***Roseovarius* sp. strain 217: aerobic taurine dissimilation via acetate kinase and acetate-CoA ligase**

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Abstract

The genome sequence of *Roseovarius* sp. strain 217 indicated that many pathway enzymes found in other organisms for the degradation of taurine are represented, but that a novel, apparently energy-dependent pathway is involved in the conversion of acetyl phosphate to acetyl CoA. Thus, an ABC transporter for taurine could be postulated, while inducible taurine: pyruvate aminotransferase, alanine dehydrogenase, sulfoacetaldehyde acetyltransferase and sulfite dehydrogenase could be assayed. Whereas phosphate acetyltransferase has been found in other organisms, none was indicated in the genome sequence and no activity was found in cell-free extracts. Instead, acetate kinase was active as was acetate-CoA ligase.

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Keywords

acetate:CoA ligase (AMP-forming); acetate
kinase; *Burkholderia xenovorans* LB400;
genome sequences; *Roseovarius* sp. strain 217;
taurine dissimilation.

Introduction

The first two research groups to work on the biochemistry of taurine dissimilation found evidence for different pathways leading to the desulfonation of sulfoacetaldehyde (Kondo *et al.*, 1973; Shimamoto & Berk, 1979). It has now been demonstrated that two uptake systems for taurine (ABC [TC 3.A.1.17.1] or TRAP [TC 2.A.56.4.1] transporters), two deamination systems (taurine dehydrogenase [EC 1.4.2.-] or the combination of taurine:pyruvate aminotransferase (Tpa) [EC 2.6.1.77] and alanine dehydrogenase (Ald) [EC 1.4.1.1]) and three subgroups of the desulfonative sulfoacetaldehyde acetyltransferase (Xsc) [EC 2.3.3.15] are widespread and can be found in different combinations in different organisms (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004; Denger *et al.*, 2006; Gorzyska *et al.*, 2006). The excess ammonium ion from the deamination reaction is believed to be exported by the ammonium-methylammonium transporter AmtB (Gorzyska *et al.*, 2006). The fate of the sulfite released in aerobic bacteria by Xsc seems to involve different sulfite dehydrogenases (e.g. SorAB) ([EC 1.8.2.1]), presumably intracellular or periplasmic, with correspondingly different sulfate (TauZ) [TC 9.B.63.1.1] or sulfite exporters (OrfX) (Rein *et al.*, 2005; Denger *et al.*, 2006; Cook *et al.*, 2007). The fate of the acetyl phosphate produced by Xsc in

aerobes has always involved phosphate acetyltransferase (Pta) [EC 2.3.1.8] to yield acetyl-CoA (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004; Denger *et al.*, 2006; Gorzyska *et al.*, 2006), but the unfinished genome sequence of *Roseovarius* sp. strain 217 (Schäfer *et al.*, 2005) indicated an alternative pathway.

Initial hypothesis of this study with these sequence data (Fig. 1a) involved an inducible ABC transporter (TauABC), the involvement of Tpa, Ald and an Xsc of subgroup 3, whereby the fate of both the sulfite and the acetyl phosphate was unclear. The presence of an acetate kinase gene (*ack*) directly downstream of *xsc* then led to the prediction that taurine served as a sole source of nitrogen for growth and that the carbon atoms from taurine would be excreted as acetate. This would be a novel variant of established excretion of a carbon moiety from taurine (Denger *et al.*, 2004b; Styp von Rekowski *et al.*, 2005; Weinitschke *et al.*, 2005). However, when Schäfer and Murrell supplied the organism, they informed us that taurine was a good carbon source. An unknown (or unsequenced) Pta or an alternative activation pathway to yield acetyl-CoA from the acetate generated by Ack was thus required, with which the organism could supply carbon for fatty acid synthesis, the Krebs cycle and the alternative glyoxylate shunt (see Alber *et al.*, 2006).

Analytical methods

Taurine was quantified by HPLC after derivatization with 2,4-dinitrofluorobenzene (Denger *et al.*, 1997). Acetate was determined by GC (Laue *et al.*, 1997). Sulfate was measured as a suspension of BaSO₄ (Sörbo, 1987). Protein in whole cells was assayed in a Lowry-type reaction (Cook & Hütter, 1981); protein in crude extract was assayed by dye-binding (Bradford, 1976). The ammonium ion was determined enzymically (Bergmeyer & Beutler, 1984).

Enzyme assays

Tpa was assayed discontinuously by following taurine disappearance and alanine formation (Denger *et al.*, 2004a). Ald was assayed photometrically at 365 nm (Denger *et al.*, 2004a). Xsc or Ack was assayed discontinuously in a spectrophotometer as the formation of the iron complex of acetylhydroxamate (Rose, 1955; Ruff *et al.*, 2003). Pta was assayed photometrically as the formation of acetyl-CoA (Bergmeyer *et al.*, 1983). Sulfite dehydrogenase (Sor) was assayed photometrically as the oxidation of ferricyanide (Reichenbecher *et al.*, 1999). Acs was assayed discontinuously using HPLC analysis to quantify reaction products. The reaction mixture contained 50 mM triethanolamine buffer, pH 8.0 (containing 2.5 mM MgCl₂), 0.2 mM ATP, 0.2 mM CoA, 10 mM acetate and c. 0.25 mg protein. Samples of 500 µL were taken at intervals and added to 500 µL methanol, to stop the reaction. The formation of acetyl CoA and AMP and the decrease of ATP and CoA were followed by HPLC: the mobile phase was 50 mM potassium phosphate buffer, pH 2.2, and a linear gradient of methanol to 30% was applied.

Results and discussion

Quantitative aspects of growth

Roseovarius sp. strain 217 grew exponentially with taurine as the sole source of carbon and energy (Fig. 2a); the growth rate (μ) was 0.045 h⁻¹ (representative of three experiments). The utilization of taurine was concomitant with growth (Fig. 2b) and the molar growth yield (6.5 g protein mol C⁻¹) was in the range consistent with quantitative utilization of the substrate (Cook & Hütter, 1981): no release of acetate was detected (Fig. 2b). The specific utilization rate of taurine was calculated from these data to be 3.8 mkat kg⁻¹ of protein. A net release of about 9 mM NH₄⁺ from about 12 mM taurine was observed (Fig. 2b); the balance of the nitrogen was calculated to be in cell polymers. The formation of about 10 mM SO₄²⁻ was found (Fig. 2b), which was presumed to represent quantitative release of sulfate from the sulfonate moiety. There was, thus, mass balance for the

Fig. 2. Kinetics of growth with taurine of *Roseovarius* sp. strain 217 (a), and substrate utilization and product formation as a function of growth (b). ●, turbidity; ▲, taurine; ■, sulfate; ○, ammonium ion; □, acetate.

carbon, nitrogen and sulfur moieties during the catabolism of taurine.

Taurine could serve as a sole source of nitrogen for the growth of *Roseovarius* sp. strain 217 with malate as the source of added carbon (not shown). There was no excretion of acetate under those conditions, either.

Roseovarius sp. strain 217 was tested for growth with different aliphatic sulfonates and was shown to grow well with isethionate, cysteate, sulfolactate, *N*-methyltaurine and *N*-acetyltaurine as sole sources of carbon and energy. In all cases, sulfate was detected in the medium, indicating substrate utilization. The organism did not grow with methanesulfonate (Schäfer *et al.*, 2005), sulfoacetate, sulfo-propane or 1,3-disulfopropane.

Enzyme activities

Roseovarius sp. strain 217 could be grown in gram-amounts and crude cell extracts were generated. Extracts of malate-

Table 1. Specific activities of enzymes relevant to taurine dissimilation in extracts of induced and noninduced cells of *Roseovarius* sp. strain 217

Enzyme	Specific activity (mkat kg ⁻¹ of protein) in extracts of cells grown with	
	Malate	Taurine
Taurine:pyruvate aminotransferase (Tpa)	ND	1.9
Alanine dehydrogenase (Ald)	0.4	10
Sulfoacetaldehyde acetyltransferase (Xsc)	ND	3.8
Sulfite dehydrogenase (Sor)	0.3	5.3
Phosphate acetyltransferase (Pta)	ND	ND
Acetate kinase (Ack)	0.2	9.4
Acetate-CoA ligase (Acs)	0.07	0.8

ND, not detected.

grown cells contained negligible levels of enzyme activities, which are directly involved in sulfonate metabolism (Table 1). In contrast, extracts of taurine-grown cells contained the predicted activities of Tpa, Ald, and Xsc, as well as Sor (Table 1). These data are interpreted to confirm the induction of the enzymes of the degradative pathway for taurine, including the ABC transporter (TauABC; Fig. 1), for which no direct evidence is available. The frequent occurrence of the *tauR*-gene in a cluster involving a taurine pathway (Brüggemann *et al.*, 2004; Denger *et al.*, 2004a, b, 2006; Gorzynska *et al.*, 2006; T.H.M. Smits, unpublished) leads us to postulate that TauR is the transcriptional regulator for expression of the taurine genes. TauR is a MocR-type regulator in the GntR-family, and the conservation level within this large group is $\geq 40\%$.

No activity of phosphate acetyltransferase was found (Table 1), in contrast to work in other organisms (Ruff *et al.*, 2003; Brüggemann *et al.*, 2004; Denger *et al.*, 2006; Gorzynska *et al.*, 2006). However, as predicted from the partial genome sequence (Fig. 1), inducible acetate kinase (Ack) was detected in significant amounts (Table 1) relative to the specific turnover rate of taurine (3.8 mkat kg⁻¹ protein, above). Correspondingly, 11-fold induction of the activity of Acs was detected (Table 1). The reaction was followed by HPLC, where the formation of acetyl CoA was confirmed by cochromatography with authentic material (not shown). Similarly, the formation of AMP from ATP was detected. The activity was apparently that of EC 6.2.1.1 [acetate:CoA ligase (AMP-forming)].

It was of concern that the assay of Pta might be inadequate, so another organism was used as a positive control. Inducible Pta was found in *B. xenovorans* LB400, which was previously shown to utilize taurine (Ruff *et al.*, 2003). No Ack was formed under these conditions, though Acs (0.6 mkat kg⁻¹ protein) was observed (not shown).

Roseovarius sp. strain 217 is the first aerobic organism to be shown to contain an alternative pathway to the Pta observed previously (cf. Cook & Denger, 2002; Denger *et al.*, 2006; Gorzynska *et al.*, 2006), but recent data from genome sequencing indicate other aerobes with a similar pathway. *Roseobacter* sp. strain MED193 contains a cluster containing *tauR*, *xsc*, a gene encoding a DUF81 protein (DUF, domain of unknown function), *ack* and *tauZ* but no *pta* gene; given candidate *tpa*- and *acs*-genes, a pathway similar to that in Fig. 1b, but with a known sulfate exporter (TauZ) (Rein *et al.*, 2005; Denger *et al.*, 2006), is presumed to be present. Similarly, in *Dinoroseobacter shibae* DFL 12, one of the three *xsc*-paralogues is flanked by *tauR*- and *ack*-genes.

Conclusions

Roseovarius sp. strain 217 is able to generate acetyl CoA from acetyl phosphate, but instead of the energetically neutral reaction of Pta, a two-step sequence is used in which the high-energy phosphate bond from acetyl phosphate is conserved as ATP in Ack, but two high-energy bonds are expended in Acs. This alternative route to generate acetyl CoA for biosynthesis of fatty acids, for entry into the tricarboxylic acid cycle and into the newly found alternative glyoxylate anaplerotic pathway (Alber *et al.*, 2006), is apparently not limited to *Roseovarius* sp. strain 217, because a similar set of genes is found in two other organisms in the *Roseobacter* clade. There would appear to be very considerable diversity in the pathways of taurine dissimilation, which suggests that the substrate must be widespread in many ecosystems.

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