida* (43).

Typically, the transformation of 2.2 mM sulfopropanal by 3-sulfopropanal dehydrogenase yielded about 2.0 mM sulfopropanoate. Photometrically, 0.05 mM succinate semialdehyde allowed the formation of 0.05 mM NADH in the presence of excess NAD⁺; similarly, 0.05 mM NADP⁺ yielded 0.04 mM NADPH in the presence of excess succinate semialdehyde. The data were interpreted to represent unit stoichiometry. The enzyme was almost inactive at pH 4.5, but the specific activity

increased steadily to a broad optimum between pH values 8.0 and 10.5, above which no assays were done. 3-Sulfopropanal dehydrogenase was subject to substrate (succinate semialdehyde) inhibition with a K_m^{app} value of $41 \pm 15 \mu\text{M}$ (mean $\pm$ standard deviation) and a K_i^{app} value of $700 \pm 200 \mu\text{M}$. The K_m^{app} value for NAD⁺ (NADP⁺) was $230 \pm 110 \mu\text{M}$ ($350 \pm 190 \mu\text{M}$). These properties are in general agreement with the results of work on GabD in the crude extract of a different strain of *Cupriavidus necator* (34).

3-Sulfopropanal dehydrogenase is encoded by the gene with locus tag H16_B0982 (*gabD1*). It is downstream from and contiguous with H16_B0981 (*gabT*), encoding homotaurine:2-oxoglutarate aminotransferase. The gene at locus H16_B0980 encodes a hypothetical GntR-type regulator on the reverse strand, which we term GabR' (3) (Fig. 1B).

Relevance of sulfoacetaldehyde acetyltransferase (Xsc; EC 2.3.3.15). During the purifications of GabT and GabD1, a strongly induced (~10% of cell protein) 63-kDa protein was observed in the crude extract (Fig. 2A). The suspicion that it represented Xsc in the lower pathway of, e.g., taurine degradation, was confirmed when enzyme assays were positive and the peptide mass fingerprint confirmed the encoding locus as H16_B1870. Xsc is highly specific (42), so we speculated that another gene in the three-gene cluster might be relevant, namely H16_B1872 (Fig. 1B), encoding the sulfite exporter TauE (54). This class of exporters (DUF81) is associated not only with sulfite export (54) but also with export of sulfoacetate (30) and isethionate (Z. Krejčík and A. M. Cook, unpublished). So a candidate exporter was available.

Reverse transcription experiments. The enzyme data identify the metabolic enzymes and the corresponding genes, as shown in Fig. 1A and B, and allow the hypothesis that GabR' is involved in their regulation. There is no information on the uptake of homotaurine, but given the relevance of the *gabRTD* cluster, we used the BLAST algorithm to check for the presence of a *gabP* orthologue and located H16_B1890. Experience with the excretion of sulfite and sulfonates (11, 30, 54) led us to consider orthologues of TauZ (none detected) and TauE (represented solely by the protein encoded at locus H16_B1872) as potential exporters of sulfopropanoate.

gabT transcript was detected inducibly in both homotaurine- and GABA-grown cells (Table 2; also see Fig. S2 in the supplemental material), which confirmed both the appropriate data in Table 1 and the presumption that GabT is both GABA transaminase and homotaurine transferase. *gabD1* transcript was also detected inducibly in both homotaurine- and GABA-grown cells (Table 2), which again confirmed both the data from enzyme assays (Table 1) and the presumption that GabD1 is both succinate-semialdehyde dehydrogenase and 3-sulfopropanal dehydrogenase. It was then shown that *gabT* and *gabD1* were cotranscribed.

The paralogues of *gabD1* (*gabD2*, *-D3*, and *-D4*) were either not transcribed detectably or transcribed constitutively (*gabD3*) (Table 2) to give low activity (Table 1). The gene products are thus of little significance in homotaurine or GABA metabolism.

The *gabP* gene was expressed constitutively (Table 2), so we presume that GabP is always available to transport homotaurine or GABA into the cell. Both the *xsc* and the *tauE* genes were expressed inducibly when *C. necator* H16 was utilizing

TABLE 2. Transcription of genes involved or putatively involved in assimilation of homotaurine nitrogen in *C. necator* H16, and assignment of annotations to enzymes

Gene annotation	Enzyme encoded	Transcription ^a in cells grown with:			Type of expression
		Homotaurine	GABA	Ammonium	
<i>gabT</i>	Homotaurine:2-oxoglutarate aminotransferase	+	+	ND	Inducible
<i>gabD1</i>	Sulfopropional dehydrogenase	+	+	ND	Inducible
<i>gabD3</i>	Sulfopropional dehydrogenase	+	+	+	Constitutive
<i>gabD2</i>	Unknown	ND	ND	ND	
<i>gabD4</i>	Unknown	ND	ND	ND	
<i>gabR'</i>	Putative regulator of <i>gabTD1</i>	(+)	(+)	(+)	Constitutive
<i>gabP</i>	Putative homotaurine transporter	+	+	+	Constitutive
<i>xsc</i>	Sulfoacetaldehyde acetyltransferase	+	+	ND	Inducible
<i>tauE</i>	Putative sulfopropionate exporter	+	+	ND	Inducible

^a +, strong band; (+), weak band; ND, no (or negligible) band detected.

homotaurine or GABA (Table 2), so the hypothesis that TauE is a sulfopropionate exporter is viable.

Transcription of the putative regulator gene *gabR'* was detected in noninduced cells (Table 2), as is usual for this class of regulators (e.g., see reference 56). No increase in levels of transcript was detected in either homotaurine- or GABA-grown cells (Table 2), although this would be expected (56).

Biodiversity in GABA (and homotaurine) utilization. Two different regulators of GABA metabolism are already known, GabC in some enteric bacteria and GabR in spore formers (3, 15), and a third became probable when we generated a dendrogram of representative candidates for GabC and GabR (see Fig. S3 in the supplemental material). A second class of GntR-type regulators, GabR', representing the candidates in *C. necator* H16, *B. xenovorans* LB400, and *D. acidovorans* SPH-1, became obvious. There is presumably at least a fourth class, because the alphaproteobacteria and the marine gammaproteobacteria shown above to utilize homotaurine are not represented in Fig. S3 in the supplemental material. Several different classes of potential transporters (e.g., ABC transporters) were clustered with different *gabTD* genes. The biodiversity of GABA aminotransferases seems high: the GABA aminotransferase of *P. fluorescens* has a substrate spectrum that is completely different from GabT of strain H16 (9; see also reference 57), and some organisms (e.g., *Roseovarius* sp. strain 217) do not use a 2-oxoglutarate-coupled enzyme in homotaurine utilization (J. Mayer, unpublished data). The only gene which seemed to be consistent throughout the candidate pathways was *gabD*.

Whereas the regulation, uptake, and conversion of homotaurine to sulfopropionate seem to include proteins also involved in GABA metabolism, the excretion of an organosulfonate is attributed to a protein in sulfonate metabolism, TauE. Most of the organisms listed in the first paragraph of Results and Discussion express TauE, and we speculate that it is always TauE which exports the organosulfonates, as seen in *Neptuniibacter caesariensis* (30) and *Chromohalobacter salexigens* (Z. Krejčík and A. M. Cook, unpublished). The involvement of part of a different degradative pathway and its control are beyond the scope of the present work, but research on the topic is in progress.

Conclusions. Homotaurine, which is present in marine and terrestrial environments from both natural and anthropogenic sources, was shown to serve as a sole source of nitrogen for a

variety of bacteria. *C. necator* H16, as well as, e.g., *Burkholderia* sp. strain N-APS2, thereby excrete 3-sulfopropionate, which can be further degraded by other organisms.

The discovery that enzymes from GABA metabolism, GabT and GabD1, are involved in the assimilation of homotaurine nitrogen led us to the genes of the putative regulator GabR', clustering with *gabTD*, and the transporter GabP. There is evidence supporting the idea that 3-sulfopropionate is excreted by TauE, a transporter known from the metabolism of other sulfonates (30, 54). Therefore, we term this nitrogen-scavenging pathway, which is constituted by enzymes from GABA and sulfonate metabolism that are encoded by noncontiguous genes, a patchwork pathway.

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