Phototrophic utilization of taurine by the purple nonsulfur bacteria *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*

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Taurine metabolism by two phototrophically grown purple nonsulfur bacteria enrichment isolates has been examined. *Rhodopseudomonas palustris* (strain Tau1) grows with taurine as a sole electron donor, sulfur and nitrogen source during photoautotrophic growth. *Rhodobacter sphaeroides* (strain Tau3) grows on the compound as sole electron donor, sulfur and nitrogen source, and partial carbon source, in the presence of CO$_2$ during photoheterotrophic growth. Both organisms utilize an inducible taurine–pyruvate aminotransferase and a sulfoacetaldehyde acetyltransferase. The products of this metabolism are bisulfite and acetyl phosphate. Bisulfite ultimately was oxidized to sulfate, but this was not an adequate source of electrons for photometabolism. Experiments using either $[^{14}C]$taurine or $^{14}$CO$_2$ demonstrated that *Rb. sphaeroides* Tau3 assimilated the carbon from approximately equimolar amounts of taurine and exogenous CO$_2$. The taurine-carbon assimilation was not diminished by excess non-radioactive bicarbonate. Malate synthase (but not isocitrate lyase) was induced in these taurine-grown cells. It is concluded that assimilation of taurine carbon occurs through an intermediate other than CO$_2$. Similar labelling experiments with *Rp. palustris* Tau1 determined that taurine is utilized only as an electron donor for the reduction of CO$_2$, which contributes all the cell carbon. Photautotrophic metabolism was confirmed in this organism by the absence of either malate synthase or isocitrate lyase in taurine+$^{14}$CO$_2$-grown cells. Culture collection strains of these two bacteria did not utilize taurine in these fashions.

**INTRODUCTION**

Taurine (2-aminoethanesulfonate) is characterized by a carbon-linked sulfur atom at an oxidation state of $+5$, and is found in many different environments. In marine habitats, for example, a diverse group of organisms such as algae, oysters, mussels, copepods, diatoms, vestimentiferan worms and flounder utilize taurine as an osmolyte (Benyajati & Renfro, 2000; Burton & Feldman, 1982; Jackson *et al*., 1992; Pierce *et al*., 1992; Sansone *et al*., 1987; Walther, 2002; Yin *et al*., 2000). While sulfonate compounds are found in many environments, many eukaryotic organisms are unable to attack the carbon–sulfur bond. Therefore, bacteria play an integral role in the metabolism of these compounds and the recycling of global sulfur.

The purple nonsulfur bacteria (PNS) are exceptionally versatile in the range of organic compounds that they can utilize (Dutton & Evans, 1969, 1978; Harwood & Gibson, 1986, 1988; Imhoff & Trüper, 1992; Madigan & Gest, 1979; Pfennig, 1978). These bacteria are unique in the ability to grow by a variety of modes of metabolism. However, it is their versatile photometabolism that distinguishes them from other phototrophic bacteria. PNS are able to use a variety of organic acids, alcohols, sugars and aromatic compounds as carbon sources and/or electron donors during photoheterotrophic growth (Harwood & Gibson, 1986). Their metabolic and physiological diversity makes them an important component of wastewater treatment, photoassimilating and mineralizing a variety of organic compounds (Kobayashi & Nakanishi, 1971).

The ability of the PNS to use carbon-linked sulfur compounds as sources of sulfur and as a source of carbon and electron donors was first demonstrated with thiols (Visscher & Taylor, 1993). In that study, isolates of *Rhodopseudomonas* were able to use these compounds in a more conventional way, cleaving the thiol group of mercaptomalate and using it as a source of electrons for the reduction of CO$_2$. One isolate was also able to grow photoorganotrophically using the thiol sulfur and assimilating the residual carbon for biosynthesis. The utilization of thiol sulfur is consistent...
with the reported ability of the PNS to use low levels of \( \text{H}_2\text{S} \) as a source of electrons (Imhoff & Trüper, 1992). Recently, it was demonstrated that \textit{Rhodobacter capsulatus} was able to grow phototrophically with taurine as sulfur source (Masepohl et al., 2001), but this work did not address the possible utilization of taurine either as a carbon source or as an electron donor; no mention was made of the desulforative abilities of other PNS.

Previous reports have demonstrated the anaerobic desulfonation of taurine (Chien et al., 1995; Denger et al., 1996, 1997a). Subsequent research has established the importance of inducible enzyme activities for the anaerobic metabolism of this compound (Chien et al., 1997; Lau & Cook, 2000; R. T. Novak and others, unpublished results).

Also, the utilization of taurine as a source of carbon and electrons under anoxic, nitrate- and sulfate-respiratory growth conditions has been established (Denger et al., 1997a; Lie et al., 1999). We now report a new aspect of anaerobic taurine metabolism: two strains of PNS utilize the sulfonate taurine as an electron donor for phototrophic growth. In addition, we will show that these organisms can utilize the sulfonate as a sole source of nitrogen and sulfur and, in one strain, as a partial source of carbon.

**METHODS**

**Isolation and growth of taurine-utilizing PNS.** Enrichment cultures were grown in a defined minimal salts plus vitamins (MSV) medium used for phototrophic growth, with taurine as the electron donor. The medium contained 50 mM taurine as a sole electron donor, nitrogen and sulfur source, 30 mM sodium bicarbonate, 0-1 M potassium phosphate (pH 6.8) and 1% (v/v) mineral base, where chloride salts replaced sulfate salts (Cohen-Bazire et al., 1957). Following autoclaving, 2 ml 1⁻¹ of filter-sterilized vitamin solution was added, containing (per 100 ml): for \textit{Rhodopseudomonas palustris} (\textit{Rp. palustris}), p-aminobenzoic acid (4 mg); for \textit{Rhodobacter sphaeroides} (\textit{Rb. sphaeroides}), thiamin (50 mg), biotin (2 mg) and nicotinic acid (100 mg); for \textit{Rhodobacter capsulatus} (\textit{Rb. capsulatus}), thiamin (50 mg). The vitamins provided reflected the needs of the PNS sought (van Niel, 1971). The sterile media were stored anaerobically under CO\(_2\)/argon gas (20:80, v/v). Portions (10 ml) were transferred to sterile screw-capped tubes filled to capacity. The inocula (1%, v/v) were untreated sediment slurry samples from Swan Lake on the University of Connecticut campus. Solid media were prepared by addition of 1% (w/v) Bacto Agar.

Enrichment cultures were incubated at 27°C under incandescent light. Cultures deemed to be phototropic bacteria (light dependence, development of pigments) were subcultured twice in the above medium. Pure cultures were obtained following growth on agar media at 27°C in glass Brewer jars under incandescent light. GasPak H\(_2\) and CO\(_2\) gas generators (BBL) with palladium catalyst were used to produce anoxic conditions. Culture purity was checked microscopically and by growth on complex medium (1%, w/v, yeast extract, 1%, w/v, Bacto Agar).

Phototrophic growth experiments with the pure cultures were done with 20 mM taurine (unless otherwise indicated for individual experiments) in minimal salts medium (9-5 ml) added to modified Hungate tubes (Miller & Wollin, 1974); the headspace of the tubes was initially 100% argon. Following autoclaving, filter-sterilized vitamin mix (described above) was added (2 ml 1⁻¹). Filter-sterilized, anoxic sodium bicarbonate (concentration dependent on individual experiment) was then added to each tube. In some experiments, cells were grown in a non-sulfonate medium [0-1 M potassium phosphate buffer, pH 6.8, 30 mM sodium bicarbonate, 20 mM sodium acetate, 10 mM ammonium chloride, 100 mM sodium sulfate, mineral base and vitamin mix, with a head space of CO\(_2\)/argon (20:80, v/v)]. Aerobic (dark) growth studies were conducted using a similar medium without added CO\(_2\), with 30 mM succinate as a carbon and energy source and taurine as a sole nitrogen and sulfur source, and cultures were incubated at 27°C in Erlenmeyer flasks on an orbital shaker (New Brunswick Scientific) at 140 r.p.m.

**Taxonomic status of strains Tau1 and Tau3.** Cell lysis, amplification of 16S rDNA genes and 16S rRNA gene sequencing procedures are described in detail elsewhere (Dewhurst et al., 1999). For identification of closest relatives, the sequences from strains Tau1 and Tau3 were compared to the 16S rDNA gene sequences of over 4000 microorganisms in the Forsyth Institute’s database, 16000 sequences in the Ribosomal Database Project (RDP; Maikad et al., 2000), and GenBank.

**Chemicals.** All of the chemicals used in this study were of the highest purity from Fisher Scientific, Sigma/Aldrich Chemical Co. (Taurine ~99%, no. T 0625), Difco and Roche-Boehringer. Sulfonate/acytelylde hydrosulfite adduct (SABA) was synthesized as described by Kondo et al. (1971). The structure was confirmed by electrospay mass spectrometry (Gritzer et al., 2003). Gas (argon and CO\(_2\)/argon) was ultrahigh purity grade supplied by Airgas East.

**Phototrophic growth physiology and analytical methods.** The consumption of substrate (taurine) and the formation of one product (sulfate) were routinely assayed by HPLC, using refractometric methods following chromatography on an ion-exclusion and partition/adsorption column (Shodex KC811), with a conductivity detector (Waters) used in conjunction with a Shodex KC-810P precolumn; the mobile phase was 0-1% phosphoric acid at 70°C, 1 ml min⁻¹ flow rate, and 400 p.s.i. (2760 kPa). Products were identified by comparison to the elution profiles of authentic reference compounds and quantified using standard curves. Sulfate ion was assayed using a modification of the BaCl\(_2\) technique (Schauder et al., 1986). Because of interference by some low-molecular-mass species that were potential metabolites, we were unable to identify definitively one of the products of taurine metabolism (which we suspected to be sulfate) simply by a comparison to a reference compound. Cultures were grown using media with 0-1 M Tris buffer (pH 6-8) replacing phosphate buffer, which, like sulfate and sulfite, is precipitated by barium and hence interferes with this determination. The culture supernatants were assayed by HPLC following treatment with BaCl\(_2\) and centrifugation; where this caused disappearance of a peak with an elution time essentially that of sulfate (which was well separated from sulfite) we identified that peak as sulfate.

**Cell extracts and enzymology.** Cells were grown in MSV medium with 20 mM taurine and 20 mM bicarbonate in 500 ml screw-capped bottles with butyl rubber septa (which allowed introduction of substrates or withdrawal of samples with syringes) and
an initial headspace of 100% argon. Mid-exponential-phase cells were harvested by centrifugation and washed with ‘breaking buffer’ (50 mM potassium phosphate, 100 μM pyridoxal 5’-phosphate, pH 7.5) three times. After resuspension in a small volume of buffer (0.4 g wet weight cells ml⁻¹), the cells were broken using a French pressure cell at 15 000 p.s.i (104 MPa). The preparation was then centrifuged (12 000 g, 20 min, 4°C) to remove whole cells and cell debris, and the supernatant was subsequently ultracentrifuged (63 000 g, 60 min, 4°C) to sediment cell membranes. Crude cell extracts were stored on ice and used for enzyme assays on the same day. Protein concentrations were measured by the bicinchoninic acid method (Smith et al., 1985), with BSA as a protein standard.

**In vitro assay of aminotransferase activity.** The enzyme assay system contained 10 μmol of an amino donor (taurine, β-alanine, 2-aminoethanesphosphate, 1-butyramine, 1-aminobutyric acid, 1-butanesiamide, spermidine, spermine, 1,6-hexanediame or 1,7-heptanediame), 20 μmol of an amino acceptor (pyruvate, oxalacetate or 2-oxoglutarate), 100 μmol of potassium phosphate (pH 8.5), 4 μg of pyridoxal 5’-phosphate and 100 μl cell extract (0–1–0.2 mg protein) in a final volume of 400 μl. The reaction mixture was incubated at 37°C for 60 min, and then inactivated by heat (90°C, 10 min). Following heating, denatured proteins were removed by centrifugation (10 000 g, 5 min, 25°C). In some experiments, amino acids formed in the reaction mixture were separated by TLC on cellulose by isobutyric acid/water (4:1, v/v), along with the amino acid standards alanine, aspartate and glutamate. The amino acids were detected using ninhydrin spray (0.5% ninhydrin in 95% ethanol).

**Characterization and quantitative determination of taurine–pyruvate aminotransferase.** The calculated activity of taurine–pyruvate aminotransferase (EC 2.6.1.77) was based on a two-part fixed-time assay, as described by Chien et al. (1997). In the first part of the assay, alanine was formed from the transamination of taurine and pyruvate. In the second part of the assay, the alanine formed was quantitatively determined using commercial alanine dehydrogenase (Sigma) (Yoshida & Freese, 1965). Various amino donors and amino acceptors (listed above) were tested as replacements for taurine and pyruvate.

**In vitro assay of sulfoacetaldehyde desulfonation.** Desulfonation of sulfoacetaldehyde by sulfoacetaldehyde acetyltransferase (EC 2.3.3.15), forming bisulfite and acetyl phosphate, by cell extracts, was examined. The enzyme system contained 5 mM taurine and 5 mM pyruvate, or 5 mM SABA, plus 0·1 M potassium phosphate buffer, pH 7·5, 0·1 mM pyridoxal 5’-phosphate (omitted when SABA was the substrate), 1 mM thiamin pyrophosphate, 5 mM MgCl₂ and 1 mg protein ml⁻¹. The reaction mixture was incubated at 30°C for 60 min, and the enzymes then chilled in an ice bath. Controls contained boiled extract (100°C, 15 min), or were without substrate. The presence of desulfonation activity was based on the measurement of bisulfite and acetyl phosphate produced (liberated from sulfoacetaldehyde, the transamination product of taurine, or SABA) during the fixed-time assay. Sulfoacetaldehyde is unstable in solution, but SABA (the stable bisulfite adduct) functions as a substrate in this assay (Gréziter et al., 2003).

**Determination of bisulfite.** Assays for the determination of bisulfite formed in the desulfonation assay contained 10 μg of Ellman’s reagent [5,5’-dithiobis-(2-nitrobenzoic acid)] and 10 μl of assay mixture containing reaction product formed in the complete standard assay system (described above), in a final volume of 1 ml. In this latter assay system, bisulfite formed from desulfonation activity was determined by measuring the A₄₁₅ at 0₉ and at 60 min. Controls showed that loss of bisulfite due to oxidation to sulfate was negligible during the assay period. In controls where boiled extracts were used, small amounts of bisulfite were produced chemically, and values for other samples were corrected accordingly. When SABA was used as an assay substrate, the reagent reacted with the adduct bisulfite, yielding a background that was subtracted from experimental values (for assays in which desulfuration of the sulfonates produced additional Ellman reactivity).

**Determination of acetyl phosphate.** One of the products of sulfoacetaldehyde acetyltransferase has been identified previously as acetyl phosphate (Ruff et al., 2003). This product was carefully preserved and identified in our extracts of taurine-grown cells by the procedure (reaction with hydroxylamine to form hydroxamic acids which are then complexed with ferric ion to form coloured products) of Stadtman (1957). The assay volume was reduced to 1·5 ml, and succinic anhydride was used as standard.

**Assay of glyoxylate cycle enzymes.** Cell extracts were also assayed for the presence of (inducible) glyoxylate cycle enzymes (malate synthase, isocitrate lyase), following the procedure of Dixon & Kornberg (1959).

**Incorporation of taurine carbon into cell material: radio-labelling experiments.** Rb. palustris Tau1 and Rb. sphaeroides Tau3 were grown in 20 mM taurine (taurine-limiting)/20 mM CO₂/MSV medium to an OD₆₅₀ of ~ 1·0. Two separate sets of tubes were labelled with either [U-¹⁴C]taurine or [¹⁴C]CO₂ at a specific activity of 600 c.p.m. (μg-atom carbon)⁻¹. Samples (1 ml) were removed and collected on filters (25 mm diameter × 0·45 μm pore size; Millipore) and washed three times with 5 ml portions of distilled, deionized water. The filters were placed in 5 ml scintillation cocktail (Optifluor, Packard Instruments). The radioactivity incorporated into cells was quantified with an ISOCAP/300 liquid scintillation counter (Searle) and expressed as μg-atom carbon (OD₆₅₀ unit) of culture⁻¹. The number of OD₆₅₀ units was obtained by multiplying the OD at 650 nm by the sample volume in ml.

The same cultures (tubes) were then acidified with concentrated HCl to release dissolved CO₂. The culture was bubbled with N₂ for 20 min (10 ml min⁻¹) into 14CO₂ traps consisting of two 7 ml scintillation vials in series filled with 0·5 ml β-phenethylamine in 4 ml scintillation cocktail (Brune et al., 1995).

**Phototrophic growth requirement for CO₂.** These organisms would not grow phototrophically using taurine in the absence of CO₂. To assess the proportion of cellular carbon derived from each source, a medium was used that contained [¹⁴C]taurine (20 mM) at a specific activity of 3000 c.p.m. (μg-atom carbon)⁻¹ and variable amounts of non-radioactive bicarbonate (10–200 mM). Cells were harvested, and the CO₂ from the supernatant was trapped and counted as described above. Incorporation of radioactivity into cell material was quantified as described in the previous section.

In order to assess whether the CO₂ fixation during phototrophic growth of Rh. sphaeroides Tau3 occurred over the entire course of growth or only at a particular growth phase, a medium was constructed as above with 20 mM taurine and 60 mM bicarbonate, the latter labelled with [¹⁴C]CO₂ at a specific activity of 600 c.p.m. (μg-atom carbon)⁻¹, in a total volume of 400 ml in 500 ml screw-capped serum bottles with butyl rubber septa. Samples were removed (0·5 OD₆₅₀ units of cells, e.g. 5 ml of a culture of OD₆₅₀ 0·1) at various times during growth (OD₆₅₀ ~ 0·1, 0·2, 0·3, 0·4, 0·6, 1·0 and 1·2) and counted; the supernatant was acidified and CO₂ trapped as described above. Radioactivity incorporated into cell material was quantified as described above.
RESULTS

Isolation and identification of strains
Combinations of physiological, morphological and phylogenetic analysis were used to identify the environmental isolates. Of 10 tertiary enrichments on taurine, 4 were positive; our desire to have two different species represented led to the selection of strains Tau1 and Tau3 for further study. Pigment profiles with ATCC strains as references, cell morphology and electron microscopy suggested that the identity of Tau3 was characteristic of *Rb. sphaeroides*, and that of Tau1 characteristic of *Rp. palustris*. In addition, the growth of Tau1 on benzoate as a source of carbon and electrons suggested that this isolate was indeed a *Rp. palustris* strain. The full 16S rRNA genes of both strains were sequenced, and a BLAST search of the ribosomal database (Maidak *et al.*, 2000) confirmed that Tau3 was in fact *Rb. sphaeroides*, and that Tau1 was *Rp. palustris*.

Phototrophic growth of organisms with taurine
*Rb. sphaeroides* ATCC 17023T and isolate Tau3, and *Rp. palustris* ATCC 17001T and isolate Tau1 were examined for their ability to grow phototrophically using taurine. None of these organisms demonstrated significant growth with taurine as sole carbon, nitrogen and sulfur source and electron donor. Only the environmental isolates Tau1 and Tau3 were able to grow (cf. Fig. 1 for Tau1: the results for Tau3 were virtually identical) with taurine as sole electron donor, sulfur and nitrogen source, and only when CO2 was provided as well. Neither organism was able to grow with sulfate as sole electron donor, but both were able to utilize acetate as electron donor.

Growth physiology of phototrophically grown *Rp. palustris* Tau1 and *Rb. sphaeroides* Tau3
The isolates *Rp. palustris* Tau1 and *Rb. sphaeroides* Tau3 were able to grow exponentially with taurine as a sole electron donor and source of sulfur and nitrogen. These isolates had essentially identical doubling times (170 min) in this medium; this doubling time was identical to that observed when cells were grown in non-sulfonate medium. *Rp. palustris* Tau1 had a significantly longer lag time (data not shown) than *Rb. sphaeroides* Tau3 (Fig. 1) when grown in taurine + CO2 medium, regardless of whether cells were transferred from non-sulfonate medium or taurine medium. Using HPLC, it was found that 20 mM taurine was completely consumed over the period of growth by both organisms. Data for *Rb. sphaeroides* Tau3 are shown in Fig. 1; the results for *Rp. palustris* Tau1 are not shown but were virtually identical. No detectable carbon products were found in the supernatant following growth. However, sulfate (Fig. 1) and ammonium [1 mol (mol taurine consumed)]−1; data not shown] were present in the supernatant following growth.

Enzymology of growth with taurine
Extracts of *Rp. palustris* Tau1 and *Rb. sphaeroides* Tau3 grown phototrophically using taurine as sole electron donor and sulfur and nitrogen source, and also of cells grown aerobically in the dark using succinate as a carbon and energy source and taurine as sole sulfur and nitrogen source, were assayed for the presence of enzyme activities (below) known to be involved in taurine metabolism (Chien *et al.*, 1997; Denger *et al.*, 2001; Laue & Cook, 2000; Ruff *et al.*, 2003).

Taurine–pyruvate aminotransferase. Those organisms grown phototrophically using taurine contained inducible aminotransferase activity; this was specific for the transamination of taurine or its carboxyl analogue, β-alanine, with pyruvate as amino acceptor (Table 1; only data for *Rb. sphaeroides* aminotransferase are shown; results were essentially identical for *Rp. palustris* cells). Growth with β-alanine + sulfate also induced an aminotransferase activity in a similar amount and with similar substrate specificity. This induction was also observed in *Clostridium pasteurianum* C1 grown in the presence of β-alanine, and may reflect the structural similarity of β-alanine to the sulfonate (unpublished data). As we describe below, the acetyltransferase was induced by β-alanine as if it and the aminotransferase were co-induced by the amino donor. These observations are consistent with the idea that the aminotransferase activities induced by either compound represent the same enzyme, but more detailed studies are needed to establish this point. In contrast, cells grown aerobically in the dark, with taurine as sole sulfur and nitrogen source, contained an aminotransferase that was not specific for taurine (results not shown). Activity was not detected when either taurine or pyruvate were left out of the assay, and was also absent when heat-inactivated.

Fig. 1. Phototrophic growth of *Rb. sphaeroides* strain Tau3 with 20 mM taurine and 20 mM bicarbonate (1 OD unit = 2.5 mg protein ml−1). Taurine disappearance (●) and sulfate formation (▲) are shown as a function of time in relation to growth (●) measured as OD650. Data are representative values.
**Table 1.** Substrates for the taurine–pyruvate aminotransferase activity in cell extracts of phototrophically grown *Rb. sphaeroides* Tau3

Aminotransferase activity is given as a percentage of total product formed *in vitro* from phototrophically grown, taurine-utilizing cell extracts when taurine was used as a substrate in the complete assay system.

<table>
<thead>
<tr>
<th>Amino-group donor</th>
<th>Aminotransferase activity</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Pyruvate = amino acceptor)</td>
<td>Sulfur source + electron donor for growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Taurine*</td>
<td>Sulfate + β-alanine</td>
</tr>
<tr>
<td>Taurine</td>
<td>100 (0-5)†</td>
<td>103</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>108</td>
<td>116</td>
</tr>
<tr>
<td>2-Aminoethanephosphonate</td>
<td>3-1</td>
<td>9-2</td>
</tr>
<tr>
<td>1-Butylamine</td>
<td>12</td>
<td>7-9</td>
</tr>
<tr>
<td>1-Amylamine</td>
<td>8-1</td>
<td>4-6</td>
</tr>
<tr>
<td>Spermidine</td>
<td>4-7</td>
<td>2-8</td>
</tr>
<tr>
<td>Spermine</td>
<td>4-2</td>
<td>2-6</td>
</tr>
<tr>
<td>1,4-Diaminobutane</td>
<td>3-1</td>
<td>7-4</td>
</tr>
<tr>
<td>1,6-Diaminohexane</td>
<td>2-6</td>
<td>6-5</td>
</tr>
<tr>
<td>1,7-Diaminoheptane</td>
<td>2-1</td>
<td>3-1</td>
</tr>
<tr>
<td>Taurine (no PLP ‡)</td>
<td>101</td>
<td>97</td>
</tr>
<tr>
<td>None</td>
<td>0-2</td>
<td>0-3</td>
</tr>
<tr>
<td>Heat-inactivated extract</td>
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<table>
<thead>
<tr>
<th>Amino-group acceptors</th>
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</thead>
<tbody>
<tr>
<td>(Taurine = amino donor)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>100</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>0</td>
</tr>
<tr>
<td>Oxalacetate</td>
<td>56</td>
</tr>
</tbody>
</table>

*Phototrophically grown cells using taurine as a sole electron donor, and nitrogen, sulfur and partial carbon source.
†Value in parentheses is μmol alanine (mg protein)^−1.
‡PLP, pyridoxal 5′-phosphate.

Extracts of β-alanine + sulfate-grown cells also contained desulfonation activity (Table 2). Thus, induction of the ability to deaminate β-alanine seems also to induce the desulfonation activity, despite the absence of a sulfonate.

### Incorporation of taurine and CO₂ carbon into cell material

Taurine and bicarbonate dose–response curves for both organisms demonstrated that 20 mM taurine and bicarbonate were sufficient to support extensive growth (OD₆₅₀nm 1±2). By labelling each compound (in separate experiments) to a known specific activity, it was possible to estimate the proportion of each incorporated into cell material.

[U-14C]Taurine incorporation versus 14CO₂ incorporation during phototrophic growth. In order to determine whether taurine was used as a carbon source, as opposed to merely as an electron donor to provide the reducing power for CO₂ reduction, separate tubes containing 20 mM taurine and 60 mM bicarbonate were labelled with either [U-14C]taurine or [14C]bicarbonate to a specific
activity of 3000 c.p.m. (μg-atom carbon)$^{-1}$. *Rh. sphaeroides* Tau3 and *Rp. palustris* Tau1 were inoculated and harvested (OD$_{650}$ 1-2), and the cellular radioactivity was quantified (Table 3). In *Rp. palustris* Tau1, almost all of the cellular carbon was derived from CO$_2$. *Rh. sphaeroides* Tau3, on the other hand, derived a majority of its cellular carbon from taurine, but also incorporated some CO$_2$. As shown earlier, in both organisms, taurine is completely consumed, and there were no detectable carbon products of its metabolism excreted into the supernatant. Thus, we surmised that any carbon was either incorporated into cell material or oxidized to CO$_2$. The supernatants from cultures grown with $[^{14}C]$taurine were examined for the presence of either labelled taurine or CO$_2$ (Table 3). Following acidification of the media and measurement of the trapped $^{14}$CO$_2$, the culture supernatant contained negligible label. Thus, in both organisms, all of the taurine carbon was either incorporated into cell material or oxidized to CO$_2$. 

[$^\text{U}$-$^{14}$C]Taurine incorporation as a function of bicarbonate concentration during phototrophic growth. In order to establish whether taurine carbon was assimilated as a reduced metabolic intermediate or only as CO$_2$, *Rh. sphaeroides* Tau3 and *Rp. palustris* Tau1 were grown in MSV medium with 20 mM taurine labelled at a specific activity of 3000 c.p.m. (μg-atom carbon)$^{-1}$ together with varying amounts of non-radioactive bicarbonate (10–200 mM). If all of the carbon were assimilated in the form of CO$_2$, then increasing amounts of bicarbonate

Table 3. Fate of labelled taurine or bicarbonate in *Rp. palustris* Tau1 and *Rh. sphaeroides* Tau3

Each labelled compound was labelled with a specific activity of 3000 c.p.m. (μg-atom C)$^{-1}$. ODU, optical density unit, obtained by multiplying OD$_{650}$ of the culture by volume sampled (ml). Each cell mass value is the mean of triplicate assay, standard deviation ± 1%. Expected values are those which would be obtained if all $^{14}$C not incorporated into cell mass were present as CO$_2$.

<table>
<thead>
<tr>
<th>Labelled compound</th>
<th>Organism</th>
<th>Fate of labelled carbon (μg-atoms carbon ODU$^{-1}$)</th>
<th>Cell mass</th>
<th>CO$_2$</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine (20 mM)</td>
<td><em>Rp. palustris</em> Tau1</td>
<td></td>
<td>0:12</td>
<td>31:2</td>
<td>39:9</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rh. sphaeroides</em> Tau3</td>
<td></td>
<td>7:3</td>
<td>24:3</td>
<td>32:7</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (60 mM)</td>
<td><em>Rp. palustris</em> Tau1</td>
<td></td>
<td>22:0</td>
<td>31:5</td>
<td>38:0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rh. sphaeroides</em> Tau3</td>
<td></td>
<td>4:0</td>
<td>44:6</td>
<td>56:0</td>
<td></td>
</tr>
</tbody>
</table>
would dilute any $^{14}$CO$_2$ arising from [U-$^{14}$C]taurine, and the observed amount of $^{14}$C (OD$_{650}$ unit)$^{-1}$ would decrease as the bicarbonate concentration increased. In *Rb. sphaeroides* Tau3, the amount of labelled carbon in cell material decreased from 14.6 to 7.0 μg-atoms from taurine (OD$_{650}$ unit)$^{-1}$ as the bicarbonate concentration was increased from 10 to 60 mM (Fig. 2), but then levelled off at about 7.0 μg-atoms from taurine (OD$_{650}$ unit)$^{-1}$ between 60 and 200 mM bicarbonate. The higher proportion of labelled carbon in cell material at low concentrations of bicarbonate was most likely the result of $^{14}$CO$_2$ generation from taurine which was not much diluted by exogenous bicarbonate but was assimilated for growth. In contrast, the amount of labelled taurine diluted by exogenous bicarbonate but was assimilated in Tau3. The mean of at least two independent determinations that agreed to within 5%. Growth (•) was measured as a function of OD$_{650}$ (1 OD unit = 2.5 mg cellular protein ml$^{-1}$). The medium contained 20 mM taurine and 60 mM bicarbonate. OD data are representative values. Data for $^{14}$CO$_2$ OD unit$^{-1}$ are from duplicate, independent determinations that agreed to within 5%.

**Kinetics of $^{14}$CO$_2$ incorporation into cell material in *Rb. sphaeroides* Tau3**

*Rb. sphaeroides* Tau3 was further examined to determine if the requirement for CO$_2$ was a result of incorporation at a particular point in growth or was consistent throughout phototrophic growth. MSV was employed containing 20 mM non-radioactive taurine and 60 mM bicarbonate labelled at a specific activity of 600 c.p.m (μg-atom carbon)$^{-1}$. The higher concentration of bicarbonate was used in order to minimize the effect on the specific activity of $^{14}$CO$_2$ generated from taurine. Cells were harvested at various points throughout the growth of the organism (OD$_{650}$ 0.1–1.1), and μg-atoms of carbon from $^{14}$CO$_2$ (OD$_{650}$ unit)$^{-1}$ was determined (Fig. 3). Throughout growth, the amount of $^{14}$CO$_2$ incorporated was constant, at about 4 μg-atoms from $^{14}$CO$_2$ (OD$_{650}$ unit)$^{-1}$. Thus, a fixed proportion of CO$_2$ carbon is required at all times for growth with taurine.

**Glyoxylate cycle enzymes**

To determine enzyme activities possibly involved in the assimilation of taurine carbon, we assayed both *Rp. palustris* Tau1 and *Rb. sphaeroides* Tau3 for the two glyoxylate cycle enzymes malate synthase (MS) and isocitrate lyase (ICL) (Table 4). The cells were grown phototrophically with either 20 mM acetate or taurine + CO$_2$. *Rp. palustris* Tau1 grown with acetate contained a complete glyoxylate cycle (ICL and MS), while similarly grown *Rb. sphaeroides* Tau3 only contained MS. *Rb. sphaeroides* has not been reported to contain a complete glyoxylate cycle (Albers & Gottschalk, 1976), and genomic information has been used for confirmation (Joint Genome Institute, 2003). When cells were grown with taurine + CO$_2$, neither enzyme activity was

**Table 4. Levels of malate synthase and isocitrate lyase in cell extracts of *Rp. palustris* Tau1 and *Rb. sphaeroides* Tau3 grown phototrophically with acetate or taurine + CO$_2$**

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Organism</th>
<th>Malate synthase* (U mg$^{-1}$)</th>
<th>Isocitrate lyase† (U mg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td><em>Rp. palustris</em> Tau1</td>
<td>0.55</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td><em>Rb. sphaeroides</em> Tau3</td>
<td>0.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Taurine</td>
<td><em>Rp. palustris</em> Tau1</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td><em>Rb. sphaeroides</em> Tau3</td>
<td>0.51</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

*1 unit is defined as 1 μmol coenzyme A consumed min$^{-1}$.
††1 unit is defined as 1 μmol glyoxylate formed min$^{-1}$.
present in cell extracts of *R. palustris* Tau1, while only MS was induced in *Rb. sphaeroides* Tau3. Cells grown photo- trophically with succinate did not contain either enzyme activity (data not shown).

**DISCUSSION**

Although the sulfonate taurine is known to be metabolized by various aerobic and anaerobic organisms (Chien *et al.*, 1999; Denger *et al.*, 1997a, b; Laue *et al.*, 1997; Shimamoto & Berk, 1979), this is believed to be the first demonstration of the sulfonate serving as an electron donor for phototrophic metabolism. This occurred in environmental isolates obtained by enrichment on taurine; the failure of stock cultures of *R. palustris* and *Rb. sphaeroides* to utilize taurine in this way may reflect the loss of this metabolic capability during prolonged cultivation on other substrates, as was found for *Comamonas acidovorans* (Seitz *et al.*, 1993). Nonetheless, both type-culture strains, as well as both enrichment isolates, were able to use taurine as sole sulfur source for both phototrophic and respiratory growth (our unpublished results).

Both *R. palustris* and *Rb. sphaeroides* are known to be able to grow using acetate either as a source of carbon (photoheterotrophic) or only as an electron donor (photautotrophic) (Albers & Gottschalk, 1976; Barbosa *et al.*, 2001; Berg *et al.*, 2002; Schauder *et al.*, 1986; VigenSchow *et al.*, 1986; Yang *et al.*, 2002). Both isolates Tau1 and Tau3 grown with taurine + CO2 contained the core enzyme activities of anaerobic taurine metabolism: taurine–pyruvate aminotransferase and sulfoacetaldehyde acetyltransferase. Interestingly, our own (unpublished) searches of available sequence databases reveal an ORF with high sequence similarity to a known taurine–pyruvate aminotransferase in *Rb. sphaeroides*, and one with high similarity to omega amino acid aminotransferase in *R. palustris*. The products of this taurine metabolism are bisulfite and acetyl phosphate. Acetate (derived from acetyl phosphate) is the most likely candidate for an intermediate electron donor for photometabolism, since sulfite does not serve as sole electron donor in either organism (data not shown). However, the pathway between acetate phosphate and cell material apparently is complicated and has not been completely elucidated either by us or by others (Albers & Gottschalk, 1976; Berg *et al.*, 2002), although a ‘citramalate cycle’ has been proposed. In this cycle, citramalate would be formed by condensation of pyruvate and acetyl-CoA and ultimately converted to glyoxylate and regenerated pyruvate; the glyoxylate could be converted to malate by malate synthase and thus be assimilated into cellular metabolites without involvement of either isocitrate or its lyase.

*R. palustris* strain Tau1 grew with taurine as sole electron donor, sulfur and nitrogen source in the presence of CO2 (Fig. 4a). Studies of dose–response, with varying amounts of non-radioactive bicarbonate, that demonstrated linear growth yield over the range 1–20 mM (data not shown), suggested that the limiting nutrient for growth was CO2; hence growth is photoautotrophic (data not shown). Experiments where either the taurine or the CO2 carbon was labelled in taurine+CO2 medium demonstrated that the carbon in cell mass was derived entirely from CO2.

*Rb. sphaeroides* strain Tau3 also grew with taurine as sole electron donor, sulfur and nitrogen source in the presence of CO2; but, in contrast to the *R. palustris* strain, it derived a majority of its cellular carbon from taurine (Fig. 4b). Dose–response studies with varying amounts of non-radioactive bicarbonate suggested that the limiting nutrient for growth was taurine (data not shown). Experiments where either the taurine or the CO2 carbon was labelled in taurine+CO2 medium demonstrated that the carbon in cell mass was derived from both taurine and CO2 carbon.

‘Bicarbonate dilution curves’ in the presence of labelled taurine supported this observation. [14C]Taurine carbon was assimilated at a constant level when the bicarbonate concentration was at a level that greatly diluted any 14CO2 possibly generated from acetate (from taurine) oxidation. When the incorporation of 14CO2 was measured during growth, we found that CO2 was incorporated at a constant level, regardless of growth phase. This suggests that CO2 assimilation is not limited to early stages of the growth cycle, as might be the case for cells growing photoheterotrophically in this phase, then switching to photoheterotrophic growth. Instead, the constant incorporation of CO2 per unit cell mass suggests that it is a required component of a carboxylation reaction during the assimilation of acetyl phosphate or another intermediate of taurine metabolism. The incorporation of approximately 1 µg-atom of carbon from CO2 per 2 µg-atoms from taurine (Table 3 and Figs 2 and 3) suggests the involvement of a single carboxylation of each two-carbon unit from taurine.

The two isolates differed in their ability to assimilate taurine carbon, even though some similar taurine metabolic enzymes and (presumably) transport mechanisms are present. Why then is only *Rb. sphaeroides* Tau3 capable of assimilating taurine carbon? In *Alcaligenes defragns* the actual carbon-containing product of sulfoacetaldehyde acetyltransferase is acetyl phosphate (Ruff *et al.*, 2003). We assume that acetyl phosphate is converted to acetyl-CoA, and subsequently acetate, in *Rb. sphaeroides*. Therefore, the answer to the question posed may relate to the apparent induction of the glyoxylate-cycle enzyme activities. *R. palustris* Tau1 grown photoheterotrophically with acetate contained both glyoxylate cycle enzymes. However, growth with taurine + CO2 resulted in induction of neither enzyme. Taurine (20 mM) was completely metabolized in cultures of this organism. Therefore, it may be that the acetate generated from taurine is readily consumed for the reduction of CO2 to cell material, and that acetate does not accumulate in the cell to a level sufficient to induce the glyoxylate cycle. It is somewhat surprising that taurine is not used as a substrate for photoheterotrophic growth by *R. palustris* Tau1 in the absence of CO2; this may be a reflection of transport efficiency.
On the other hand, *Rhodobacter sphaeroides* Tau3 assimilates taurine carbon for a majority of cell mass. The metabolism of acetate in this organism is complex, and to date has not been completely elucidated (Birgit Albers, personal communication; Albers & Gottschalk, 1976; Berg et al., 2002). Our isolate contained an inducible malate synthase in both acetate- and taurine-grown cells, but no isocitrate lyase was detected; this is consistent with a genomic analysis of this organism (Joint Genome Institute, 2003). There have been suggestions that pyruvate carboxylase is involved in the synthesis of C₄ dicarboxylic acids from acetate and CO₂, but a mutant strain of *Rhodobacter sphaeroides* devoid of pyruvate carboxylase was still able to grow with acetate and CO₂ (Payne & Morris, 1969), so there must be additional mechanisms for acetate assimilation in the presence of CO₂ which do not involve pyruvate carboxylase. Regardless of the mechanism involved, the data presented here indicate that taurine-carbon assimilation during phototrophic growth of *Rhodobacter sphaeroides* Tau3 requires CO₂; nonetheless, the phototrophic utilization of taurine carbon suggests a new aspect of sulfonate carbon cycling.

This work describes the first example of a sulfonate utilized as a sole electron donor, sulfur and nitrogen source by members of the purple nonsulfur bacteria, but this occurs only under phototrophic conditions. In addition, it also demonstrates that *Rhodobacter sphaeroides* Tau3 can derive a majority of its cellular carbon directly from the sulfonate taurine. The difference in sulfonate metabolism between *Rhodopseudomonas palustris* Tau1 and *Rhodobacter sphaeroides* Tau3 demonstrates another aspect of the metabolic diversity within the purple nonsulfur bacteria.

**ACKNOWLEDGEMENTS**

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