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ORIGINAL PAPER

(*R*)-Cysteate-nitrogen assimilation by *Cupriavidus necator* H16 with excretion of 3-sulfolactate: a patchwork pathway

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Abstract *Cupriavidus necator* H16 grew exponentially with (*R*)-cysteate, a structural analogue of aspartate, as sole source of nitrogen in succinate-salts medium. Utilization of cysteate was quantitative and concomitant with growth and with the excretion of the deaminated product (*R*)-sulfolactate, which was identified thoroughly. The deaminative pathway started with transport of (*R*)-cysteate into the cell, which we attributed to an aspartate transporter. Transamination to sulfopyruvate involved an aspartate/(*R*)-cysteate:2-oxoglutarate aminotransferase (Aoa/Coa) and regeneration of the amino group acceptor by $NADP^+$-coupled glutamate dehydrogenase. Reduction of sulfopyruvate to (*R*)-sulfolactate was catalyzed by a (*S*)-malate/(*R*)-sulfolactate dehydrogenase (Mdh/Sdh). Excretion of the sulfolactate could be attributed to the sulfite/organosulfonate exporter TauE, which was co-encoded and co-expressed, with sulfoacetaldehyde acetyltransferase (Xsc), though Xsc was irrelevant to the current pathway. The metabolic enzymes could be assayed biochemically. Aoa/Coa and Mdh/Sdh were highly enriched by protein separation, partly characterized, and the relevant locus-tags identified by peptide-mass fingerprinting. Finally, RT-PCR was used to confirm the transcription of all appropriate genes. We thus demonstrated that *Cupriavidus necator* H16 uses a patchwork pathway by recruitment of 'housekeeping' genes and sulfoacetaldehyde-degradative genes to scavenge for (*R*)-cysteate-nitrogen.

Keywords Sulfonate metabolism · Cysteate aminotransferase · 3-sulfopyruvate dehydrogenase · Sulfolactate excretion · Transport

Abbreviations

RT-PCR	Reverse-transcription PCR
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
MALDI-TOF	Matrix-assisted laser desorption time of flight (mass spectrometry)
Mdh/Sdh	(*S*)-malate/(*R*)-sulfolactate dehydrogenase
Aoa/Coa	Aspartate/(*R*)-cysteate:2-oxoglutarate aminotransferase
Gdh	Glutamate dehydrogenase
TauE	Sulfite/organosulfonate exporter
Xsc	Sulfoacetaldehyde acetyltransferase
Pta	Phosphotransacetylase

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Introduction

(*R*)-Cysteic acid (L-cysteate, 2-amino-3-sulfopropionic acid) is a naturally occurring amino-sulfonic acid, which was discovered in weathered wool as residues of oxidized cystine (Consden et al. 1946; Benning 2007). Various marine (Ito 1969) and freshwater algae (Shibuya et al. 1963) contain cysteate, and spiders' webs are extracellular sources of this sulfonate (Fischer and Brander 1960). The

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compound is also relevant in bacteria and archaea: in *Bacillus subtilis*, it is formed during sporulation in the presence of 3-sulfolactate (Koshikawa et al. 1981), which contributes 5 % to the dry weight of the endospores (Bonsen et al. 1969); in some archaea, a cysteate synthase functions in an alternative biosynthetic pathway for coenzyme M (Graham et al. 2009). Capnine is a bacterial sulfolipid, whose polar head group is synthesized from cysteate (Godchaux and Leadbetter 1980; White 1984).

Cysteate, as a structural analogue of aspartate, was shown to be deaminated by aspartate aminotransferase (EC 2.6.1.1) in both archaea and mammals (Weinstein and Griffith 1988; Helgadóttir et al. 2007). Several types of aspartate transporters were reported to interact with the sulfonic or sulfinic analogue (Schellenberg and Furlong 1977; Palmieri et al. 1979).

Cysteate is a versatile substrate for bacteria. Under oxic conditions, it is degraded as a carbon and energy source (Stapley and Starkey 1970; Rein et al. 2005; Denger et al. 2006) and used as a sulfur source (Stapley and Starkey 1970; Seitz et al. 1993). Under anoxic conditions, it is utilized as carbon and energy source in respirations with nitrate or iron(III) as electron acceptors (Denger et al. 1997; Mikosch et al. 1999), or as an electron acceptor (Lie et al. 1996; Laue et al. 1997b). In fermentations, cysteate was found to serve as sulfur source (Chien et al. 1995) or as carbon source, with the dismutation of the sulfonate moiety to sulfide and sulfate (Laue et al. 1997a).

The utilization of cysteate as a source of both carbon and nitrogen was reported 40 years ago (Stapley and Starkey 1970). But no organism has been reported, which utilized the cysteate-nitrogen and excreted an organosulfonate (Stapley and Starkey 1970; Rein et al. 2005; Denger et al. 2006), whereas this phenomenon is well-known with taurine (2-aminoethanesulfonate) (Krejčík et al. 2008, 2010) and homotaurine (3-aminopropanesulfonate) (Mayer and Cook 2009). The homotaurine pathway was elucidated in *Cupriavidus necator* (formerly *Ralstonia eutropha*) H16, a metabolically versatile β-proteobacterium, whose genome is completely sequenced (Pohlmann et al. 2006). The desulfonation pathways in *C. necator* H16 involve the shared three final steps in desulfonation, namely *xsc* (encoding the desulfonative sulfoacetaldehyde acetyltranserase (Xsc, [EC 2.3.3.15])), *pta* (encoding phosphotransacetylase, [EC 2.3.1.8]) and *tauE* (encoding sulfite/sulfonate exporter TauE, [TC 2.A.102.2.1]) (Weinitschke et al. 2007, 2010a, b; Denger et al. 2011). *C. necator* H16 catalyzes the deamination of homotaurine in a patchwork pathway involving the 4-aminobutyrate (GABA) pathway and the *xsc-pta-tauE* cluster (Mayer and Cook 2009).

We now report that *C. necator* H16 can utilize (*R*)-cysteate as a sole source of nitrogen and excrete sulfolactate. The patchwork involves (1) 'housekeeping' enzymes, which make the ammonium ion available for assimilation into biopolymers and (2) the *xsc-pta-tauE* cluster, which provides an exporter of sulfolactate and thereby maintains constant osmolarity in the cell.

Materials and methods

Chemicals

3-Sulfopyruvate (Denger et al. 2001) and racemic 3-sulfolactate (Roy et al. 2003) were synthesized as described elsewhere. Commercial chemicals were of the highest purity available from Sigma-Aldrich, Merck, Roth, Fluka or Biomol.

Organism, growth conditions, preparation of extracts and enzyme separations

Cupriavidus necator (formerly *Ralstonia eutropha*) H16 (DSM 4058) (Pohlmann et al. 2006) was grown aerobically at 30 °C in a phosphate-buffered mineral salts medium, pH 7.2 (Thurnheer et al. 1986). The sole source of nitrogen was either 2 mM (*R*)-cysteate or 2 mM ammonium; the carbon source was 10 mM succinate. Precultures (3 ml) were grown in 30-ml screw-cap tubes in a roller. Growth experiments were done on the 50-ml scale in a 300-ml Erlenmeyer flask shaken at 30 °C. Samples were taken at intervals to measure optical density at 580 nm (OD_{580}) and to quantify concentrations of protein, cysteate, sulfate, ammonium, sulfopyruvate and sulfolactate. Cultures for enzyme assays and for protein purification (1 l in 5-l Erlenmeyer flasks) were grown on a shaker and harvested at an OD_{580} of $\sim$ 0.6 by centrifugation (20,000$\times g$ 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 30- to 200-fold concentrated suspensions. Disruption was done by four to five passages through a chilled French pressure cell at 140 MPa (Junker et al. 1994) in the presence of DNase (50 µg ml^{-1}). Cell debris and the membrane/particulate fraction were removed by ultracentrifugation (170,000$\times g$ for 30 min at 4 °C). The supernatant fluid was designated the soluble fraction. Soluble fractions of cysteate-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 (0–75 mM in 10 min followed by 75–150 mM in 45 min) in 25 mM potassium phosphate buffer (pH 7.2) was applied. Active fractions were salted up to 1.7 M ammonium sulfate, the precipitate spun off, and the supernatant fluid subjected to hydrophobic interaction chromatography on Phenyl Superose HR 10/10

(Pharmacia); a linear decreasing gradient of ammonium sulfate was applied.

Analytical methods

Growth was followed as turbidity at 580 nm (OD_{580} = 1.0 = 157 μg protein ml^{-1}) or quantified as protein in a Lowry-type reaction (Cook and Hütter 1981). Sulfate was quantified turbidimetrically as a suspension of $BaSO_4$ (Sörbo 1987). The ammonium ion was assayed enzymatically by the reaction with glutamate dehydrogenase (Bergmeyer and Beutler 1984). Cysteate and glutamate were determined after derivatization with 2,4-dinitrofluorobenzene (DNFB) (Sanger 1945) and separation by reversed-phase HPLC as described previously (Mayer et al. 2008). 3-Sulfopyruvate and 3-sulfolactate were quantified by ion chromatography as described for sulfoacetate (Denger et al. 2004). Protein in extracts was measured by dye-binding (Bradford 1976). Denatured proteins were separated by SDS-PAGE (13 % gels) and stained with Coomassie brilliant blue R250 (Laemmli 1970).

Peptide-mass fingerprints were generated by the Proteomics-Facility of the University (http://www.proteomics-facility.uni-konstanz.de). The protease used was trypsin, and the analyses were done on the Bruker Esquire 3000+ machine after separation on the Agilent 1100 HPLC.

Enzyme assays

Aspartate/(*R*)-cysteate:2-oxoglutarate aminotransferase (Aoa/Coa) activity was assayed discontinuously at 30 °C as the formation of 3-sulfopyruvate and glutamate, and the concomitant depletion of cysteate. The reaction mixture contained 5–10 mM (*R*)-cysteate, 10 mM 2-oxoglutarate, 0.1 mM PLP and 30–300 μg protein ml^{-1} in 50 mM potassium phosphate buffer (pH 7.2). Samples (50 μl) were taken for the derivatization of cysteate and glutamate with DNFB and stopped by the addition to 1 ml of 0.1 M $NaHCO_3$. To follow sulfopyruvate formation discontinuously by ion chromatography, samples (20 μl) of the reaction were injected directly on to the column at intervals, where the eluent at pH 11 stopped the reaction.

(*R*)-Sulfolactate dehydrogenase was assayed photometrically as the sulfopyruvate-dependent oxidation of NADH as described previously for (*S*)-sulfolactate dehydrogenase (Denger and Cook 2010). Sulfopyruvate could be replaced as a substrate by oxaloacetate. The reverse reaction was measured with NAD^+ and malate in a glycine/hydrazine buffer (Denger and Cook 2010). The dehydrogenase reaction could also be followed discontinuously by ion chromatography in the presence of NADH as an increase in substrate concentration and a decrease in product concentration.

Glutamate dehydrogenase (Gdh) was assayed photometrically as the formation of NADPH (Schmidt 1974). Sulfoacetaldehyde acetyltransferase (Xsc, EC 2.3.3.15) was assayed discontinuously as the formation of acetyl phosphate from sulfoacetaldehyde (Denger and Cook 2010).

Transcriptional analyses

Cells for the preparation of total RNA were grown in the appropriate selective medium and harvested in the mid-exponential growth phase ($OD_{580nm} \approx 0.3$) by centrifugation (13,000×*g*, 10 min, 4 °C); the cell pellets were stored in RNA-*later* solution (Ambion) at −20 °C. Total RNA was prepared using the E.Z.N.A. Total RNA Miniprep Kit (Omega Bio-Tek), and this RNA preparation (40 μl) was treated with RNase-free DNase I (Fermentas), following the manufacturer's instructions. The M-MuLV Reverse Transcriptase kit (Fermentas) was used for reverse transcription of RNA (0.5 μg) according to the manufacturer's instructions. The cDNA from these preparations was used as template for PCR (1 μl in 20-μl reactions) using *Taq* DNA polymerase (Fermentas). The primers used for RT and subsequent PCR are listed in Table 1. The absence of residual DNA in the total RNA preparations was confirmed with PCR primer pair aoa, and chromosomal DNA was used as PCR template for the positive controls.

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. Orthologous neighborhood regions were compared with the IMG system of the DOE Joint Genome Institute (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi).

Results and discussion

Nitrogen-limited growth of *Cupriavidus necator* H16

Cupriavidus necator H16 grew exponentially in nitrogen-limited mineral salts medium with 2 mM (*R*)-cysteate as sole source of nitrogen and 10 mM succinate as carbon source. Utilization of cysteate was complete and concomitant with growth as was the excretion of a deaminated

Table 1 Sequences of PCR primers used for the transcriptional analysis of genes attributed to cysteate-N assimilation in *C. necator* H16

Primer name	Primer sequence (5′–3′)	Amplicon length (bp)
aoa-for	TTGCCGGCATGGTGGAATC	389
aoa-rev*	CGTCGGCGGGTTGGAGTAGT	
mdh-for	TCCGCGCAGCAAGGGTATG	400
mdh-rev*	GACGGTCGGCAGGAACACG	
xsc-for	ACCGACATCGGCAACATCAACTC	295
xsc-rev*	GGTTGTAGAAGTCCACCTGGTTCT	
pta-for	TGGTGTCGAGCTTCTTCCTGAT	395
pta-rev*	GCCGGCTTCCAGGCTGGGAA	
tauE-for	GGCACCTATTTCCAGACGGTGAC	261
tauE-rev*	CAGGGTGGCTGCGCTGGAACTC	

* primer used for reverse transcription

sulfonate, which was tentatively identified by ion chromatography as 3-sulfolactate; the conversion was stoichiometric. The identity of the product was confirmed by MALDI-TOF MS in the negative-ion mode. In out-grown cultures, the signal for cysteate ($m/z = 167.8 = ([M–H]^{-1}$) was absent and a new signal at $m/z = 168.8 = [M–H]^{-1}$ was observed, which was identical with that of authentic 3-sulfolactate ($M_{calc} = 170.1$). The $m/z = 168.8$ signal was not observed when the culture had been grown with ammonium as nitrogen source. During growth with cysteate-nitrogen, excretion of neither the ammonium nor the sulfate ion was detected. However, traces of 3-sulfopyruvate were excreted transiently as detected by MALDI-TOF MS ($m/z = 167.0 = [M–H]^{-1}$, consistent with $M_{calc} = 168.1$ of 3-sulfopyruvate). The growth rate was about 0.11 h^{-1}, and the molar growth yield was about 65 g protein (mol N)$^{-1}$, consistent with complete assimilation of cysteate-nitrogen into cell material (Cook 1987). The calculated specific degradation rate was 0.45 mkat (kg protein)$^{-1}$. Cultures with ammonium chloride as nitrogen source grew faster but showed the same molar growth yield. Finally, no growth was observed with cysteate as carbon source in the presence of a nitrogen source, or with succinate-carbon in the absence of a nitrogen source. These results led us to hypothesize the degradative pathway depicted in Fig. 1a.

Enzymes involved in the release of cysteate-nitrogen

The activities of the three metabolic enzymes in the hypothetical pathway (Fig. 1a) could be observed in the soluble fraction of cell extracts of cysteate-grown and ammonium-grown *C. necator* H16 (Table 2). Firstly, activity of an aspartate/(*R*)-cysteate:2-oxoglutarate aminotransferase was detected in cysteate-grown cells (1.6 mkat (kg protein)$^{-1}$), and a similar specific activity was detected in ammonium-grown cells (1.9 mkat (kg protein)$^{-1}$), indicating a constitutive enzyme. Secondly, $NADP^+$-dependent glutamate dehydrogenase activity (Gdh; EC 1.4.1.4), attributed to the regeneration of the amino group acceptor 2-oxoglutarate, was found in both cysteate-grown (0.4 mkat (kg protein)$^{-1}$) and ammonium-grown (0.6 mkat (kg protein)$^{-1}$) cells. The enzyme was considered to be constitutive, but it was unstable and it was not examined further. Thirdly, the reduction of 3-sulfopyruvate to 3-sulfolactate was assayed photometrically with soluble fractions of both cysteate-grown (2.5 mkat (kg protein)$^{-1}$) and ammonium-grown (2.7 mkat (kg protein)$^{-1}$) cells; thus, this enzyme was also constitutively expressed. The specific activities observed for these three enzymes were sufficient to explain the calculated specific degradation rate of 0.45 mkat (kg protein)$^{-1}$.

Separation, identification and some properties of Aoa/Coa and Mdh/Sdh

The soluble fraction of cysteate-grown *C. necator* H16 was subjected to anion-exchange chromatography. The Aoa/Coa eluted at sodium sulfate concentrations between 45 and 90 mM with an average specific activity of 90 mkat (kg protein)$^{-1}$ and an enrichment factor of about 50. A second purification step by hydrophobic interaction chromatography failed due to apparent inhibition by ammonium sulfate and interference of the ammonium ion with the detection method. Nevertheless, after anion-exchange chromatography, a prominent protein band on SDS-PAGE at about 43 kDa was observed in the active fractions only, and the band was excised and subjected to peptide-mass fingerprinting. The locus-tag, H16_A1151 (73 % coverage) (encoding a 43.2-kDa protein) (Fig. 1b), is annotated as an aspartate/tyrosine/aromatic aminotransferase gene (COG1448). The enriched enzyme preparation exhibited no transaminase activity with tyrosine as substrate, but cysteate was a substrate, and aspartate gave a fivefold higher activity. We thus concluded that the aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.57) of *C. necator* H16 (H16_A1151) accepts (*R*)-cysteate as substrate, as has been observed for other aspartate aminotransferases (Weinstein and Griffith 1988; Helgadóttir et al. 2007) and that it is responsible for the first scalar reaction (Aoa/Coa) in the hypothesized pathway (Fig. 1a).

The Coa reaction was followed discontinuously by HPLC and ion chromatography as the consumption of cysteate. This occurred concomitantly with the formation of 3-sulfopyruvate and glutamate (Fig. 2). The transaminase also accepted 3-aminopropanesulfonate (homotaurine) as amino group donor with a lower specific activity, but not racemic 2-aminobutyrate, taurine, β-alanine or glycine.

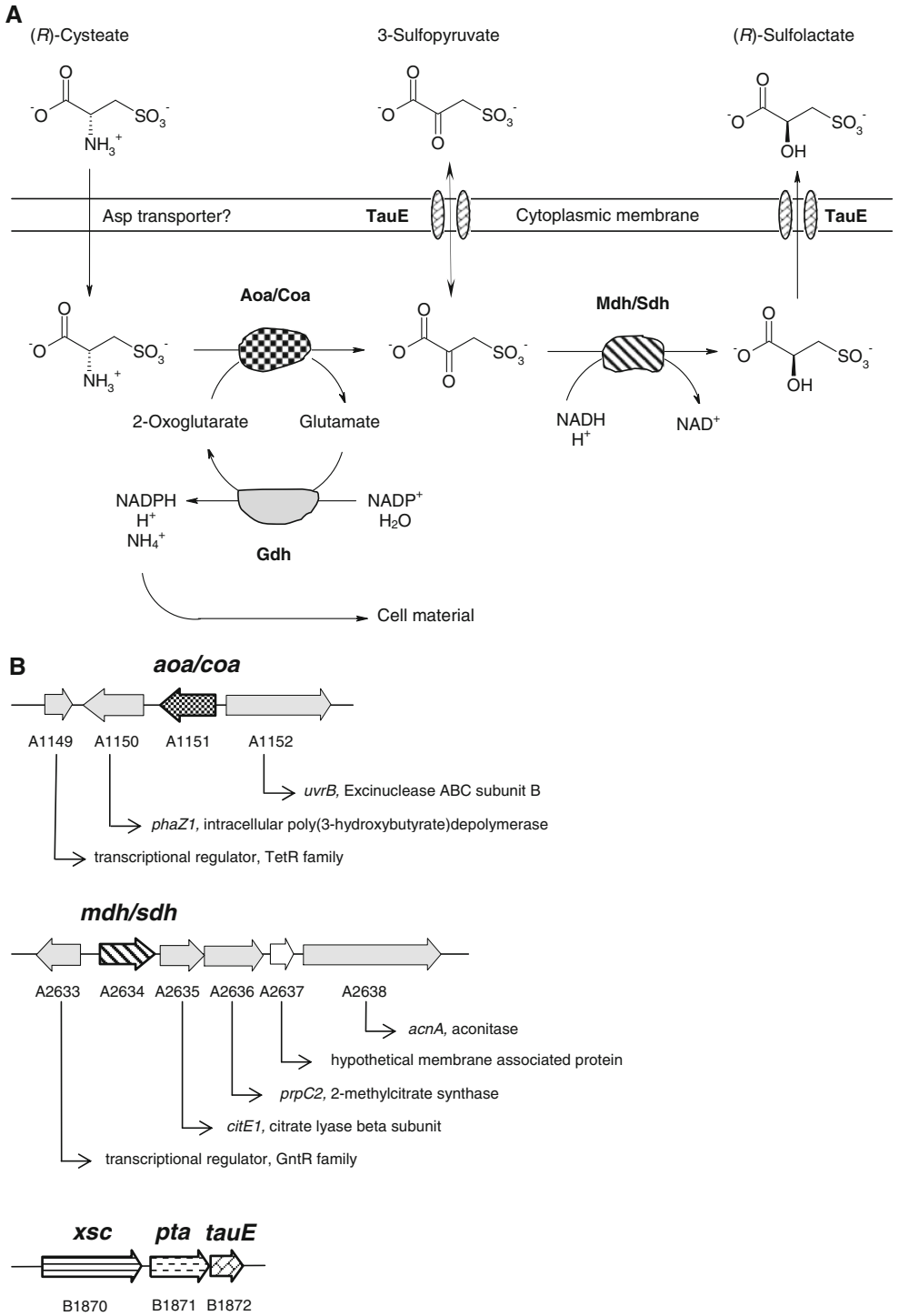

Fig. 1 Hypothesized pathway for the deamination of cysteate-nitrogen by *C. necator* H16 (**a**) and gene clusters encoding the hypothesized patchwork pathway proteins (**b**). The numbers in **b** represent locus-tags. *Asp* aspartate, *Aoa/Coa* (*S*)-aspartate: 2-oxoglutarate aminotransferase/(*R*)-cysteate:2-oxoglutarate aminotransferase, *Mdh/Sdh* (*S*)-malate dehydrogenase, *Gdh* (*S*)-glutamate dehydrogenase. Further abbreviations: see main text

The amino group acceptor 2-oxoglutarate could be replaced by pyruvate or oxaloacetate, but not by 2-oxobutyrate or glyoxylate. The enriched enzyme was stable and could be stored at 4 or −20 °C for many weeks.

Reduction of 3-sulfopyruvate to (*R*)-sulfolactate by Sdh could be measured photometrically with NADH as co-substrate. Sdh eluted from the anion-exchange column in fractions at sodium sulfate concentrations between 15 and

Table 2 Specific enzyme activities (mkat (kg protein)$^{-1}$) in soluble fractions of *C. necator* H16 under different growth conditions

Enzyme	Cysteate-N grown	Ammonium-N grown
Aspartate:2-oxoglutarate aminotransferase EC 2.6.1.1 with cysteate as amino group donor	1.6	1.9
Glutamate dehydrogenase EC 1.4.1.3	0.4	0.6
Malate dehydrogenase EC 1.1.1.37 with sulfopyruvate as substrate	2.5	2.7
Sulfoacetaldehyde acetyltransferase EC 2.3.3.15	3.1	>0.1

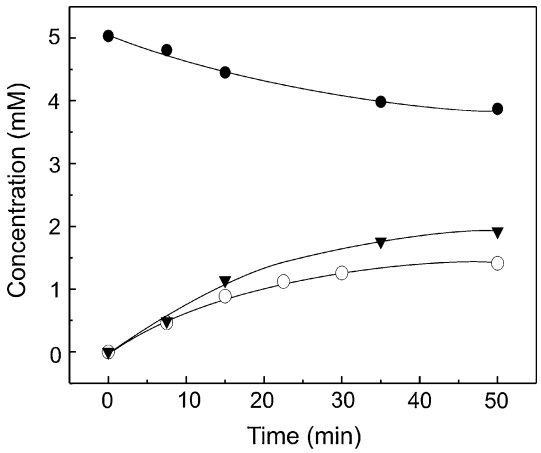

Fig. 2 Conversion of (*R*)-cysteate (*filled circle*) to 3-sulfopyruvate (*filled inverted triangle*) and glutamate (*empty circle*) by Aoa/Coa from *C. necator* H16. Enriched enzyme preparation free of sulfolactate dehydrogenase was used

80 mM, which partially overlapped the Coa fractions. The activity was enriched about 100-fold. A second purification step by hydrophobic interaction chromatography achieved a further enrichment as visualized on SDS-PAGE (Fig. 3, lane 4) with an enrichment factor of about 150. The prominent band with a molecular mass of about 35 kDa was excised and subjected to peptide-mass fingerprinting. The locus-tag, H16_A2634 (49 % coverage) (encoding a 35.1 kDa protein) (Fig. 1b), is annotated as a NAD-dependent (*S*)-malate dehydrogenase (EC 1.1.1.37) gene (COG0039). We concluded that the malate dehydrogenase H16_A2634 accepts sulfopyruvate as substrate and is responsible for the second scalar reaction (Mdh/Sdh) in the hypothesized pathway in *C. necator* H16 (Fig. 1a).

The conversion of sulfopyruvate to sulfolactate was followed discontinuously by ion chromatography (Fig. 4). NADH could not be replaced by NADPH. Oxaloacetate was a 40-fold better substrate for Mdh/Sdh than sulfopyruvate. An apparent K_m value of 0.16 ± 0.03 mM for oxaloacetate was calculated, whereas the K_m value for

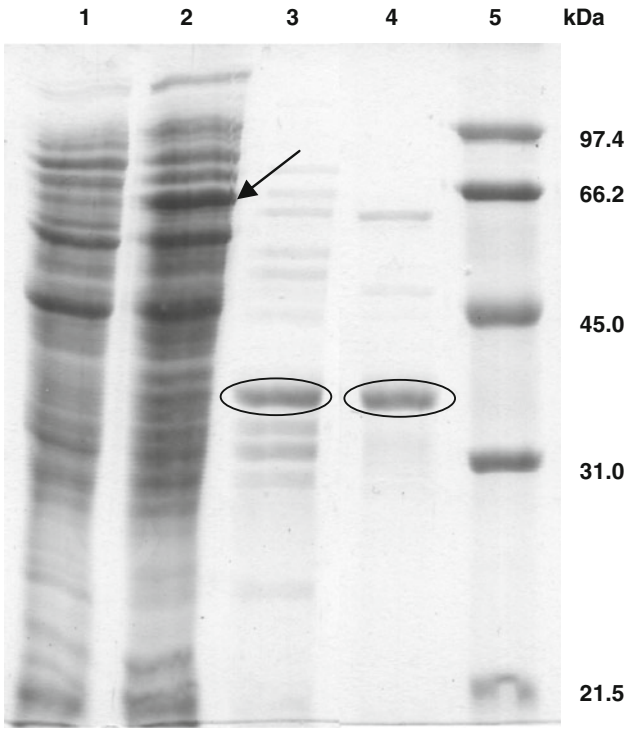

Fig. 3 SDS-PAGE of denatured protein fractions of *C. necator* H16. Key: lane 1, soluble fraction from cells grown with ammonium-nitrogen (non-induced); lane 2, soluble fraction from cells grown with cysteate-nitrogen (induced); lanes 3 and 4, Mdh/Sdh (marked by *circles*) in the active fraction obtained from anion-exchange chromatography (lane 3) and after hydrophobic interaction chromatography (lane 4); lane 5, molecular mass marker. The *arrow* indicates the induced band at 66 kDa (Xsc, see text)

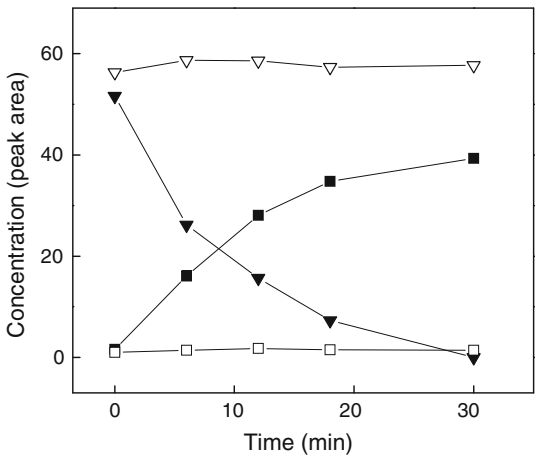

Fig. 4 Conversion of 3-sulfopyruvate (*filled inverted triangle*) to (*R*)-sulfolactate (*filled square*) by malate dehydrogenase from *C. necator* H16. 3-Sulfopyruvate (*empty triangle*) and (*R*)-sulfolactate (*empty square*) in a control experiment with boiled enzyme

sulfopyruvate was ≥10 mM. Pyruvate was not a substrate. The pH range for the reaction was broad (similar activity between pH 4.5 and 10.5). The reverse reaction could be measured with (*S*)-malate as substrate. (*R*)-Malate was not

a substrate. These findings and the known selectivity of malate dehydrogenases of EC 1.1.1.37 for the (*S*)-enantiomer led us to conclude that the sulfopyruvate-reducing enzyme produces (*R*)-sulfolactate, corresponding to the configuration of (*S*)-malate. The enzyme was stable and could be stored at 4 or −20 °C for many weeks.

Xsc, Pta, TauE and their involvement in scavenging cysteate-nitrogen

Sulfoacetaldehyde acetyltransferase, Xsc [EC 2.3.3.15], the key enzyme in C_2-sulfonate degradation (Cook and Denger 2002), was found to be expressed inducibly during growth with cysteate as a nitrogen source (see Table 2). Xsc was visible when comparing soluble fractions of induced and non-induced cells by SDS-PAGE (Fig. 3, lanes 1 and 2), that is, as a prominent band at about 66 kDa, the molecular mass of all known Xscs. During separation by anion-exchange chromatography, Xsc eluted at about 200 mM sodium sulfate, but the activity was unstable. The band at 66 kDa was excised and subjected to peptide-mass fingerprinting, which identified the Xsc gene, locus-tag H16_B1870 (40 % coverage) in the *xsc-pta-tauE* gene cluster (Fig. 1b). The gene directly downstream of the Xsc gene, H16_B1871 (Fig. 1b), encodes for phosphotransacetylase (Pta, 36.0 kDa, [EC 2.3.1.8]), which is also involved in C_2-sulfonate degradation (Cook and Denger 2002). This gene was found to be expressed inducibly during growth with cysteate as a nitrogen source (see below), and the (inactive) protein was found serendipitously (68 % coverage).

The neighboring gene downstream of *pta*, H16_B1872 (Fig. 1b), is known to encode for the sulfite/sulfopropanoate-exporter TauE (Mayer and Cook 2009). Orthologues of *tauE* export sulfoacetate or isethionate (Krejčík et al. 2008, 2010). We hypothesized that TauE is also inducibly expressed during growth with cysteate as a nitrogen source and is responsible for the export of (*R*)-sulfolactate and the traces of 3-sulfopyruvate (Fig. 1a). Xsc and Pta, however, which have no evident function for the assimilation of cysteate-nitrogen, are co-expressed as one *xsc-pta-tauE* regulon.

Transcription of genes involved in cysteate-nitrogen assimilation

The transcription of the *xsc, pta* and *tauE* genes when cysteate served as nitrogen source, but not when ammonium ion was utilized, was confirmed by RT-PCR (Fig. 5). Thus, our hypothesis of TauE being the sulfite/organosulfonate exporter with a wide substrate range, now including also 3-sulfolactate and 3-sulfopyruvate, is viable. The transcription of the *coa/aoa* and *mdh/sdh* genes was confirmed in both cysteate- and ammonium-grown cells (Fig. 5), which is in agreement with the constitutively expressed enzyme activities observed (Table 2). The constitutive expression of these enzymes is unsurprising, since they are known 'housekeeping' enzymes and apparently have cysteate and sulfopyruvate in their substrate range (Weinstein and Griffith 1988; Helgadóttir et al. 2007). The genes surrounding *mdh* (Fig. 1b) encode housekeeping enzymes, such as aconitase and the *β*-subunit of citrate lyase downstream, or succinate dehydrogenase and citrate synthase upstream (not shown). Gene *aoa/coa* is also located in a cluster with housekeeping genes, for poly(3-hydroxybutyrate) depolymerization and DNA repair (Fig. 1b).

Transport of (*R*)-cysteate

Cysteate is a charged molecule at physiological pH (Andrews and Schmidt 1927) and therefore requires transport across the cell membrane (Graham and White 2002). Considering the deamination of cysteate by aspartate transaminase (present work; see also Weinstein and Griffith 1988; Helgadóttir et al. 2007), we attributed transport of cysteate to an aspartate transporter. Evidence that amino acid transporters also translocate sulfonates such as cysteate or homocysteate is available (Schellenberg and Furlong 1977; Essenberg 1984), and several transport systems for aspartate are known and characterized, for example, from *E. coli*, *Pseudomonas putida* or *Bacillus subtilis*

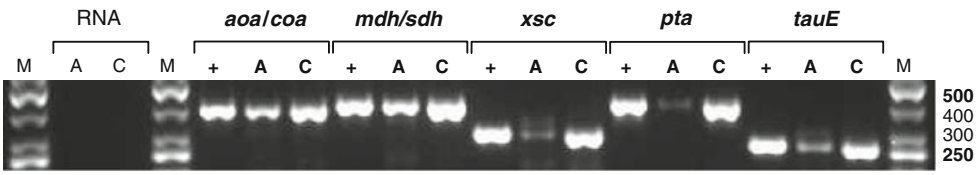

Fig. 5 Transcriptional analysis of the attributed genes for cysteate-nitrogen assimilation in *C. necator* H16 cells grown with ammonia-nitrogen or cysteate-nitrogen. Primers used for reverse-transcription PCR are given in Table 1. Lanes: (M), length marker; (+), PCR-positive controls with chromosomal DNA as template; (A), PCR with cDNA as template derived from reverse transcription of total RNA of ammonium-grown cells; (C), PCR with cDNA as template derived of cysteate-grown cells; (RNA), PCR control reactions for residual DNA contamination with total RNA as template from ammonium- and cysteate-grown cells, respectively, using the primer pair for gene *aoa/coa* for PCR. Relevant marker sizes (in kb) are indicated

(Schellenberg and Furlong 1977; Lorca et al. 2003; Singh and Röhm 2008). BLASTP searches with these sequences in *C. necator* H16 yielded several candidates for aspartate/cysteate transporters in the ABC superfamily, the amino acid-polyamine-organocation family, and the dicarboxylate/amino acid:cation symporter family. Strain H16 did, indeed, grow with aspartate as carbon or nitrogen source, thus at least one transporter for aspartate is functional.

Conclusion

This is the second patchwork pathway for an amino-C_3-sulfonate to be established in *C. necator* H16 to scavenge organic nitrogen for growth (*cf.* Mayer and Cook 2009). We presume that an undefined aspartate uptake system and three housekeeping enzymes for aspartate metabolism are recruited to release the amino group of (*R*)-cysteate. The use of constitutively expressed housekeeping enzymes (Fig. 1a) encoded in different gene clusters (Fig. 1b) differs from the first patchwork pathway (Mayer and Cook 2009), where a single, inducible gene cluster for γ-aminobutyrate metabolism (GabP, GabT and GabD1) is recruited to deaminate homotaurine. In this situation also, the sulfite/organosulfonate exporter TauE is expressed at the expense of an unprofitable co-expression of two proteins that are encoded in the same regulon, Xsc and Pta, at high levels (see Fig. 3, arrow; Mayer and Cook 2009). The excretion of the sulfonate, in this case sulfolactate, which will allow a constant osmotic pressure in the cell to be maintained, is probably worth the metabolic cost of synthesizing, for example, large amounts of Xsc (Fig. 3). The assembly of new pathways with enzymes from different pre-established pathways enables an organism to adapt quickly to nutrient limitation, here ammonium depletion by accessing alternative nitrogen sources such as cysteate and homotaurine. The excreted residues, in this case C_3-sulfonates, are good carbon sources for a wide range of other bacteria (Denger et al. 2009; Mayer and Cook 2009).

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